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Parazitace štěnicemi u netopýrů a člověka

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Poděkování

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Obsah

8.1 Využívání a střídání úkrytů u netopýrů

(1) BARTONIČKA T. & ŘEHÁK Z., 2007: Influence of the microclimate of bat boxes on their occupation by the soprano pipistrelle *Pipistrellus pygmaeus*: possible cause of roost switching. Acta Chiropterologica, 9(2): 517–526. (IF=0,831)

(2) BARTONIČKA T., BIELIK A. & ŘEHÁK Z., 2008: Roost switching and activity patterns in the soprano pipistrelle, *Pipistrellus pygmaeus*, during lactation. Annales Zoologici Fennici 45(6):503-512. (IF=1,03)

8.2 Životní cyklus štěnic a přežívání v úkrytech netopýrů

(3) BARTONIČKA T. & GAISLER J., 2007: Seasonal dynamics in the numbers of parasitic bugs (Heteroptera, Cimicidae): a possible cause of roost switching in bats (Chiroptera, Vespertilionidae). Parasitology Research, 100:1323–1330. (IF=2,327)

(4) BARTONIČKA T., 2010: Survival rate of bat bugs (*Cimex pipistrelli*, Heteroptera) under different microclimatic conditions. Parasitology Research, 107:827–833. (IF=2,327)

(5) BARTONIČKA T. & RŮŽIČKOVÁ L., 2013: Recolonization of bat roost by bat bugs (*Cimex pipistrelli*): could parasite load be a cause of bat roost switching? Parasitology Research, 112:1615–1622. (IF=2,327)

8.3 Vliv štěnic na chování netopýrů

(6) BARTONIČKA T., 2008: *Cimex pipistrelli* (Heteroptera, Cimicidae) and the dispersal propensity of bats: an experimental study. Parasitology Research, 104:163–168. (IF=2,327)

(7) BARTONIČKA T. & RŮŽIČKOVÁ L., 2012: Bat bugs (*Cimex pipistrelli*) and their impact on non-dwelling bats. Parasitology Research, 111:1233–1238. (IF=2,327)

8.4 Hostitelská specifita štěnic

(8) WAWROCKA K. & BARTONIČKA T., 2013: Two different lineages of bedbug (*Cimex lectularius*) reflected in host specificity. Parasitology Research, 112: 3897-904. (IF=2,327)

9. Seznam dalších publikací k tématu

1. Struktura habilitační práce a její cíle

Předložená habilitační práce se skládá z osmi vědeckých publikací. Jednotícím tématem habilitační práce je výzkum úkrytových strategií netopýrů s následným posunem k antiparazitárnímu chování vůči jejich nejběžnějším úkrytovým ektoparazitům – štěnicím. Habilitační práce má mezioborový vertebratologicko-parazitologický charakter a je doplněna o úvod do problematiky. Shrnující úvod je koncipován jako představení zásadních poznatků publikovaných v přiložených studiích, které jsou zasazeny do širšího kontextu problematiky životních strategií a ekologicko-biologických charakteristik studovaného modelu štěnice - netopýr.

Hlavní cíle habilitační práce:

1. U vybraných štěrbinových druhů netopýrů popsat změny v sezónním využívání úkrytů.
2. Definovat faktory, které mohou hrát roli ve změnách využívání úkrytů.
3. V terénních i laboratorních podmínkách testovat vybrané faktory ovlivňující využívanost úkrytů.
4. Popsat a sledovat antiparazitární strategie netopýrů s ohledem na jejich úkrytové parazity.
5. Sledovat vliv dočasného opouštění úkrytů netopýry na denzity štěnic.
6. Zjistit rychlost rekolonizace netopýřích úkrytů štěnicemi.
7. Testovat hostitelskou specifitu štěnice domácí (*Cimex lectularius*) parazitující na netopýrech a člověku.

2. Úvod

Studium vztahu parazit – hostitel patří nepochybně k vděčným biologickým tématům. V tomto směru však netopýři nepatřili nikdy k typické modelové skupině hostitelů. Většina studií věnovaná jejich ektoparazitům byla zaměřena spíše faunisticky a vzájemné interakci se věnovala pouze okrajově. Tradičně studovaní ektoparazité netopýrů tráví na těle hostitele většinu svého ontogenetického vývoje a na chování hostitele související s úkrytovými strategiemi mají pouze omezený vliv. Zcela odlišná je však situace u štěnic, které na těle hostitele tráví pouze krátkou dobu během sání krve a většinu života žijí mimo hostitele. Díky rychlému vývoji a velkému reprodukčnímu potenciálu na jedné straně a značné mobilitě na straně druhé patří štěnice nepochybně k významným ektoparazitům nejen netopýrů, ale i člověka. Štěnice v úkrytech netopýrů však žijí skrytým způsobem a snad i proto jim byla v minulosti věnována pouze malá pozornost. Štěnice si netopýři na svých tělech přenášejí pouze ojediněle na rozdíl od ostatních permanentních ektoparazitů (např. muchulí a roztočů), a to z nich činí jedinečný model při výzkumu adaptivních úkrytových strategií netopýrů. Vztah netopýrů a úkrytových parazitů, jako jsou štěnice, byl doposud z velké části založen pouze na předpokladech. Přiložené publikace, vycházející z terénního pozorování a následných experimentů, objasňují, jak významným stresorem štěnice pro netopýry jsou, jakým způsobem jsou provázány reprodukční cykly netopýrů a štěnic, a částečně také objasňují hostitelskou specifitu štěnic ve vztahu k člověku.

3. Problematika

3.1 Model parazit a hostitel

Podrobné studie o mezi- a vnitro-druhových mechanismech jsou nezbytné pro správné pochopení celkové biodiverzity. Vzhledem k rozmanitosti a složitosti životních strategií a vztahů k hostiteli jsou parazitické organismy velmi zajímavými a hodnotnými modely v evolučně-ekologických studiích. Prezentují celou škálu různých typů speciace a koevoluce (Huyse et al. 2005). Úroveň speciace a diverzifikace parazita pramení ze stupně závislosti na hostiteli a jeho životních strategiích. Dále je pak závislá na počtu preferovaných hostitelů, oblastí jejich výskytu, populační struktuře hostitelů a charakteru jejich rozptýlení v čase a prostoru (Esch et al. 1975). V populacích parazita se pak setkáváme s určitými podjednotkami - démy (populace parazita na sociální skupině hostitele více či méně izolovaná od jiných hostitelských démů) a infrapopulacemi (populace parazita z jednoho hostitelského jedince), které představují různý stupeň izolace mezi sebou (Mayr 1963). U štěnic parazitujících na netopýrech nebo ptácích, kteří většinu života tráví mimo tělo hostitele, je však termín infrapopulace obtížně použitelný. Je proto vhodnější u nich používat termín dém. Ten je zde reprezentován populací parazita v jednom úkrytu nebo hnízdě.

Jestliže předpokládáme, že polymorfismus v populaci parazita souvisí s preferencí určitých životních podmínek (určitá část těla hostitele nebo část jeho úkrytu), je pravděpodobné, že parazit bude mít tendenci lépe přežívat jen v některých z nich. V podmínkách, kde bude vykazovat vyšší fitness, bude hledat i svého sexuálního partnera a bude se tedy pářit s dostupnými jedinci podobných genotypů. To však logicky povede ke snížení genového toku mezi jednotlivými démy parazita. Schopnost parazita přežívat na větším spektru hostitelských druhů vede nejen k vyššímu polymorfismu (Brooks & McLennan 1993), ale též v případě dalšího omezování genového toku k sympatrické speciaci (např. v případě adaptace jedné sub-populace parazita na jiný hostitelský druh), která následně vede opět k obnovení úzké specificity vůči (novému) hostitelskému druhu (Combes & Théron 2000). Ačkoliv je sympatrická speciace (bez existence geografické bariéry) ekology tradičně odmítána, podmínky ve vztahu parazit a hostitel ji umožňují a často je označována termínem alloxenická speciace (Melhorn 2008).

Lokální diferenciace zdůrazňuje závody ve zbrojení (host races) mezi hostitelem a parazitem (Drès & Mallet 2002). Koncept závodů ve zbrojení bývá často definován z více směrů 1) střídání hostitelských druhů a z ní vyplývající různá věrnost a závislost na hostiteli, 2) soužití v sympatrii s jediným hostitelem nebo 3) vztah mezi vyhledáváním sexuálního partnera a změnou hostitele (Funk 2012). Důsledkem toho jsou pak například genetické diferenciace nebo obecné ovlivnění toku genů v rámci populace parazita či mezi nimi. S ohledem na stupeň diferenciace mezi jednotlivými entitami parazitů, je pak pravděpodobnost opakování těchto změn v časoprostoru vyšší. Na druhou stranu je pravděpodobnost fixace nové charakteristiky parazita závislá na efektivní velikosti jeho populace, jeho životním cyklu, plodnosti, stupni závislosti na hostiteli, migralitě hostitele či kolonizaci nových hostitelských skupin (Gandon & Michalakis 2002, Barrett et al. 2008).

3.2 Využívání a střídání úkrytů u netopýrů

Věrnost úkrytům není u mnoha druhů savců příliš vysoká a v případě druhů netopýrů využívajících úkryty ve stromech je dokonce poměrně nízká (Lewis 1995). Terénní pozorování dokládají, že druhy netopýrů přes den využívající štěrbinové úkryty, je střídají několikrát za reprodukční sezónu, dokonce i každých několik dní (Lausen & Barclay 2002, Mazurek 2004, Rancourt et al. 2005). Takové chování bylo pozorováno právě u druhů využívajících úkryty ve stromech (např. *Myotis californicus*, Barclay & Brigham 2001; *Eptesicus fuscus*, Willis & Brigham 2004). Štěrbinové úkryty ve stromech využívá i řada zástupců evropských rodů *Nyctalus*, *Pipistrellus* a *Myotis*; z posledně jmenovaného rodu pak především malé druhy jako *M. bechsteinii*, *M. daubentonii*, *M. nattereri*, *M. mystacinus* a *M. brandtii* (Meschede & Heller 2000, Kerth et al. 2001, Meschede 2001). K dalším druhům netopýrů rodící svoje mláďata ve stromových úkrytech dále patří i *Plecotus auritus* a *Barbastela barbastellus*.

Úkryty ve stromech jsou často různého typu, může jít o dutiny, vzniklé činností některých datlovitých ptáků nebo vyhníváním, štěrbiny a pukliny v rozsochách větví nebo za odchlípnutou borkou (Vonhof & Barclay 1996). Dnes existují již práce věnované tomu, jaký typ dutin netopýři upřednostňují (teplota, vlhkost, velikost, tvar, výška a orientace vletového otvoru, hloubka dutiny, mocnost stěny dutiny), aby byl pro netopýry atraktivní (Sedgeley & O'Donnell 1999, Gibbons et al. 2002, Russo et al. 2004, Willis & Brigham 2005, Spada et al. 2008, Ruczyński et al. 2010).

U výše zmíněných druhů se však v řadě případů setkáme s letními koloniemi i v náhradních typech antropogenních úkrytů (Russo et al. 2004). Situace, kdy jeden druh netopýra využívá jak přirozené typy úkrytů, tak štěrbinové typy úkrytů v budovách, je stále častější. S rostoucí synantropizací některých druhů roste jejich vazba na úkryty v budovách a postupně klesá využívanost úkrytů ve stromech. Do této skupiny patří např. netopýr rezavý (*Nyctalus noctula*) (Boonman 2000), ale i dvojice druhů *Pipistrellus pipistrellus* a *Pipistrellus* *pygmaeus* (Thompson 1992, Park et al. 1996, Ceľuch et al. 2006). Netopýři využívají jak dřevěné stavby (posedy, seníky, sruby), tak se mohou ukrývat za dřevěným obložením stěn, za okenicemi nebo ve šterbinách pod střešní krytinou běžných lidských stavení. Ale některé druhy využívají i štěrbiny mezi panely nebo větrací šachty v panelových domech (*N. noctula*, *P. pipistrellus*, Ceľuch et al. 2006).

Řada z přirozených úkrytů má krátkou životnost (odchlípnutá kůra, Grindall 1996, Grindall 1999) a nedává netopýrům jinou volbu, než úkryt změnit (Grindal & Brigham 1998). Naopak úkryty v dutinách a zejména budovách jsou plně dostačující po více roků a přesto je netopýři pravidelně střídají. Jarní osidlování úkrytů je dočasné a jsou využívány na cestě ze vzdáleného zimoviště do místa reprodukce (Dzal et al. 2009). V létě jsou stromové dutiny, půdy budov či různé štěrbiny využívány samicemi jako místo úkrytu reprodukční kolonie. Shromažďují se v nich různě početné skupiny, které zde rodí a odchovávají svá mláďata. Samci tráví letní období solitérně. Ve druhé polovině léta, kdy se po osamostatnění mláďat rozpadají reprodukční kolonie, a počátkem podzimu, jsou dutiny ve stromech nebo obložení domů využívány jako úkryty, kde se netopýři dočasně shromážďují při přesunu na místo zimování (Dzal et al. 2009). Pohlavně dospělí jedinci některých druhů se v dutinách páří. U některých z nich se projevuje teritoriální chování, kdy samci po obsazení dutiny hájí tento úkryt před jinými samci. V té době vznikají i nestabilní harémové skupiny (např. netopýři rodu *Pipistrellus*), tvořené nejčastěji jedním samcem a několika samicemi (Jahelková et al. 2000).

Dalším faktorem ovlivňujícím obsazenost úkrytu v určité části vegetační sezóny je blízkost bohatého potravního stanoviště (Boonman 2000). Blízký výskyt vodního biotopu je obvykle zárukou vysoké potravní nabídky. Ta je též vysoká na okrajích porostů a v úzkých průletových koridorech (Kusch et al. 2004). Netopýři se také během reprodukční sezóny mohou přesunout do jiného úkrytu v závislosti na změně potravní nabídky (Davidson-Watts & Jones 2006).

Řada studií ukazuje, že samice některých druhů nejsou omezeny pouze na úkryt v konkrétním stromě, ale jedna kolonie se může rozdělit do více úkrytů (Kerth & König 1999, Willis & Brigham 2004). Tato sociální struktura, popsaná jako fission-fusion model, může pomoci udržet řadu výhod spojených s životem ve velké kolonii a současně omezit některé jeho nevýhody. Fission-fusion model byl u evropských netopýrů popsán detailně na druhu *Myotis bechsteinii* (Kerth & König 1999). Podskupiny vzájemně velmi příbuzných samic střídají úkryty na poměrně malém území velmi často, obvykle každý 2. - 4. den (Kerth & König 1996). Podobné chování bylo zjištěno i u řady běžných severoamerických druhů netopýrů jako např. *Eptesicus fuscus* nebo *Myotis lucifigus* (Barclay et al. 1979, Willis & Brigham 2004). Rozptyl jediné kolonie mezi více úkrytů se může na první pohled jevit jako intenzivní střídání úkrytů více koloniemi. U druhů tvořících početné kolonie v budovách (např. *M. myotis*) dochází též k výměně jedinců. Samice se však nepřesouvají všechny naráz a do stejného úkrytu. Nedochází tedy pravidelně k situacím, kdy všichni netopýři úkryt opustí během jediné noci, jako je to pozorováno u štěrbinových druhů osidlujících stromy.

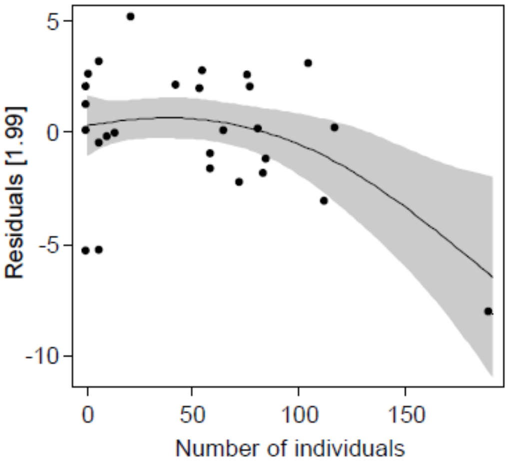
Pravděpodobně nejčastěji uváděným důvodem střídání úkrytů jsou odlišné teplotní preference a požadavky na prostor se zvyšujícím se počtem jedinců ve skupině v průběhu roku (Whitaker 1998, Lefebvre et al. 2003). Teplotní a vlhkostní preferendum se liší jednak podle druhu netopýra (Harmata 1969) a jednak podle fáze reprodukčního cyklu (gravidita, laktace, postlaktační období, harémové úkryty) (např. Gaisler 1970, Lourenço & Palmeirim 2004). Netopýři si vybírají pro každé období úkryt s optimálními mikroklimatickými podmínkami (Kerth et al. 2001, Lourenço & Palmeirim 2004). Nepochybně znají alternativní úkryty, včetně jejich mikroklimatu v předstihu, jelikož pátrání po novém úkrytu až v případě potřeby souvisí s dalšími energetickými náklady a mohlo by znamenat značné riziko např. úhynu mláďat, potratu apod. (Lewis 1995).

V roce 2002 byl na PřF MU, PřF UK a AVČR zahájen výzkum komplexu hemi-synantropních netopýrů rodu *Pipistrellus* (projekty GAČR 206/06/0954 *Intraspecific variability of populations of two cryptic bat species of genus Pipistrellus in Central Europe*, GAČR 206/02/0961 „*Situation Pipistrellus pipistrellus superspecies in Czech Republic*“). Výzkum byl zaměřen zejména na rozšíření a vztahy dvou středoevropských druhů *Pipistrellus pipistrellus* a tehdy čerstvě popsaného *Pipistrellus pygmaeus*. Před rokem 2000 oba druhy nebyly na území České republiky a většiny Evropy rozlišovány a v této práci i publikacích následujících jsou u starších údajů uváděny jako *P. pipistrellus* sensu lato (s.l.). V souvislosti s intenzivním výzkumem těchto netopýrů byla zjištěna i řada behaviorálně-ekologických poznatků souvisejících zejména s obsazováním úkrytů. Z našeho pozorování (Bartonička 2004, Bartonička & Řehák 2007) vyplývá, že samice *P. pygmaeus*, podobně jako samice *P. pipistrellus*, mění úkryty během letního období několikrát. Počátkem června gravidní samice sledované úkryty opuštěly a vracely se do něj i se svými vzletnými mláďaty teprve v polovině července. Thompson (1992) zjistil, že většina jím pozorovaných kolonií *P. pipistrellus* s.l. během reprodukční sezóny využívá 2 až 3 úkryty a pouze 20% kolonií navštěvovalo úkrytů více. Feyerabend & Simon (2000) sledovali pomocí telemetrie u jediné kolonie *P. pipistrellus* s.l. přesuny mezi osmi úkryty během dvou let. Bartonička et al. (2008) telemetricky zjistili, že označené kojící samice *P. pygmaeus* přeletovaly mezi 2-3 úkryty. Společné však většině pozorování je, že samičí kolonie obou druhů několik dní před obdobím porodů opustí dosavadní úkryt a přesunou se do hlavního porodního úkrytu (Swift 1980, Webb et al. 1996). Bartonička et al. (2008) nikdy nezjistili, že by všechny samice opustily hlavní porodní úkryt v budově v průběhu laktace, zatímco satelitní úkryty, kam se přesouvala vždy pouze část kolonie, ano. Bohužel telemetricky byly samice sledovány pouze v průběhu laktace a není tedy známo, zda změnily úkryty využívané v graviditě. Z výše uvedeného je tedy zřejmé, že netopýři rodu *Pipistrellus* střídají úkryty častěji než synantropní druhy osidlující půdy budov (např. *M. myotis*). Ačkoliv jsme v článku Bartonička et al. (2008) toto chování viděli jako analogické s chováním definovaným výše jako fission-fusion model, pozdější naše studie ukázaly, že netopýři rodu *Pipistrellus* využívají jeden úkryt na dobu výrazně delší než je uváděna ve studii Kerth & König (1999).

Výše je nastíněno několik aspektů, které ovlivňují střídání úkrytů netopýry. Přestože je zřejmé, že u různých druhů jsou důvody odlišné a všechny nejsou doposud uspokojivě vysvětleny (Lewis 1995, Vonhof & Barclay 1996, Brigham et al. 1997), lze je dle dostupné literatury shrnout do čtyř možných skupin:

- náhlé změny mikroklimatických podmínek související s omezenou cirkulací vzduchu ve štěrbinovém úkrytu (Whitaker 1998, Lourenço & Palmeirim 2004) nebo změna v nárocích na mikroklima úkrytu s ohledem na reprodukční cyklus (gravidita versus laktace) (např. Thompson 1990, Hamilton & Barclay 1994, Kerth et al. 2001).
- vyhnutí se vzrůstající parazitaci s cílem udržení tělesné kondice nebo snížení rizika postnatální mortality mláďat (Wolz 1986, Lewis 1996).
- rušení lidskou činností, predátorem nebo jiné neznámé vlivy (Lewis 1995).
- dostupnost potravní nabídky v blízkosti úkrytů (Willis & Brigham 2004, Fleischmann & Kerth 2014)

V naší studii Bartonička & Řehák (2007) jsme testovali hypotézu, která předpokládala střídání úkrytů v důsledku potřeby jiného mikroklimatu v průběhu gravidity, kdy se samice snaží využívat maximálně stav denní strnulosti, a laktace, kdy naopak při častém kojení mláďat samice upadat do strnulosti nemohou (Kerth et al. 2001). Ve vnitřní teplotě zkoumaných netopýřích budek jsme nezjistili žádné rozdíly mezi budkami obsazenými a neobsazovanými, jak v průběhu gravidity, tak během laktačního období. Vnitřní teplota negativně korelovala s větším počtem netopýrů, zejména během gravidity (Obr. 1). V období před odletem z obsazených budek (v období gravidity) nebyly zjištěny žádné extrémní výkyvy ve vnější teplotě, které by mohly souviset s opuštěním úkrytu v souvislosti s jeho přehřátím. Nebyly zjištěny ani rozdíly ve vnitřní teplotě mezi úkryty, které netopýři před porody opustili a kde zůstali, přestože signifikantní rozdíly mezi reprodukčními obdobími nalezeny byly. Naše výsledky potvrzují, že samice před porody nejsou motivovány k přesunu výhradně mikroklimatickými podmínkami, přestože se mezi graviditou a laktací mění jejich možnost využívat denní letargie. Důvod přesunu samic v období před porodem mláďat studií spolehlivě objasněn nebyl.



Obr. 1. Negativní vztah mezi počtem netopýrů a vnitřní teplotou, grafický výstup generalizovaného aditivního modelu. Šedá zóna označuje 95% intervaly spolehlivosti a číslo v závorce na y ose udává stupně volnosti.

3.3 Úkrytoví ektoparaziti netopýrů

Obvykle jsou studována společenstva ektoparazitů různých druhů netopýrů bez ohledu na jejich životní strategie (např. Whitaker & Mumford 1978, Morkel 1999, Whitaker et al. 2000, Ritzi et al. 2001, Ritzi & Whitaker 2003). Na netopýrech bylo zjištěno více než 680 druhů parazitujícího hmyzu (Arixeniidae, Cimicidae, Polyctenidae, Nycteribiidae, Streblidae, Ischnopsyllidae, Tungidae) a dále především roztočů včetně klíšťat (Argasidae, Ixodidae, Acaridae, Glycyphagidae, Sarcoptidae, Laelapidae, Macronyssidae, Spinturnicidae, Demodecidae, Myobiidae a Trombiculidae) (Scheffler 2011). Většina výzkumů, které se zabývaly ekologickými souvislostmi vztahu ektoparazit – netopýr, je však realizována na ektoparazitech permanentních respektive takových, kteří tráví na těle netopýra většinu svého života (např. Hůrka & Povolný 1968, Gannon & Willig 1995, Sasse & Pekins 2000, Moura et al. 2003). Některé publikace řeší přímo problematiku koevoluce ektoparazita a hostitelského druhu netopýra (např. Patterson et al. 1998). Specifita ektoparazita je závislá na historicko-fylogenetických, fyziologických, biochemických nebo behaviorálně morfologických faktorech (Ryšavý et al. 1988, Volf & Horák 2007). Extrémně dlouhá doba koexistence blech (Ischnopsyllidae) a muchulí (Nycteribiidae) s netopýry je mezi savci ojedinělá a poskytla oběma skupinám dostatek času na koadaptaci (Horáček 1986). Není tedy divu, že vysoká úroveň závislosti na hostiteli byla opakovaně v centru zájmu výzkumníků (např. Grulich & Povolný 1955, Rosický 1957).

Ektoparazitům trávícím na svém hostiteli pouze krátký čas potřebný k sání krve, tzv. úkrytovým parazitům, však byla dosud věnována pouze omezená pozornost. K nejvýznamnějším úkrytovým ektoparazitům netopýrů patří zástupci roztočů (Acari), klíšťáci čeledi Argasidae (např. klíšťák netopýří *Carios vespertilionis*) a zejména štěnice čeledi Cimicidae. Tito obligátní ektoparazité recentně téměř výhradně asociovaní s ptáky, netopýry a člověkem mají dorzoventrálně zploštělé tělo a silně sklerotizovaný integument, což snižuje poškození při pohybu ve štěrbinách a usnadňuje tak únik před hostitelem (Usinger 1966, Marshall 1980). Čeleď Cimicidae zahrnuje asi 110 druhů zařazených do 24 rodů a šesti podčeledí (Henry 2009). Zástupci této čeledi jsou přítomni na všech kontinentech; pouze z Austrálie nejsou známy původní druhy (Usinger 1966).

Omezení štěnic na letouny je poměrně úzké a netradiční ve srovnání s jinými krevsajícími skupinami hmyzu. Navíc se zdá, že netopýři jsou pravděpodobně původní hostitelé štěnic (Horváth 1913), zejména pak čeledi Vespertilionidae a Molossidae (Marshall 1982). Asi dvě třetiny z celkového počtu druhů uvedených výše, parazituje na netopýrech a z nich některé příležitostně sají i na člověku. Nejméně tři taxony z podčeledi Cimicinae parazitují však na lidech pravidelně a vytváří na nich stabilní populace: *Cimex lectularius* (Linnaeus, 1758), *Cimex hemipterus* (Fabricius, 1803) a *Leptocimex vespertilionis* (Ferris & Usinger, 1957) (Usinger 1966, Marshall 1981, Balvín 2013).

Vysoká mobilita a schopnost dlouhodobé existence mimo hostitele staví štěnice mezi význačné modelové ektoparazity (Usinger 1966). Tělo hostitele využívají pouze k sání krve a k transportu do dalších hostitelských úkrytů.

3.4 Stručný přehled systematiky rodu Cimex

V úvodu do problematiky je třeba zmínit, že kompletní systematika čeledi Cimicidae dosud není zcela ujednocena a v literatuře se setkáváme s jejím různým pojetím. Druhy rodu *Cimex* jsou podle tradičních morfologických kritérií děleny do čtyř skupin (Usinger 1966).

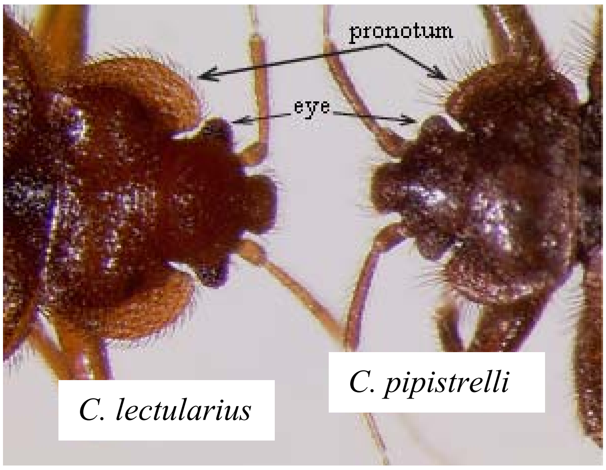
1. Skupina kolem druhu *Cimex pilosellus* (Horváth, 1910) zahrnuje několik druhů žijících na netopýrech a občas i na člověku v Severní a Střední Americe.

2. Do skupiny druhů *Cimex lectularius* náleží druh *Cimex lectularius* (Obr. 2a, 3), který parazituje na netopýrech, člověku, domestikovaných a (hemi)synantropních druzích savců, a dále *Cimex columbarius* Jenyns, 1839 vyskytující se na drůbeži a holubech. Posledním druhem skupiny je *Cimex emarginatus* známý pouze z netopýra brvitého (*Myotis emarginatus*).
3. Skupina druhů kolem *Cimex pipistrelli* parazituje pouze na netopýrech (Obr. 2b, 3). Přestože občas napadají i člověka, nejsou na něm schopny dlouhodobě přežívat. Skupina zahrnuje tři druhy popsané z Evropy a tři druhy popsané z Asie.
4. Druh *Cimex hemipterus* je vázaný především na tropické oblasti a parazitující na lidech, netopýrech a drůbeži.

Zde je však potřeba zmínit jisté nejasnosti v taxonomii štěnic parazitujících na netopýrech (Wendt 1941, Povolný 1957, Usinger 1966, Péricart 1972, Péricart 1996). V podmínkách střední Evropy se lze setkat jak s druhy skupiny *Cimex lectularius*, tak zde lze najít zástupce všech druhů skupiny *C. pipistrelli* (Tab. 1). Příležitostně jsou nalézáni zřejmě i zavlečení jedinci druhu *Cimex hemipterus*. O taxonomických nejasnostech v obou skupinách v posledních letech vznikla řada studií, které za použití standardních morfometrických přístupů i genetických znaků přehodnocují doposud publikované taxony. S ohledem na středoevropskou problematiku vynikají zejména práce O. Balvína (Balvín 2005, 2008, 2010 a 2013).

Z Evropy (v rámci Palearktu) byly popsány tři druhy štěnic žijících na netopýrech (*C. pipistrelli, C. dissimilis, C. stadleri*) (Tab. 1). Obvykle jsou shrnovány do skupiny *C. pipistrelli*. Pro klasifikaci evropských štěnic vyskytujících se na netopýrech různí autoři využívali omezený počet morfologických znaků či nedostatečný počet vyšetřených jedinců bez ohledu na časoprostorovou distribuci štěnic (např. Povolný 1957, Lansbury 1961, Péricart 1972). Snaha o klasifikaci na základě morfologických znaků však nevedla k přílišnému vyjasnění taxonomické problematiky. Navíc Usinger (1966) pomocí hybridizačních experimentů popsal určitou reprodukční bariéru mezi štěnicemi *Cimex pipistrelli* s. str. z Britských ostrovů a populace *C. stadleri* z tehdejšího Československa. Je však zjevné, že právě tyto morfologické rozdíly, způsobené snad adaptacemi na úkrytové chování jednotlivých hostitelských druhů netopýrů, vedly k popisu více druhů.

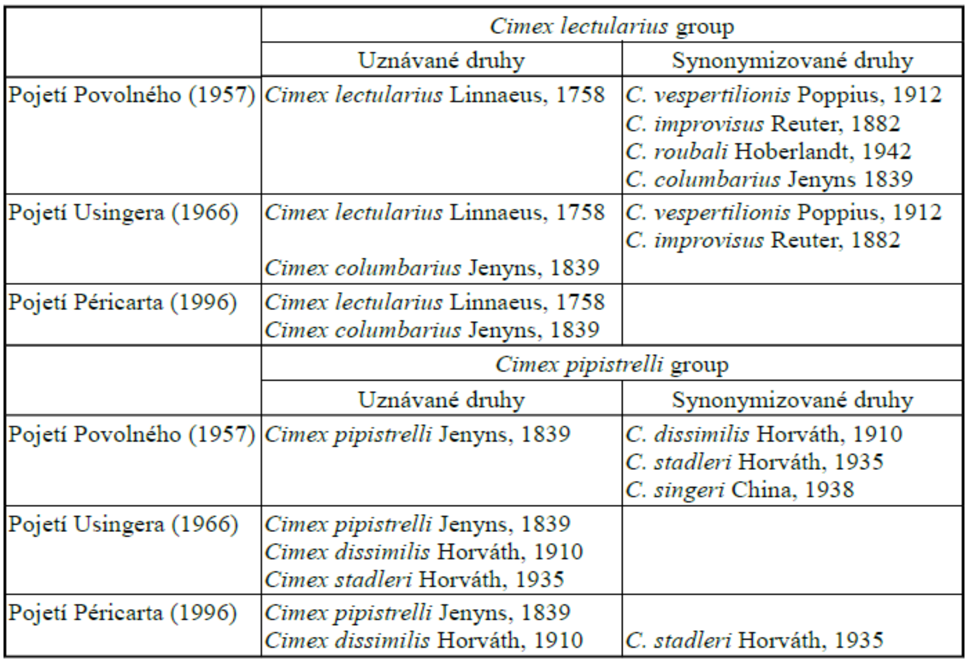
Obr. 2. Portréty modelových druhů štěnic a) štěnice *Cimex lectularius*, adultní samice, b) štěnice *C. pipistrelli*, adultní samec (foto V. Káňa).



Obr. 3. Srovnání tvaru štítu u druhu *C. lectularius* se zaoblenými výběžky a krátkými chlupy a *C. pipistrelli* s výběžky ostrými a dlouhým ochlupením (foto N.T. Gallagher).

Teprve v současnosti Balvín et al. (2013) doložili, že rozdíly v mitochondriální DNA neukázaly souvislost s morfologickými znaky ukazujícími značnou fenotypovou variabilitu štěnic mezi jednotlivými hostitelskými druhy netopýrů (Balvín et al. 2013). V následujícím textu se proto přidržím taxonomického označení druhů skupiny *Cimex pipistrelli* popsaných z Evropy jako *Cimex pipistrelli* s. lat., které vidím jako historicky oprávněné, ačkoliv recentní studie Balvín et al. (2013) již dokonce ukazuje i na neplatnost zbývajících dvou druhů (*C. dissimilis* a *C. stadleri*).

Tab. 1.: Seznam druhů skupin *Cimex lectularius* a *C. pipistrelli* jak jej charakterizují vybrané studie (převzato z Růžičková 2012).



	Cimex lectularius group	
	Uznávané druhy	Synonymizované druhy
Pojetí Povolného (1957)	*Cimex lectularius* Linnaeus, 1758	*C. vespertilionis* Poppius, 1912 *C. improvisus* Reuter, 1882 *C. roubali* Hoberlandt, 1942 *C. columbarius* Jenyns 1839
Pojetí Usingera (1966)	*Cimex lectularius* Linnaeus, 1758 *Cimex columbarius* Jenyns, 1839	*C. vespertilionis* Poppius, 1912 *C. improvisus* Reuter, 1882
Pojetí Péricarta (1996)	*Cimex lectularius* Linnaeus, 1758 *Cimex columbarius* Jenyns, 1839	
	Cimex pipistrelli group	
	Uznávané druhy	Synonymizované druhy
Pojetí Povolného (1957)	*Cimex pipistrelli* Jenyns, 1839	*C. dissimilis* Horváth, 1910 *C. stadleri* Horváth, 1935 *C. singeri* China, 1938
Pojetí Usingera (1966)	*Cimex pipistrelli* Jenyns, 1839 *Cimex dissimilis* Horváth, 1910 *Cimex stadleri* Horváth, 1935	
Pojetí Péricarta (1996)	*Cimex pipistrelli* Jenyns, 1839 *Cimex dissimilis* Horváth, 1910	*C. stadleri* Horváth, 1935

V minulosti popsaná diverzita souvisí spíše s adaptacemi na konkrétního hostitele, než se vznikem nových druhů. Patrně až na jedinou výjimku, která souvisí s významnou změnou hostitele, z tradičního hostitele (netopýra) na velmi vzdáleného hostitele (člověka) a to u druhu *C. lectularius*. Balvín et al. (2012a) popsali značnou divergenci v mitochondriální i jaderné DNA a morfologii mezi *C. lectularius* parazitující na člověku a netopýrech a ukazují, že se obě linie štěnic oddělily již před desítkami tisíc let v době posledního interglaciálu v souvislosti s disperzí moderního člověka z Afriky (Armitage et al. 2011). Další studie (Booth et al., in press), kdy autoři využili jaderné markery, mikrosatelity, nezjistila žádný genetický tok mezi liniemi štěnic parazitujících na člověku a na netopýrech. Mohlo by se tak jednat o případ sympatrické (alloxenické) speciace, pravděpodobně v důsledku dlouhodobého využívání různých hostitelů. Obě linie jsou od sebe též rozpoznatelné morfologicky.

Balvín et al. (2012a) dokládají, že úkryty synantropních netopýrů jako *M. myotis* a *M. emarginatus* poskytují v současnosti jediný typ úkrytu původním divokým populacím štěnice *C. lectularius* na většině území Evropy. Synantropizace těchto druhů netopýrů, stará několik století, umožnila štěnicím snadné šíření mezi lidskými příbytky. Lze předpokládat, že by se obě hostitelské linie mohly opakovaně potkávat a opětovně se pářit. Na druhou stranu opakovaná a stále se prodlužující oddělenost obou linií, prohlubovaná rostoucí “civilizovaností” člověka (odstartovaná např. již používáním ohně v jeskyních, Maestre 2013), mohla vést k tvorbě pre- i post-kopulačních reprodukčních bariér již daleko dříve.

Zcela odlišné životní strategie obou hostitelů vedly k postupné adaptaci linií na jednoho nebo druhého hostitele. Balvín et al. (2012a) uvádějí, že štěnice pocházející z netopýrů jsou větší, vyznačují se větším počtem sét a mají relativně kratší tělní výběžky jako tykadla, rostrum a končetiny. O tom, že obě linie vykazují značný stupeň potravní speciace, pojednává studie založená na experimentech, kdy byly štěnice *C. lectularius*, sebrané v úkrytech netopýrů, krmeny lidskou krví a naopak štěnice z lidí byly krmeny netopýří krví (Wawrocka & Bartonička 2013).

Pro úplnost charakteristiky skupiny *C. lectularius* zbývá dodat, že Simov et al. (2006) publikovali popis nového druhu štěnice *Cimex emarginatus* Simov, 2006. Populace této štěnice byla objevena v Bulharsku již v roce 1998 v kolonii netopýra brvitého, *Myotis emarginatus* (Geoffroy, 1806), od něhož je odvozen i druhový název štěnice. Její postavení v systému zůstává pro nejednoznačnost morfologických a molekulárních dat prozatím nevyjasněno, zdá se ale, že by mohlo jít o druh příbuzný *C. lectularius* s. stricto (O. Balvín, ústní sdělení).

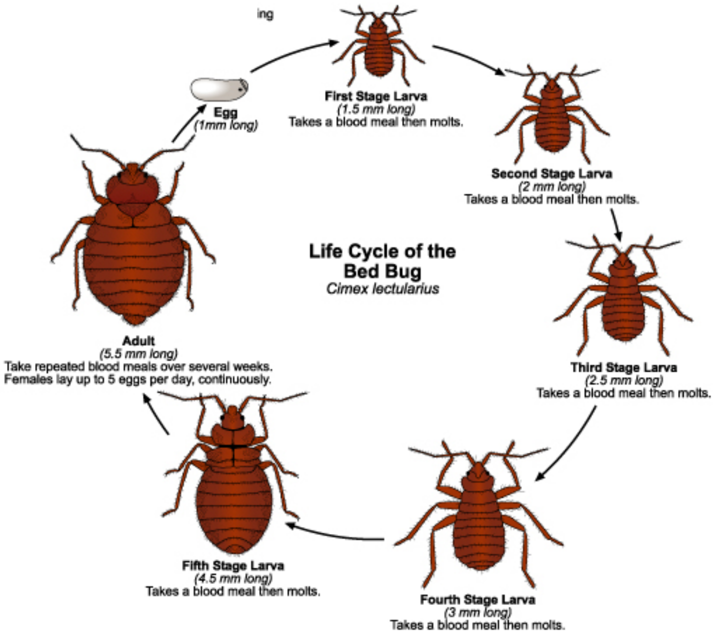
Mezi zástupci čeledi Cimicidae bylo v minulosti provedeno několik hybridizačních experimentů testujících přítomnost reprodukčních bariér (např. Ueshima 1964, Usinger 1966). V řadě případů byla u hybridů potvrzena snížená fitness. Hybridi byli získáni mezi blízkými i vzdálenými druhy (Usinger 1966), což dokládá malý význam pre-kopulačních bariér. V současnosti dokončujeme studii založenou na hybridizaci mezi hostitelskými liniemi štěnice *C. lectularius*. Přestože sporadicky dochází mezi smíšenými rodičovskými páry ke kopulacím a detekci spermatu v abdomenu samic, pářené samice nekladou vajíčka. To by ukazovalo na přítomnost post-kopulační bariéry (Wawrocka et al., in prep.). V tomto případě, kdy předpokládáme oddělení obou linií skrze prostorovou izolaci zapříčiněnou různým hostitelem, je absence pre-kopulačních bariér pochopitelná (Balvín & Bartonička, in press).

3.5 Životní cyklus štěnic a přežívání v úkrytech netopýrů

Následující informace odpovídají specifikům skupiny druhů *C. pipistrelli* a *C. lectularius*. Zástupci čeledi Cimicidae jsou oviparní. Všech pět nymfálních instarů i adultní jedinci žijí v úkrytech netopýrů nebo člověka, nikoliv přímo na těle hostitele (Usinger 1966). Samice jsou schopny klást vajíčka dokonce bez nasátí krve hostitele. Růst a vývoj štěnic je velice rychlý, při teplotě mezi 18 a 25°C se larvy za 30 dní pětkrát svléknou (Obr. 4). Poslední instary nepotřebují k dalšímu vývoji žádné speciální podněty a jsou-li přítomni v úkrytu i samci, mohou dospělé samice produkovat první vajíčka již do 5 dní po pohlavním dospění. V experimentálních chovech však k prvním snůškám dochází 14 – 20 dní po metamorfóze na imago (T. Bartonička, vlastní pozorování). Rychlý vývoj umožňuje štěnicím v úkrytu dosáhnout vysokých abundancí záhy po jeho osídlení netopýry. Samice je schopna denní produkce 2 - 4 vajíčka. Celoživotní snůška pak přesahuje i 500 vajec (Davis 1964), obvykle je to však méně. Štěnice sají v intervalu několika dní a jsou schopny několik měsíců hladovět (až 1,5 roku), což jim umožňuje přečkat zimní období nebo nepřítomnost hostitele v úkrytu (Johnson 1942, Povolný 1957, Overal & Wingate 1976, Marshall 1982). Při vhodné teplotě a optimální dostupnosti hostitele se však štěnice dožívají kolem jednoho roku. Usinger (1966) sledoval, že teplota a vlhkost úkrytu významně ovlivňuje přežívání všech vývojových stádií a také ochotu ke kopulaci. Štěnice však preferují celkově nižší teplotu úkrytu než většina ektoparazitů, neboť se na hostiteli zdržují pouze krátkodobě po dobu krmení (Usinger 1966). Mimo dobu krmení je lze najít v početných skupinách ve škvírach trámů nebo v záhybech polstrování nábytku (Obr. 5). Vyšší teplota naopak může snižovat jejich fitness (Reinhardt 2007). V současnosti existují pouze ojedinělé informace o tom, zda změny mikroklimatických podmínek a délka hladovění působí odlišně na přežívání jednotlivých vývojových stádií, popřípadě pohlaví (srv. Funakoshi 1977, Overal 1980). Dosavadní informace o délkách hladovění (až 250 dní) dokládají značnou odolnost imág (Dubinin 1947, Southwood 1954). Zdali jsou však schopny podobného hladovění i další instary, není známo. Doba hladovění s následnou schopností sát krev po znovuobjevení se hostitele v úkrytu je však zásadní pro predikci density štěnic při opětovném osídlení úkrytu hostitelem a je klíčovým momentem pro pochopení souvislosti stupně parazitace úkrytu a následného střídání úkrytů netopýry.

Bartonička & Gaisler (2007) zjistili, že absence hostitele v úkrytech, spolu s vysokými teplotami, vede ke snížení počtu dospělých štěnic a jejich vývojových stádií na méně než polovinu původního počtu. Na přežívání štěnic má bezesporu velký vliv teplota a vlhkost (Usinger 1966). Nicméně až do současnosti jen málo výzkumů řešilo ekologické vztahy mezi teplotou a přežitím jednotlivých instarů po různě dlouhém hladovění (Funakoshi 1977, Overal 1980). Předchozí studie ukázaly u štěnic na vysokou toleranci k dehydrataci (až 40 % ztráty vody v těle) a odolnost vůči vysokým teplotám (35-40°C) (Dubinin 1947, Southwood 1954).

Rivnay (1932) testoval vliv vlhkosti v rozmezí 10-70% na ontogenezi *C. lectularius*. Nezjistil však žádné signifikantní rozdíly v růstu ani svlékání nymf, vliv vlhkosti byl zanedbatelný. Lze však očekávat, že zejména v korelaci s vysokou teplotou v přirozených úkrytech budou mít první instary s vyšším obsahem vody v těle horší přežívání (Jones 1930, Johnson 1960, Usinger 1966). Reinhardt et al. (2008a) porovnávali teploty v úkrytu kaloně egyptského (*Rousettus aegyptiacus*) a v refugiu štěnice *Afrocimex constrictus*. Zjistili, že štěnice se raději za kaloni každou noc přesouvají několik metrů, než by riskovali trávit celou dobu v úkrytu s vyšší teplotou, která se negativně projevuje na úspěšnosti jejich reprodukce. Z jejich výzkumu vyplývá, že i poměrně malý teplotní rozdíl může ovlivnit fekunditu ektoparazita.



Obr. 4. Životní cyklus štěnice domácí (*Cimex lectularius*) (převzato z Usinger 1966).

Obr. 5. Úkryty štěnice *C. lectularius* v bytech najdeme často v záhybech polstrování nábytku (foto T. Bartonička).

Přežívání štěnic (*Cimex* spp.) je daleko více ovlivňováno okolní teplotou než je tomu u permanentních ektoparazitů. Délka ontogeneze štěnic je limitována mikroklimatickými podmínkami v úkrytu hostitele a dobou, kdy je hostitel přítomen. Doba pobytu hostitele je v úkrytu omezena zejména u štěnic parazitujících netopýry. Toto omezení by mohlo ovlivnit abundanci štěnic v úkrytu a způsobit asynchronizaci mezi reprodukcí ektoparazita a hostitele. Bartonička (2010) zjistil rozdílnou pravděpodobnost přežívání u jednotlivých ontogenetických stádií v rozmezí teplot 5-35°C. Nejvyšší přežívání bylo zjištěno u samičích imag a nymf 4. a 5. instaru. *C. pipistrelli* byla též tolerantnější k vyšším teplotám ve srovnání s *C. lectularius*. Štěnice *C. pipistrelli* z netopýrů měly kratší ontogenetický cyklus než štěnice *C. lectularius* parazitující na člověku. Schopnost lépe přežívat při vysokých teplotách (35°C) u druhu *C. pipistrelli* je pravděpodobně důsledkem jejího dlouhodobého soužití s netopýry, jejichž úkryty se v letních měsících snadno přehřívají. S druhem *C. lectularius* se lze v netopýřích úkrytech setkat též a dokonce je u některých druhů častějším parazitem než *C. pipistrelli*. Nedávné studie však ukazují, že štěnice druhu *C. lectularius* tvoří dvě oddělené linie parazitující na lidech a netopýrech (Balvín et al. 2012a, Wawrocka & Bartonička 2013). Zdali je tolerance k vyšším teplotám i u štěnic *C. lectularius* z lidí než těch z netopýrů, není prozatím známo, lze to však předpokládat.

3.6 Štěnice, její šíření a jako vektor patogenů

V posledním desetiletí došlo k prudkému nárůstu počtu nového výskytu štěnic druhu *C. lectularius* parazitujících na člověku (Reinhardt & Siva-Jothy 2007). Stejní autoři uvádějí, že prudký nárůst incidence v posledních letech je charakteristický pro tzv. západní svět - Ameriku a Evropu. Příčina nárůstu je patrně velmi komplexní, ať již souvisí s novými způsoby bydlení, zálibou ve starožitném a použitém nábytku, v němž mohou být štěnice ukryty, účinnější klimatizací, novými způsoby oblékání, častější přítomnost domácích zvířat v bytech, rozvojem letecké dopravy (Dogget et al. 2004) nebo prostě pouze se skutečností, že dnešní lidé štěnice nepoznávají (Seidel & Reinhardt 2013). Např. v Angliii předkládanou štěnici správně určilo pouze 10% dotázaných z 358 osob (Reinhardt et al. 2008b).

Za další možnou příčinou návratu štěnice domácí v posledních letech byly považovány restrikce počtu insekticidů a používání insekticidů se specifickou účinností jen na určitý druh hmyzu, jiný než štěnice. U štěnice domácí odchycené v lidských obydlích několika států USA a ve Velké Britanii byla zjištěna extrémně vysoká rezistence k pyrethroidům, deltamethrinu, cyhalothrinu, cypermethrinu a ke karbamátům, které se na celém světě k jejich hubení používají nejčastěji. Publikované výsledky naznačují, že jde o fenomén rozšířený i v jiných státech (Romero et al. 2007). Tato rezistence je ovládána několika neúplně dominantními geny, takže současně se štěnicemi se šíří i jejich vysoká rezistence (Ledvinka et al. 2008).

Studie Balvín et al. (2012a) a Booth et al. (in press) ukázali, že nárůst štěnic *C. lectularius* parazitujících na lidech v posledních letech, nesouvisí s přenosem štěnic z netopýrů a obě linie jsou dobře odděleny. Štěnice na člověka přešly patrně z netopýrů, když když společně obývali jeskyně (Sailer 1952, Usinger 1966, Usinger & Povolný 1966). Odtud se štěnice s novými hostiteli přesunuly do lidských obydlí, která jim klimaticky vyhovovala ještě lépe. V tomto kontextu je velmi zajímavá kombinace ztráty ochlupení u člověka a jeho následná parazitace úkrytovými parazity jako roztoči nebo štěnicemi v souvislosti s používáním oblečení a dlouhodobých úkrytů (Rantala 1999). K výstavbě trvalejších příbytků docházelo v souvislosti s rozvojem zemědělství někdy mezi 5 - 8 tisíci lety př. n. l. Štěnice se objevily jako ektoparazité člověka již v prvních psaných dokumentech. V průběhu 20. století napomohly jejich šíření obě světové války. Štěnice expandovaly díky přesunu velkého množství lidské populace a rovněž díky pasivnímu přenosu různými dopravními prostředky (Whitfield 1939). S masivním zavedením postřiků infestovaných bytů přípravky DDT během 2. světové války a především po ní došlo k téměř úplné likvidaci štěnic, do té doby zcela běžných v lidských obydlích. Navíc po 2. světové válce docházelo k nahrazování dřevěných rámů postelí kovovými, což značně omezilo možnosti úkrytu štěnic a současně usnadnilo jejich kontrolu (Potter 2008) a tedy včasné rozpoznání jejich přítomnosti a jejich následnou likvidaci.

Hostitelé jsou obvykle štěnicemi pokousáni v noci během spánku. Štěnice probodávají kůži a do ranky vpouštějí antikoagulanty a další látky jako hydraluronidázy, proteázy a kinázy. Krev sají přímo z vlásečnic, ale mohou sát i krev z poškozených tkání. Protože sliny štěnic obsahují anestetikum, je kousnutí prakticky bezbolestné a místo vpichu není cítit mnoho hodin. V důsledku velmi pozdní reakce si lidé obvykle štěnice jako původce nepřipouštějí, což významně usnadňuje jejich šíření (Reinhardt & Siva-Jothy 2007). Do rány však při vpichu pronikají i další antikoagulační, vasodilatační (např. oxid dusnatý) a proteolytické látky (např. apyráza), které obvykle způsobují prudkou alergickou reakci (Davis et al. 2009).

Kolem vpichu se tvoří typické kožní léze - svědivé erytematózní maculopapuly, které mají 5 mm až 2 cm v průměru, s centrálním hemorhagickým váčkem. Vícečetná kousnutí štěnic často sledují přímky nebo křivky (Obr. 6). Počty vzniklých lezí se pohybují od několika do mnoha desítek za noc, v závislosti na abundanci štěnic v úkrytu. Štěnice na tělech netopýrů přednostně sají na neosrstěných blánách, u člověka na neoblečených zónách těla (Obr. 6). U člověka někdy erupce napodobují kopřivku. Léze obvykle zmizí po 2-6 týdnech, ale lokální hyperpygmentace může přetrvávat i déle (Kolb et al. 2009, Reinhardt et al. 2009). Klinický obraz je závislý na imunokompetenci a předchozí expozici napadeného člověka. Komplikace, vyplývající z případné sekundární bakteriální infekce jsou výsledkem rozškrabání a exkoriace. Kolb et al. (2009) uvádí úplnou necitlivost k bodání štěnic bez následných reakcí i při opakovaném napadení asi u 20% lidí. Ti pak mohou být spolehlivými hostiteli a úspěšnými šiřiteli štěnic. V této souvislosti je jedinou spolehlivou diagnózou nález živých štěnic. Kromě dlouhodobého svědění trpí napadení lidé často nespavostí a psychickým stresem.

Obr. 6. Ukázka umístění vpichů a sání štěnicí domácí *C. lectularius*. a) u člověka na nekrytých částech těla s jemnou kůží, obvykle v řadách za sebou (převzato z Delaunay et al. 2011) a b) štěnice sající na létacích blánách mláděte *M. myotis* (foto R. Lučan).

Štěnice, jakožto v minulosti běžný ektoparazit člověka, je tedy logicky i v centru parazitologického výzkumu jako možný vektor významných onemocnění. Delaunay et al. (2011) uvádí na 40 patogenů (bakterií, plísní a virů), které byly nalezeny v trávicím traktu, výkalech, na povrchu těla či ve slinách štěnic z volné přírody nebo z laboratorních chovů.

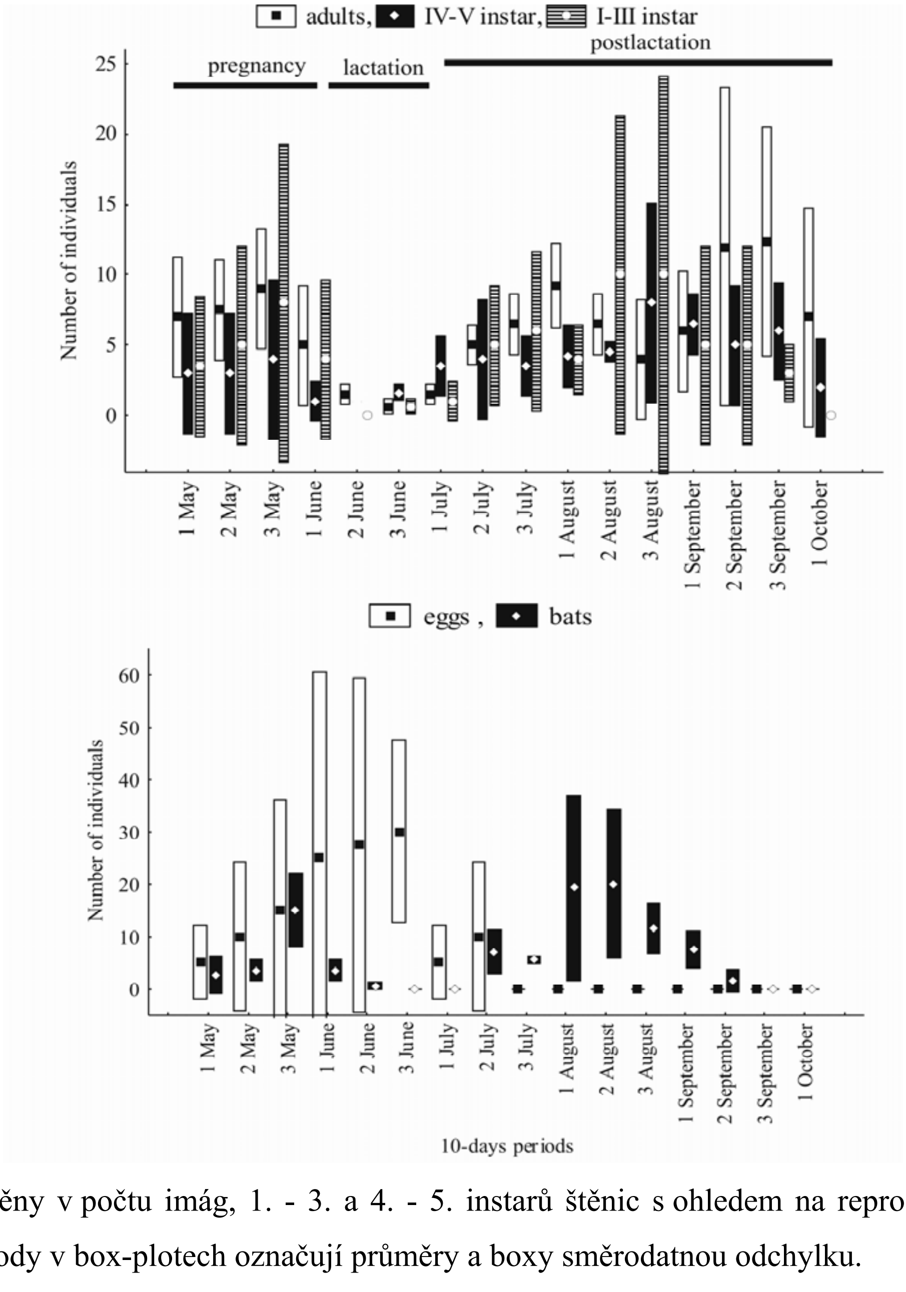
Zajímavý je však rozpor ve zjištěných vysokých hustotách patogenů v těle štěnic po jejich umělé infekci v laboratoři a současně velmi nízkých počtech přenášených patogenů při sáních. Předpokládá se, že za tímto rozporem stojí zvláštní reprodukční biologie skupiny. Štěnice jsou totiž jedinou skupinou krevsajících členovců, kteří mají traumatickou inseminaci. Opakovaný vstup patogenů do těla štěnic sice souvisí s kratší délkou života samičího imága (Reinhardt et al. 2005), ale současně může být selekčním faktorem pro optimalizaci imunitní odpovědi opakovanou stimulací systému. Přestože většina starších studií (např. Burton 1963) nedokládá, že by štěnice byly významným vektorem infekčních onemocnění, je nutno uvážit i skutečnost, že se tyto studie zaměřovaly na patogeny obecně málo přenášené krevsajícími členovci (např. HIV; methicilin-rezistentní *Staphylococcus aureus* – MRSA; hepatitis B, C, E; Goddard & deShazo 2009, Doggett et al. 2012). Současně však bylo identifikováno pouze minimum patogenů v reakci na propuknutí konkrétního lidského onemocnění. Studie na arbovirech (Adelman et al. 2013) uvádí zjištění většiny druhů až teprve intenzivním průzkumem (mezi)hostitelů. Je tedy zřejmé, že těžištěm dalších výzkumů musí být identifikace a charakterizace enzootických přenosových cyklů.

3.7 Vliv štěnic na chování netopýrů

Vysoký počet ektoparazitů nalezených na samicích a mláďatech v období po porodech může signalizovat synchronizaci populačního cyklu ektoparazit – hostitel, a tím snížení komfortu ve stávajícím úkrytu. Lewis (1996) zjistila pozitivní korelaci počtu ektoparazitů s nízkou váhou kojících samic. Na druhé straně je nutno efekt parazita chápat jako proměnnou silně závislou na jeho populační hustotě v úkrytu a je zřejmé, že s počtem roste i intraspecifická kompetice (Jaenike 1996). Obecně se proto předpokládá, že parazitace není důvodem přímého úhynu hostitele, ale spíše jeho oslabení (srv. Zahn & Rupp 2004) a zvýšené potřeby změny úkrytu vedoucí k vyššímu komfortu. Na druhou stranu tlak ze strany parazita nebude zanedbatelný, jelikož řada experimentálních i terénních výzkumů ukázala, že ektoparaziti mohou snížit hnízdní úspěšnost svých hostitelů (Loye & Carroll 1991, deLope & Møller 1993, Richner et al. 1993, Christe et al. 1996).

Synchronizace reprodukčního cyklu roztočů, nejběžnějších ektoparazitů netopýrů, byla opakovaně publikována s ohledem na různé hostitelské druhy (např. Christe et al. 2000, Lučan 2006). Podobná synchronizace byla zjištěna mezi netopýrem velkouchým (*M. bechsteinii*) a muchulí *Basilia nana* (Reckardt & Kerth 2006). Metamorfovaní dospělci této muchule přetrvávají v pupáriu týdny v úkrytech netopýrů a čekají na návrat hostitele. Po obsazení úkrytu netopýry dochází k jejich rychlému uvolnění z obalu a takřka okamžitému přichycení na těle nového hostitele. Samice netopýrů však rozpoznávají vícekrát obsazené úkryty s vysokou pravděpodobností výskytu kukel a vyhýbají se jim (Reckardt & Kerth 2007). Imága jsou už pak permanentními ektoparazity netopýrů a tělo hostitele s výjimkou kladení vajíček vůbec neopouštějí. Podobná synchronizace vývojových cyklů byla zjištěna i u blech hlodavců nebo netopýrů. Po opuštění kukly se imago blechy stejně jako muchule zdržuje ještě nějakou dobu v pupáriu a čeká na mechanický stimul ukazující na blízkost nového hostitele. Na vibrace reaguje opět velmi rychle, opouští kokon během jedné minuty a dokáže se přichytit na hostiteli až ve vzdálenosti 20 m (Rosický 1957). Schopnost vyhnout se infestovaným hnízdům byla zjištěna i u některých často ve skupinách či koloniálně hnízdících druhů ptáků (Opplinger et al. 1994, Rendell & Verbeek 1996).

V práci Bartonička & Gaisler (2007) jsme se věnovali sledování cyklu štěnic v umělých úkrytech netopýrů, v netopýřích budkách, v průběhu vegetační sezóny. Modelovými druhy byl *Pipistrellus pygmaeus* a štěnice *Cimex pipistrelli*. Po příletu gravidních samic netopýrů v květnu byly v budkách pozorovány především imága štěnic a pouze několik jedinců 1. - 3. instaru. Na začátku června netopýři některé budky opouštěli těsně před porody a období laktace většina samic trávila v kolonii v nedaleké budově (Bartonička et al. 2008).



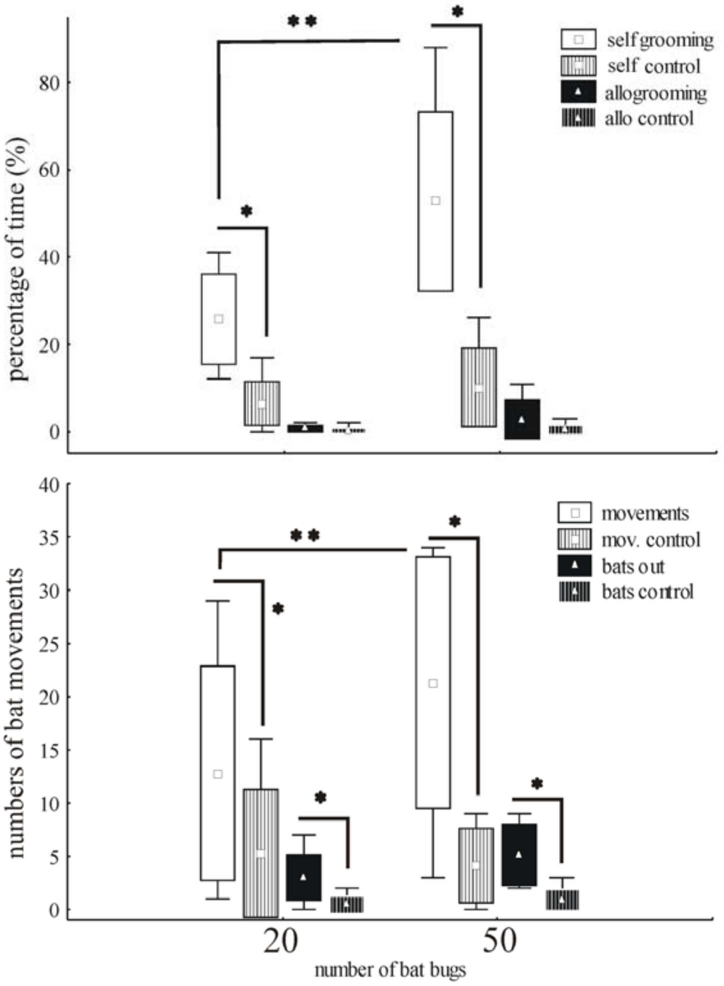
Obr. 7. Změny v počtu imág, 1. - 3. a 4. - 5. instarů štěnic s ohledem na reprodukční fáze hostitele. Body v box-plotech označují průměry a boxy směrodatnou odchylku.

Během období absence hostitele v netopýřích budkách došlo k významnému snížení počtu všech vývojových stádií, na více jak 50% původního počtu (Obr. 7). Páté instary metamorfovaly v imága během července předchozího roku, a jelikož se obvykle dožívají pouze jednoho roku (Usinger 1966), jejich úbytek koresponduje s přirozeným vymíráním. Za početní redukcí nymf, zejména 1. - 3. instarů, však stojí zjevně nepřítomnost hostitele. Rané instary metamorfují do dalších stádií během 4-7 dnů a v každém stádiu potřebují na hostiteli sát (Usinger 1966).

Ukázalo se, že krátkodobá nepřítomnost netopýrů v budkách významně omezí abundanci štěnic, ať již důvodem pro opuštění dočasných úkrytů jsou klimatické výhody prostornějšího úkrytu v budově v průběhu laktace nebo přímo antiparazitární chování. Období absence hostitele přežívají pouze vajíčka a několik jedinců starších vývojových stádií. Opětovné zvýšení počtu štěnic bylo zjištěno až po příletu samic netopýrů a čerstvě odstavených mláďat. Na počátku srpna už v budkách nebyla nalezena žádná nová vajíčka. Je tedy zřejmé, že nepřítomnost štěrbinových netopýrů v laktaci v úkrytech intenzivně využívaných během gravidity a v postlaktačním období zabrání masivnímu namnožení štěnic známému z kolonií netopýrů využívajících prostorné půdy budov (Bartonička & Růžičková 2012).

Je pochopitelné, že antiparazitární chování založené na střídání úkrytů má význam pouze u hostitelských druhů, které mohou úkryt snadno změnit (např. jsou schopni snadno přenést různě stará mláďata). Toto chování efektivně napomáhá přerušit nejen životní cyklus úkrytových parazitů, jako jsou štěnice, ale i permanentních parazitů, je-li přesun do nového úkrytu realizovan před dokončením jejich vývoje (např. Hausfater & Meade 1982; Lewis 1995, 1996; Roper et al. 2001; Kunz & Lumsden 2003; Peinke & Brown 2005). Frekvence střídání úkrytů signifikantně korelovala s abundancí parazita i u jiných savců, např. *Meles meles* (Butler & Roper 1996) nebo *Parotomys brantsii* (Roper et al. 2002). U netopýrů byla stejná korelace zjištěna např. i u druhu *Antrozous pallidus* (Lewis 1996).

Potřebu změny úkrytu v souladu s rostoucím počtem ektoparazita v úkrytech by měly doprovázet další typy chování. Takovým výrazným chováním ve vztahu k parazitaci je bezesporu grooming. Velmi málo je však známo o úrovni groomingu vyvolaným úkrytovým parazitem. Bartonička (2007, 2008) se proto věnuje významu auto a allogroomingu u netopýrů v uměle infestovaných úkrytech v experimentálních podmínkách. Ve voliéře byly instalovány dvě netopýří budky, jedna s různým počtem štěnic a druhá bez štěnic sloužící jako náhradní úkryt. Byly zjištěny významné rozdíly v úrovni autogroomingu, ve frekvenci přesunů jednotlivých netopýrů v budce a netopýrů, kteří budku opustili. Allogrooming byl pozorován pouze v několika málo případech (Obr. 8). Štěnice po nějakou dobu (přibližně 20 minut) po zahájení experimentu nejsou schopny na netopýrech sát, a to z důvodu nízké tělesné teploty hostitele. Sání je možné až tehdy, když teplota alespoň jednoho hostitele ve skupině dosáhne hodnoty, která je pro ektoparazita atraktivní. Experiment též ukázal, že štěnice napadají prvního netopýra, který se probouzí z denního torporu, a ten ve snaze zbavit se ektoparazita budí ostatní hostitele v úkrytu. Netopýři, vyrušení sáním většího počtu štěnic, kdy autogrooming a přesun v úkrytu nevede k setřesení ektoparazita, infestovaný úkryt opouštěli. Z takových úkrytů odlétali významně častěji, než z budek kam štěnice nebyly přidány.



Obr. 8. Úroveň auto- a allogroomingu, počet přesunů jednotlivých netopýrů v budce a počet netopýrů, který budku opustil ve srovnání s kontrolou, kdy do budek nebyly aplikovány štěnice. (**) ukazuje signifikantní rozdíl (p < 0,001) mezi experimenty s odlišným počtem ektoparazitů a (*) rozdíl ve sledovaných proměnných mezi experimenty s aplikací ektoparazita a bez něj. Body v box-plotech značí průměry, boxy směrodatné odchylky a „whiskers“ min-max hodnoty.

Nebyla zjištěna spolupráce dospělých samic při groomingu ani snaha zbavit vlastní mláďata štěnic, a to dokonce navzdory jejich vyšší parazitaci. Na mláďatech je však vysoký počet ektoparazitů nalézán opakovaně (např. Criste et al. 2000). Na druhé straně neexistuje přímá korelace mezi počtem ektoparazitů v úkrytu v průběhu laktace a pravděpodobnosti přežití prvního roku. Proto bude v budoucnu vhodné porovnat délku odstavu mláďat, která se obvykle používá jako hlavní ukazatel fitness (u ptáků např. Brown & Brown 1998), mezi infestovanými a neinfestovanými úkryty.

3.8 Hostitelská specifita štěnic

V případě čeledi Cimicidae nelze mluvit o výraznější hostitelské specifitě čeledi a často ani jednotlivých druhů. Spektrum štěnicemi parazitovaných druhů je obecně dosti široké. Tradičně však chybí dostatek údajů pro stanovení kompletního výčtu hostitelů konkrétního druhu. Přestože monoxenní druhy štěnic existují (např. *Primicimex cavernis* Barber, 1941 parazitující zřejmě pouze na netopýrovi *Tadarida mexicana*), daleko častěji se setkáme s druhy využívajícími více než jednoho hostitele.

Častěji však jeden druh štěnice preferuje jednoho hostitele jako hlavního a pouze příležitostně tvoří populace (démy) na jiných hostitelských druzích. Takovým příkladem je třeba štěnice *Cacodmus vicinus* Horváth, 1934, parazitující především na netopýrovi jižním *Pipistrellus kuhlii* (Quetglas et al. 2012). Naopak modelové druhy skupiny druhů *Cimex lectularius* a *C. pipistrelli* parazitují řadu druhů netopýrů zejména z čeledi Vespertilionidae, dále pak člověka, hlodavce, lasicovité šelmy a několik druhů ptáků (Wawrocka & Bartonička 2013). V čeledi Cimicidae je však obtížné najít úplné hostitelské generalisty. Jako druhy parazitující na více čeledích či dokonce řádech lze uvést *Haematosiphon indorus* Dugès, 1892 (ptáci, Usinger 1966) nebo *Stricticimex antennatus* Ferris and Usinger, 1957 (netopýři, Overal & Wingate 1976).

Jelikož v dílčích studiích habilitační práce není detailně prezentováno hostitelské spektrum studovaných štěnic *C. lectularius* a *C. pipistrelli* s.l., ale studie Bartonička & Růžičková (2012) a Wawrocka & Bartonička (2013) se spektrem hostitelů dále pracují, proto předkládám seznam hostitelských druhů netopýrů zde.

Tab. 2. Seznam hostitelských druhů netopýrů *Cimex lectularius* a skupiny *C. pipistrelli*. Vychází z literární rešerše a zahrnuje i údaje v tisku.

Druh netopýra	**Literární zdroj**
Cimex lectularius	
Pipistrellus kuhlii	Abul – Hab 1979 podle Lanza 1999
Pipistrellus pipistrellus	Rybin et al. 1989
Vespertilio murinus	Dubinij 1947 in Povolný 1957
Nyctalus leisleri	Walter 1996
Nyctalus noctula	Heise 1988
Eptesicus serotinus	Baagoe 2011
Myotis myotis	např. Povolný 1957, Usinger 1966
Myotis oxygnathus (*blythii*)	Usinger 1966 (Tagiľcev 1971 jako *Cimex* sp.)
Myotis mystacinus	Poppius 1912 in Usinger 1966
Myotis daubentonii	Wagner 1967, Bogdanowicz 1994
Myotis emarginatus	Protić & Paunović 2006, Balvín et al. 2012
Plecotus auritus	Balvín et al. 2012b
Cimex pipistrelli **s.l.**	
Pipistrellus kuhlii	Abul-Hab & Shihab 1990 podle Lanza 1999
Pipistrellus pipistrellus	Jenyns 1839
Pipistrellus pygmaeus	Bartonička 2007, Bartonička & Gaisler 2007
Pipistrellus nathusii	Heise 1988, Dietz & Walter 1995, Walter 1996
Vespertilio murinus	Horváth 1935
Nyctalus noctula	Povolný 1957, Southwood & Leston 1959, Dietz & Walter 1995
Nyctalus leisleri	Nelson & Smiddy 1997, Morkel 1999, Krištofík & Kaňuch 2006
Nyctalus lasiopterus	Balvín et al. 2012b
Eptesicus serotinus	Southwood & Leston 1959, Heise 1988
Myotis myotis	Lederer 1950, Usinger 1966
Myotis oxygnathus (*blythii*)	Tagiľcev 1971 jako *Cimex* sp.
Myotis bechsteinii	Morkel 1999
Myotis brandtii	Heise 1988, Dietz & Walter 1995, Walter 1996
Myotis daubentonii	Heise 1988, Dietz & Walter 1995, Walter 1996
Myotis dasycneme	van Rooij et al. 1982, Walter 2004
Myotis mystacinus	Kerzhner 1989
Myotis nattereri	Balvín et al. in press.
Myotis emarginatus	Usinger 1966
Rhinolophus ferrumequinum	Usinger 1966
Rhinolophus euryale	Balvín et al. in press.

V případě obou druhů vrápenců byly štěnice nalezeny ve smíšených reprodukčních koloniích se samicemi druhů *Myotis myotis* a/nebo *M. emarginatus*. Samice vrápenců si v koloniích udržují vzájemnou vzdálenost několik centimetrů (Dietz et al. 2007). Jedinou výjimkou jsou dny s nízkou teplotou v době, kdy mláďata ještě nemají vyvinutu schopnost termoregulace. Pak je možno vidět samice nahloučené ve skupině podobně jako u druhů čeledi Vespertilionidae (T. Bartonička, osobní pozorování). Rozvolněné kolonie zřejmě neposkytují dobré životní podmínky k rozvoji populací štěnic. Je tedy vysoce pravděpodobné, že štěnice byly nalezeny ve smíšených koloniích právě díky přítomnosti druhů *Myotis*, u kterých se naopak samice opakovaně shlukují. Navíc první nálezy štěnic na těchto druzích však nepocházejí přímo z kolonie, ale z letících jedinců odchycených do harfové pasti (Protić & Paunović 2006).

Podobně nevyjasněná situace je s parazitací netopýrů rodu *Plecotus*. Teprve nedávno byla nalezena štěnice na jedinci *Plecotus auritus* odchyceném mimo úkryt (Balvín et al. 2012b) (Tab. 2). Středoevropské druhy *Plecotus auritus* a *Plecotus austriacus* tvoří obvykle kolonie o několika kusech a přestože u nich byly štěnice systematicky hledány, nebyly v koloniích samic nalezeny (Heise 1988, Rupp et al. 2004). Pětina letních úkrytů netopýrů, každoročně monitorovaných na území České republiky, je sdílena druhy rodu *Plecotus* i druhy, které běžně štěnice hostí jako *M. myotis* a *M. emarginatus* (Bartonička & Gaisler 2010). Přesto však nebyla dosud zaznamenána opakovaná parazitace netopýrů *Plecotus* a *Rhinolophus* spp. a lze je tedy považovat pouze za příležitostné a spíše nevhodné hostitele štěnic. Nelze však vyloučit, že se zde potýkáme pouze s nedostatkem informací z terénu, na což mohou ukazovat výsledky diplomové práce Zedníkové (2010). Autorka prováděla experimenty hostitelské specifity štěnic skupiny *C. pipistrelli* a ukázala, že štěnice jsou schopny sát i na netopýrech rodu *Plecotus* a vrápencích *Rhinolophus hipposideros*.

I přes poměrně malou hostitelskou specifitu štěnic je zřejmé, že řada druhů netopýrů slouží štěnicím pouze jako občasní hostitelé, na kterých štěnice mohou přežívat snad i řadu generací. Odlišné přežívání parazita mezi hlavním a vedlejším hostitelem je dokladováno opakovaně z různých skupin parazitů. Jejich populace jsou schopny na vedlejších nebo příležitostných hostitelích přežívat, přestože ti jsou částečně imunní nebo jinak omezují natalitu parazita (Pulliam et al. 2007). Podobná situace pravděpodobně nastává u hostitelských druhů, které tvoří málo početné reprodukční kolonie, obvykle pod 20 jedinců (např. právě *Plecotus* spp., *Rhinolophus* spp.). Pokud se nízká početnost kolonie navíc kombinuje s častou změnou úkrytu, je pro časné instary štěnic dostupnost potravy velmi omezená. Efektivitu sání na hostiteli významně redukuje i disperze samic po půdních prostorech. Snaha dosáhnout hostitele aktivním vyhledáváním na větší vzdálenosti často selhává (Bartonička & Růžičková 2012). Z tohoto důvodu jsou štěnice nuceny vyčkat příletu hostitele zpět, do míst, které tradičně využívá. To dokládají i četná podzimní pozorování, kdy v říjnu a listopadu jsou v úkrytech pozorována převážně imaga a 4. a 5. instary ve větších počtech pouze v místech tradičního výskytu kolonie, případně přímo pod ním. Na strategii dlouhého vyčkávání jsou zjevně imága štěnic dobře vybavena a nespářené samice bez příjmu potravy přežívají až 2 roky (Usinger 1966). Tato skutečnost má zásadní význam pro ochranu kolonií netopýrů. Pokud je mi známo, doposud totiž nebyl pozorován případ, kdy by štěnice (zejména *C. lectularius*) osidlující úkryt netopýrů na půdě jinak obydlené budovy, napadaly lidi využívající další blízké prostory, a to ani v době dlouhodobé nepřítomnosti netopýrů (září - březen).

Situace v případě druhu *C. pipistrelli* je poněkud komplikovanější v souvislosti s vazbou na druhy netopýrů využívající štěrbinové úkryty. V posledních desetiletích jsou známy početné reprodukční kolonie hostitelských druhů netopýrů jako *N. noctula* a *P. pipistrellus* z panelových domů. Ty jsou však v současnosti plošně revitalizovány a původní úkryty v mnoha případech zanikají bez náhrady. Tato situace vede ke stále častějšímu výskytu netopýrů v nových často klimaticky suboptimálních úkrytech, které jsou navíc v těsné blízkosti prostor využívaných člověkem. Stav je navíc komplikován opakovanou nepřítomností hostitele v úkrytu v letním období a štěnice jsou nuceny vyhledávají alternativního hostitele. Přestože Southwood & Leston (1959) zjistili omezenou schopnost reprodukce *C. pipistrelli* krmené lidskou krví, tyto štěnice na člověku sají a dovedou založit početnou populaci (T. Bartonička, J. Šafář, osobní pozorování). To pochopitelně vede ke snaze blízký úkryt netopýrů znepřístupnit. Eradikace štěnic je však z podobných prostor obvykle nemožná a komplikuje praktickou ochranu netopýrů, jako zvláště chráněných druhů.

Dalším aspektem hostitelské specifity je přítomnost dvou reprodukčních cyklů v roce u štěnic vázaných na netopýry (zejména u druhu *C. pipistrelli*). Druh *C. lectularius*, populace vázané na jiné hostitele, zejména člověka, se rozmnožuje v průběhu celého roku bez omezení. Tato cyklicita se u linií parazitujících na netopýrech přenáší i do chovných skupin držených ve standandardních mikroklimatických podmínkách s dostatkem potravy (T. Bartonička, L. Růžičková, K. Wawrocka, osobní pozorování). Neochota samic snášet vajíčka v podzimním období byla opakovaně pozorována u dospělých samic odchycených přímo v netopýřích úkrytech. U instarů pak byla zjištěna neochota sát.

3.5 Transport štěnic netopýry

Disperze štěnic mezi úkryty hostitelů je nezbytná pro zachování genetické diverzity v jednotlivých úkrytech i diverzity celé populace. Logickou snahou štěnic je též disperze do nových, doposud neparazitovaných úkrytů. Štěnice však nemají schopnost letu, proto jejich přenos probíhá výhradně pasivním způsobem.

Na netopýrech odchycených mimo úkryty jsou štěnice zjišťovány velmi zřídka (Bartonička & Růžičková 2013). Nálezy štěnic na tělech netopýrů mimo jejich úkryty se týkají prakticky výhradně skupiny *C. pipistrelli.* Přestože byly publikovány pouze dva nálezy *C. lectularius* na netopýrech, je pravděpodobné, že netopýři musí přenášet i tento druh (Heise 1988). Z údajů uvedených v publikaci Balvín et al. (2012a), je zřejmé že nálezy štěnic *C. lectularius* na těle netopýrů na území Evropy souvisí téměř výhradně s rodem *Nyctalus*. Na druhou stranu není doposud objasněno, zda a nakolik jsou dosavadní výsledky ovlivněny nedostatkem informací. Je totiž jisté, že štěnice na svém těle přenášejí i jiní netopýři.

Bartonička & Růžičková (2013) dokumentovali rychlou kolonizaci stěnicemi u budek obývaných netopýrem nejmenším (*Pipistrellus pygmaeus*). Tento druh je druhým v pořadí po n. rezavém, na jehož těle jsou štěnice mimo úkryty nalézány opakovaně (Balvín et al. 2012b). Přenos jinými druhy je však ojedinělý a v literatuře zmínky prakticky chybí. Další, avšak velmi ojedinělé, literární záznamy přenosu štěnice jiným netopýrem než rodu *Nyctalus* ukazují na transport štěnic netopýry druhu *Vespertilio murinus* (Orlova & Pervušina 2010) a *Myotis daubentonii* (Heise 1988). Nově jsou štěnice mimo úkryt uváděny z druhů *M. dasycneme*, *M. myotis* a *Plecotus auritus* (Balvín et al. 2012b). Posledním druhem, u něhož byly štěnice mimo úkryt potvrzeny teprve nedávno, je také *M. nattereri* (A. Reiter, ústní sdělení).

Přestože je obtížné vyčíslit frekvenci přenosů štěnic netopýry, je nepoměr nálezů mezi druhy rodu *Nyctalus* a jinými druhy netopýrů jednoznačný. Heise (1988) uvádí nález 55 štěnic ze vzorku 1631 jedinců netopýra rezavého a Rupp et al. (2004) dokonce na 15% jedinců tohoto druhu (ze 221 zkoumaných jedinců). Obvykle je nalézána pouze jedna až dvě štěnice na netopýrovi. Pravděpodobně nejvyšší publikovaný počet štěnic nalezený na jednom netopýrovi je čtyři (Rupp et al. 2004). Bohužel ve většině prací není odlišeno, zda nálezy štěnic byly uskutečněny mimo úkryt kolonie či u jeho vletového otvoru, kde je logicky pravděpodobnost nálezu štěnice na odlétajícím netopýrovi vyšší než na lovišti.

Jak vyplývá z databáze soustředěné Českou společností pro ochranu netopýrů, netopýr rezavý tvoří pouze 0,9% (170 ks) z celkového počtu 18388 odchycených netopýrů v letech

2006-2013 (databáze ČESON nepubl., Bartonička & Gaisler 2010). Nepatří tedy k pravidelně odchytávaným druhům, spíše naopak.

Temperátní druhy netopýrů během dne upadají do torporu, kdy se teplota těla netopýra srovnává s teplotou vzduchu (Stones & Wiebers 1965, Stawski et al. 2014). Výjimkou je období laktace a krátké období péče o mláďata, kdy kojící samice sice upadají během dne do torporu, ale pouze na krátkou chvíli (např. Klug & Barclay 2013). Je-li však tělesná teplota netopýra nižší než 30°C (Rivnay 1930), což je teplota, nad níž je štěnice schopna hostitele najít a sát na něm, hostitel štěnici nepřitahuje (Bartonička 2008) a štěnice tak mají minimální šanci sát a být vyneseny ven z úkrytu. Tato úvaha se zdá být podpořena i zjištěním Balvína et al. (2012b). Ti zjistili, že pouze osm z 41 netopýrů, kteří na sobě měli štěnice mimo úkryt, byly kojící samice.

Jak ukázaly experimenty, netopýr nejmenší se pro štěnice stává atraktivním, když odpoledne zvyšuje tělesnou teplotu. Teprve v tom okamžiku byly štěnice schopny netopýra napadnout. K rychlému zvyšování teploty netopýrů však v experimentech docházelo až krátce před jejich výletem z budky (Bartonička 2008). Velmi pravděpodobně lze očekávat podobné chování i u netopýra rezavého. Není doposud známo, zda je probouzení z denní letargie u těchto dvou druhů netopýrů nějak odlišné v porovnání s druhy, na kterých štěnice nejsou mimo úkryt nalézány.

Dalším možným důvodem častých nálezů štěnic právě na druhu *N. noctula*, může spočívat v jeho tělesné velikosti. Jelikož patří mezi největší evropské druhy netopýrů, je tedy pravděpodobně pro štěnice více atraktivní. Může tedy záležet na velikosti plochy vhodné k přichycení a následnému sání, např. při porovnání velkého druhu *N. noctula* a drobného druhu *P. pygmaeus*. Velikost těla však pravděpodobně nebude rozhodující faktor, jelikož např. druh *M. myotis* dosahuje podobné váhy jako *N. noctula* a štěnice na něm byly nalezeny mimo úkryt pouze v jediném případě (Balvín et al. 2012b).

Významnou roli by mohla mít i tloušťka a pevnost létací membrány. Odolnost vůči zátěži je mezi membránami křídel jednotlivých druhů netopýrů odlišná a u druhu *N. noctula* byly zjištěny vysoké hodnoty momentu setrvačnosti (Thollesson & Norberg 1991). Ten pozitivně koreluje se sílou létací blány. Jelikož hodnoty momentu setrvačnosti jsou u druhu *N. noctula* dvojnásobné než u jiných druhů, lze předpokládat, že se druh vyznačuje obzvláště pevnou křídelní blánou (Quay 1970, Schwartz et al. 2006). Právě při odchytech netopýrů do sítě je dobře patrné, že je blána silnější než u velikostně srovnatelných druhů jako *M. myotis* nebo *M. dasycneme* (T. Bartonička, osobní pozorování). Štěnice sají přednostně právě na křídlech a uropatagiu. Proto lze předpokládat, že *N. noctula* může být méně citlivý na přítomnost štěnic na svém těle a může vykazovat nižší míru groomingu ve snaze se štěnic před výletem zbavit. To se v současnosti snažíme experimentálně otestovat.

Recentní studie zaměřená na genetickou strukturu populací štěnic *C. pipistrelli* navíc ukazuje, že ač jsou pozorování transportu velmi vzácná, dochází k nim opakovaně a nový dém je obvykle zakládán více samicemi (Wawrocka et al., in prep.). *N. noctula,* podobně jako druhy rodu *Pipistrellus*, jsou jako vektor ideální, jsou tažné (např. Petit & Mayer 2000, Huttere et al. 2005) a navíc opakovaně střídají úkryty i během reprodukční sezóny (Kaňuch & Celľuch 2004). Dokonce navštěvují více úkrytů během jediné noci (Feyerabend & Simon 2000). Naopak druhy rodu *Myotis* jsou ve volbě úkrytu reprodukční kolonie velmi tradiční a vrací se na jediné místo řadu let. Na druhou stranu jaderné markery, mikrosatelity, neukazují na žádnou strukturovanost populací podle hostitelského druhu (Wawrocka et al., in prep.). Nedávno jsme však realizovali sérii experimentů ve voliéře, kde jsme testovali, zda štěnice *C. lectularius* dlouhodobě parazitující na netopýrech, se nechají z umělých budek vynášet častěji netopýry druhu *N. noctula* nebo netopýry druhu *M. daubentonii*, na kterých též parazitují. I v experimentálních podmínkách se ukázalo, že si štěnice vybíraly významně více jedince druhu *N. noctula*.

Je velmi nápadné, že jsou nalézány na netopýrech mimo jejich úkryty pouze imaga a to především dospělé samice. S ohledem na signifikantně delší přežívání samic (Bartonička 2010) je pravděpodobné, že nymfy při opuštění kolonie mají mnohem menší perspektivu kolonizovat nová stanoviště. Opakovaná přítomnost dospělých samic na netopýrech je zcela nenáhodná. Doba sání navíc není u imag výrazně delší ve srovnání s nymfami, aby zvyšovala pravděpodobnost jejich vynesení z úkrytu (Bartonička 2008, 2010). Úspěšná kolonizace nového hostitelského úkrytu štěnicemi však předpokládá dvě skutečnosti. První z nich je bezpochyby schopnost přečkat v úkrytu do doby příletu většího počtu hostitelů. Tento předpoklad byl opakovaně potvrzen terénním pozorováním transportu dospělých samic, které mají vyšší přežívaní než nymfy i dospělí samci. Druhou skutečností je potřeba přenosu spářené samice. Spermie jsou pozorovatelné v těle samice pouze po dobu několika hodin, a proto nebylo možno dostupný materiál na přítomnost spermatu vyšetřit (Balvín et al. 2012b). V podmínkách voliéry byla testována i hypotéza, zda se z úkrytů nechávají častěji vynášet spářené samice než samice dosud nepářené (Bartonička, in prep.). Nicméně významně vyšší počty spářených samic na vyletujících netopýrech nebyly zjištěny. Zde bude patrně nutno více uvážit variabilitu chování imag samic v průběhu sezóny.

Tyto experimenty a řada výše uvedených okolností potvrzují, že štěnice si, pokud mohou, aktivně vybírají druh netopýra. K objasnění důvodu proč tak činí, bude ještě potřeba provést řadu pokusů. Jedním z nich by mohlo například být srovnání rychlosti probouzení z denního torporu u běžně parazitovaných druhů.

Závěrem kapitoly je však nutno pouzázat na v těchto detailech omezené znalosti v chování netopýrů a štěnic vůbec. Úroveň poznání, která by potvrdila výše uvedená tvrzení, nemusí být dostačující, a je nutno připustit jistou míru spekulativnosti. V každém případě je v současnosti obtížné rozhodnout, zda rozdílná frekvence výskytu štěnic na různých druzích netopýrů souvisí s aktivním výběrem štěnice anebo pouze s odlišným chováním a úkrytovými strategiemi jednotlivých druhů netopýrů.

4. Závěr

Shrnujícím úvodem předkládaných publikací a prací, které s tématem habilitační práce úzce souvisí, jsem se pokusil ukázat, že štěnice jsou dobrým modelem pro výzkum vztahů parazit-hostitel zejména v proximátní rovině vzájemných behaviorálních odpovědí. V posledních letech se štěnice těší nebývalé pozornosti právě díky jejich úspěšnému šíření u lidí. Štěnice jsou však původními hostiteli netopýrů a na člověka přešly později, v době využívání společných prostor. S intenzivním rozvojem laboratorních detekčních metod různých patogenů se lze stále častěji v lékařské i veterinární literatuře setkat se studiemi, které uvádějí letouny jako významné vektory a jejich populace jako refugia řady patogenů. Mnohé studie jsou však čistě laboratorní a patrně tlak ze strany oponentů nebo editorů vedou k doplňování spekulativních informací týkajících se jejich zoonotických cyklů. Tyto úvahy, do jisté míry zneužívající informační deficity v biologii letounů, však nevedou k obecnému poznání a hlubšímu pochopení závislostí ve volné přírodě, spíše naopak. Výsledkem jsou pak často až poplašné tiskové zprávy vedoucí sice ke zvýšení atraktivity dané publikace, ale také v jisté míře k matení veřejnosti. V dílčích studiích předkládané habilitační práce jsem se pokusil ukázat, že řada drobných terénních i experimentálních studií může vést k postupnému objasnění komplexní problematiky parazitace štěnicemi, od antiparazitárních strategií netopýrů, přes hostitelskou specifitu štěnic, po jistou rehabilitaci netopýrů jako hlavního přenašeče štěnic do lidských obydlí.

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Práce označené * nebyly k dispozici.

6. Komentář k publikacím

Studie v prvním tematickém celku byly vedeny spíše potřebou definovat specifické chování netopýrů v různých souvislostech jejich časoprostorových strategií. Díky terénnímu pozorování byly odhaleny specifické vzorce ve využívání úkrytů, které nebylo možno uspokojivě vysvětlit na základě doposud publikovaných výsledků. Na modelu úkrytových strategií netopýrů rodu *Pipistrellus* byly diskutovány tradičně přijímané příčiny vysvětlující střídání úkrytů. Dvě studie v tomto tematickém celku (8.1) osvětlily a částečně rozporovaly interpretace úkrytového chování běžných středoevroských netopýrů v souvislosti s dosud publikovanou literaturou.

Za nejvýznamnější však považuji studie v druhém tematickém celku (8.2), které upozornily na existenci nového mechanismu - parazitaci štěnicemi, vysvětlujícího adaptivnost střídání úkrytů (studie 3). Třetí studie v tomto tematickém bloku (5) demostrovala střídání dvou vzorců chování při přítomnosti a absenci spouštěcího faktoru, vysoké abundance štěnic v úkrytu netopýrů.

Jelikož v terénních podmínkách působí současně větší množství faktorů, kdy každý z nich může vést k velmi podobnému vzorci chování, bylo nutno připravit sérii experimentů v kontrolovaných podmínkách a ty doplnit o další pozorování. Studie z dalších dvou tematických celků více či méně potvrzují oprávněnost úvah o adaptivním významu studovaného mechanismu a dokreslují detaily ve vztahu hostitel (netopýr) a úkrytový parazit (štěnice).

V třetím tematickém celku (8.3) si pravděpodobně nejvíce cením studie 7, ve které jsme adaptivnost mechanismu střídání úkrytů potvrdili i u druhů netopýrů se značně odlišnými úkrytovými nároky.

Ve čtvrtém tematickém celku (8.4), který zahrnuje pouze jedinou publikovanou práci, ale i několik již realizovaných experimentů zmíněných v úvodním textu, byl prostor věnován problematice hostitelské specifity. Největší význam této studie spočívá v roli netopýrů jako možných vektorů štěnice *C. lectularius* do lidských obydlí. Naše výzkumy prokázaly, že tyto štěnice mohou na člověku úspěšně sát a založit dlouhobě fungující populace. Tato skutečnost, bude-li nedostatečně citlivě sdělena široké veřejnosti, může již tak komplikované vztahy mezi člověkem a netopýrem, ještě zhoršit. Lze očekávat, že pak veřejnost neuspokojí ani informace, že prozatím takové populace (založené štěnicemi z netopýrů) nebyly v lidských obydlích zjištěny (Balvín et al. 2013).

Závěrem bych chtěl poukázat na skutečnost, že publikace předkládané v habilitační práci tvoří pouze necelou třetinu mé publikační aktivity a tak logicky i mnou (spolu)řešených tematických okruhů. Práce však byly vybrány tak, aby tvořily logický tematický celek, který je však současně značně odlišný od jiných publikovaných studií.

7. Úroveň spoluautorství a hlavní přínos předkládaných studiích

S výjimkou poslední práce (8) jsem vždy prvním autorem a tedy tím kdo provedl statistické zpracování získaného materiálu a hlavním autorem textu. S výjimkou článků 1 a 2 jsem i jediným autorem nosné myšlenky konkrétní studie a navrhovatelem designu. U studií 1 a 2 jsem pak významně myšlenky studií pozměnil se zaměřením na publikaci vybraných netriviálních poznatků.

(1) BARTONIČKA T. & ŘEHÁK Z., 2007: Influence of the microclimate of bat boxes on their occupation by the soprano pipistrelle *Pipistrellus pygmaeus*: possible cause of roost switching. Acta Chiropterologica, 9(2): 517–526.

V terénu jsem získal většinu dat samostatně, po konzultacích se spoluautorem (vedoucím mé doktorské práce) je i samostatně vyhodnotil a jsem autorem textu článku.

Hlavní přínos práce: Studie porovnává mikroklimatické podmínky využívaných a nevyužívaných umělých úkrytů netopýry v průběhu gravidity, laktace a postlaktace. S ohledem na odlišné klimatické nároky reprodukujících se samic, jsme předpokládali, že podmínky budou mezi úkryty odlišné. Překvapivě se však tato hypotéza nepotvrdila. V průběhu výzkumu nebyly zjištěny ani krátkodobé výkyvy v teplotě (přehřívání, ochlazení), které by vysvětlovaly, proč reprodukující se samice netopýrů úkryt na určitou dobu opouští. Výsledky studie připouští existenci dalšího doposud nezkoumaného faktoru.

(2) BARTONIČKA T., BIELIK A. & ŘEHÁK Z., 2008: Roost switching and activity patterns in the soprano pipistrelle, *Pipistrellus pygmaeus*, during lactation. Annales Zoologici Fennici, 45(6):503-512.

Jsem spoluautorem myšlenky a designu studie (se Z. Řehákem). Na terénním výzkumu se intenzivně podílel s mnou podílel A. Bielik (diplomant Z. Řeháka), který pod mým a vedením Z. Řeháka připravil podkladovou databázi. A. Bielik se rovněž podílel na části vyhodnocení dat pro účely své diplomové práce, většinu výsledků jsem však vyhodnotil samostatně a jsem hlavním autorem textu článku.

Hlavní přínos práce: V rámci výzkumu jsme zkoumali časoprostorovou aktivitu netopýra *Pipistrellus pygmaeus*. K nejvýznamnějším zjištěním patřilo, že některé samice již koncem laktace opakovaně navštívili teritoria samců v okolí úkrytu kolonie. Přestože některé samice z původního úkrytu přenášely mláďata i do úkrytů jiných, po dobu laktace zůstala většina jedinců v původním úkrytu kolonie. Po odstavu mláďat se však začala rychle rozpadat.

(3) BARTONIČKA T. & GAISLER J., 2007: Seasonal dynamics in the numbers of parasitic bugs (Heteroptera, Cimicidae): a possible cause of roost switching in bats (Chiroptera, Vespertilionidae). Parasitology Research, 100:1323–1330.

Jsem autorem myšlenky a designu výzkumu. Data v terénu jsem získal sám, zcela samostatně je i vyhodnotil a jsem autorem text článku. Spoluautor text článku připomínkoval a upravil jeho jazykovou stránku.

Hlavní přínos práce: Studie byla zaměřena na odhalení souvislosti střídání úkrytů u netopýrů rodu *Pipistrellus* s abundancí štěnic v jejich úkrytech. Zjistili jsme, že netopýři opustili sledované úkryty na období laktace. Během této doby uhynula většina raných instarů a období nepřítomnosti netopýrů přežívala pouze vajíčka. Netopýři se do úkrytů vrátili s čerstvě vzletnými mláďaty. Dočasné opuštění úkrytu netopýry efektivně potlačuje rozvoj populace štěnic.

(4) BARTONIČKA T., 2010: Survival rate of bat bugs (*Cimex pipistrelli*, Heteroptera) under different microclimatic conditions. Parasitology Research, 107:827–833.

Jsem autorem myšlenky článku a designu experimentu. Na laboratorních experimentech se částečně podílela i K. Zedníková (moje diplomantka). Po částečných výsledcích (pro účely diplomové práce) jsem objem laboratorní práce rozšířil asi na trojnásobek a dokončil ji samostatně. Jsem autorem vyhodnocení dat a jediným autorem textu článku.

Hlavní přínos práce: Řada informací týkajících se přežívání v různých mikroklimatických podmínkách v detailech ontogenetického vývoje je známa pouze od štěnice *C. lectularius*. V experimentálních podmínkách jsem testoval přežívání štěnic *C. pipistrelli*, běžnějšího druhu u netopýrů, v různých teplotách a následně jejich schopnost sání na hostiteli. Ukázalo se, že na netopýry lépe adaptovaná štěnice *C. pipistrelli* lépe snáší vyšší teploty a má kratší vývojový cyklus než štěnice *C. lectularius*.

(5) BARTONIČKA T. & RŮŽIČKOVÁ L., 2013: Recolonization of bat roost by bat bugs (*Cimex pipistrelli*): could parasite load be a cause of bat roost switching? Parasitology Research, 112:1615–1622.

Jsem autorem myšlenky výzkumu a jeho designu. Též jsem provedl většinu terénních šetření samostatně. L. Růžičková se k terénnímu výzkumu připojila až v posledním roce. Provedl jsem statistické zpracování výsledků a připravil text publikace.

Hlavní přínos práce: Z umělých netopýřích úkrytů byli opakovaně odstraňováni úkrytoví paraziti. V letech, kdy byly úkryty infestovány vysokými populačními hustotami štěnic, netopýři se v nich nerozmnožovali. Naopak v letech, kdy došlo k odstranění štěnic, k porodům mláďat zde opakovaně došlo. Též bylo zjištěno, že štěnice kolonizují nové úkryty hostitele poměrně rychle již v období jeho gravidity.

(6) BARTONIČKA T., 2008: *Cimex pipistrelli* (Heteroptera, Cimicidae) and the dispersal propensity of bats: an experimental study. Parasitology Research, 104:163–168.

Jsem jediným autorem celého výzkumu.

Hlavní přínos práce: V kontrolovaných podmínkách voliéry jsem sledoval reakce netopýrů na vysoké abundance štěnic v jejich úkrytech. Jednoduchý experiment ukázal, že netopýři zvyšují grooming při vysokých denzitách štěnic, a pokud se nejsou schopni štěnic zbavit, opouští takový úkryt záhy po probuzení z denního torporu. Přítomnost štěnic v úkrytu vede k předčasnému opouštění úkrytu a tak změnám v úkrytovém chování hostitele.

(7) BARTONIČKA T. & RŮŽIČKOVÁ L., 2012: Bat bugs (*Cimex pipistrelli*) and their impact on non-dwelling bats. Parasitology Research, 111:1233–1238.

Jsem autorem myšlenky i designu. L. Růžičková (moje diplomantka) se podílela zásadní měrou na sběru dat v terénu. Společně jsme data vyhodnotili. Jsem autorem textu článku.

Hlavní přínos práce: Předchozí naše studie ukázala, že netopýři dočasným opuštěním úkrytu mohou redukovat masivní gradaci štěnic ve štěrbinových úkrytech, proto jsme chtěli ověřit podobnou strategii u netopýrů tradičně osidlujících pouze jediný, ale prostorný úkryt. Sledovali jsme úkryt s vysokou abundancí štěnic. Zjistili jsme, že vysoce gravidní samice se těsně před porody přesunou do předtím neobsazené části úkrytu, kde štěnice doposud nebyly. Současně na opakovaně osidlovaném místě úkrytu poklesne abundance raných instarů štěnic, které nemají dostatek potravy. Tímto způsobem mohou i netopýři osidlující dlouhodobě rozsáhlé půdní prostory efektivně redukovat abundanci úkrytových parazitů jako jsou štěnice.

(8) WAWROCKA K. & BARTONIČKA T., 2013: Two different lineages of bedbug (*Cimex lectularius*) reflected in host specificity. Parasitology Research, 112: 3897-904.

Jsem autorem myšlenky výzkumu a jeho designu. K. Wawrocka (moje doktorandka) odvedla většinu práce v laboratoři. Společně s doktorandkou jsme získaná data vyhodnotili a oba jsme se stejnou měrou podíleli na textu článku.

Hlavní přínos práce: V předchozích studiích bylo zjištěno, že se ve střední Evropě vyskytují dvě hostitelské linie štěnice *Cimex lectularius*. Jedna parazituje převážně na člověku a druhá na netopýrech. Laboratorní experimenty v chovech štěnic ukázaly na snížené přežívání skupin krmených krví nespecifického hostitele. Výrazný rozdíl byl zjištěn především u linie parazitující na člověku, kdy tyto populace nebyly schopny přežívat na netopýrech. Naopak štěnice z netopýrů krmené lidskou krví měly dostatečně vysoké přežívání na udržení populace. Toto zjištění má zásadní význam pro hodnocení netopýrů jako vektorů štěnic do lidských sídel, zejména v souvislosti se současnou expanzí štěnic u člověka.

8. Seznam publikovaných vědeckým prací k tématu habilitační práce

8.1 Využívání a střídání úkrytů u netopýrů

(1) BARTONIČKA T. & ŘEHÁK Z., 2007: Influence of the microclimate of bat boxes on their occupation by the soprano pipistrelle *Pipistrellus pygmaeus*: possible cause of roost switching. Acta Chiropterologica, 9(2): 517–526. (IF=0,831)

(2) BARTONIČKA T., BIELIK A. & ŘEHÁK Z., 2008: Roost switching and activity patterns in the soprano pipistrelle, *Pipistrellus pygmaeus*, during lactation. Annales Zoologici Fennici 45(6):503-512. (IF=1,03)

8.2 Životní cyklus štěnic a přežívání v úkrytech netopýrů

(3) BARTONIČKA T. & GAISLER J., 2007: Seasonal dynamics in the numbers of parasitic bugs (Heteroptera, Cimicidae): a possible cause of roost switching in bats (Chiroptera, Vespertilionidae). Parasitology Research, 100:1323–1330. (IF=2,327)

(4) BARTONIČKA T., 2010: Survival rate of bat bugs (*Cimex pipistrelli*, Heteroptera) under different microclimatic conditions. Parasitology Research, 107:827–833. (IF=2,327)

(5) BARTONIČKA T. & RŮŽIČKOVÁ L., 2013: Recolonization of bat roost by bat bugs (*Cimex pipistrelli*): could parasite load be a cause of bat roost switching? Parasitology Research, 112:1615–1622. (IF=2,327)

8.3 Vliv štěnic na chování netopýrů

(6) BARTONIČKA T., 2008: *Cimex pipistrelli* (Heteroptera, Cimicidae) and the dispersal propensity of bats: an experimental study. Parasitology Research, 104:163–168. (IF=2,327)

(7) BARTONIČKA T. & RŮŽIČKOVÁ L., 2012: Bat bugs (*Cimex pipistrelli*) and their impact on non-dwelling bats. Parasitology Research, 111:1233–1238. (IF=2,327)

8.4 Hostitelská specifita štěnic

(8) WAWROCKA K. & BARTONIČKA T., 2013: Two different lineages of bedbug (*Cimex lectularius*) reflected in host specificity. Parasitology Research, 112: 3897-904. (IF=2,327)

8.1 Využívání a střídání úkrytů u netopýrů

BARTONIČKA T. & ŘEHÁK Z., 2007

Influence of the microclimate of bat boxes on their occupation by the soprano pipistrelle

Pipistrellus pygmaeus: possible cause of roost switching.

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Influence of the microclimate of bat boxes on their occupation by the soprano pipistrelle *Pipistrellus pygmaeus*: possible cause of roost switching

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Between April and October 2003–2004, the changes in occupation of three bat boxes used by *Pipistrellus pygmaeus* were studied using a passive IR monitors and data loggers. Bat boxes were situated in a floodplain forest in south-eastern Moravia. Generalized additive models indicated that internal humidity described better the fluctuation in bat numbers during pregnancy and lactation than did changes in the internal temperature. Three variables (internal humidity, external temperature, and number of bats) described 87% of the variability in internal roost temperature, while the number of bats described only 29% of the variability. A negative correlation was found between the internal temperature and the number of bats roosting in a bat box the next day during pregnancy and lactation. The number of bats was also positively correlated with the internal humidity. The internal temperature of a roost with bats was biased by temperature strategies induced by the bats during particular reproductive periods. Mean temperature of occupied bat boxes was higher during pregnancy than during lactation. Females were able to go into torpor even during lactation period.

Key words: *Pipistrellus pygmaeus*, roost changing, microclimate, bat boxes

INTRODUCTION

Roost fidelity is low in many species, especially among tree-dwelling bats (Lewis, 1995). Field observations (Lausen and Barclay, 2002; Mazurek, 2004; Rancourt *et al.*, 2005) suggest that species of bats dwelling in holes and crevices switch their roosts several times during a season, even every few days. This behaviour was observed mainly in species roosting in tree hollows (Barclay and Brigham 2001; Kerth *et al.*, 2001; Willis and Brigham, 2004). However, several studies suggest that roost switching was registered even in *Pipistrellus pipistrellus* s.l., usually roosting in buildings (e.g., Thompson, 1992; Park *et al.*, 1996). Thompson (1990) or Feyerabend and Simon (2000) found large numbers of roosts exploited by one colony of pipistrelles. Small groups of female pipistrelles often leave their respective roosts and move into a main nursery roost a few days prior to parturition, probably to reduce costs associated with thermoregulation (Swift, 1980; Webb *et al.*, 1996; authors' personal observations).

Recent studies show that females of species using tree cavities are not restricted to individual trees and one colony may be spread among multiple trees on a given night, conforming with a fission-fusion model (Kerth and König, 1999; Willis and Brigham, 2004). The motivation underlying roost switching and reasons why groups disperse among several roosts is not well understood (Lewis, 1995; Vonhof and Barclay, 1996). This social structure may help maintain a number of potential benefits linked to living in a large colony. Roost switching might force social relationships among the small 'subgroups' that comprise a larger group. However, dispersion of colonies among tree cavities and bat boxes may result from a complex of different constraints. Roost switching and dispersion may reflect differences in group size between bats forming large colonies in caves and buildings and those roosting in smaller colonies in trees, perhaps due to thermal differences or to competition for space when the number of individuals is increasing and one cavity is too small (Whitaker, 1998; Lefebvre *et al.*, 2003). Roost switching could be a good mechanism by which bats select roost site with optimal microclimatic conditions (Kerth *et al.*, 2001; Lourenço and Palmeirim, 2004). Bats may know alternative roosts including their microclimate in advance, since the search for a new roost might be costly in terms of energy output (Lewis, 1995). Benefits associated with roost switching could also be positively correlated with decreasing the mortality of juveniles due to the impact of parasites (Wolz, 1986; Lewis, 1996).

We attempted to answer the following questions. (i) Are differences in microclimatic parameters among bat boxes dependent on particular parts of the reproductive season? (ii) Do reproductive requirements influence roost switching or roost sharing behaviour? (iii) Can internal temperature or other microclimatic parameters be used to predict the presence of bats in a roost?

MATERIALS AND METHODS

Study Area and Data Collection

The changes in microclimate and occupancy of bat boxes used by *P. pygmaeus* were studied in a floodplain forest in south-eastern Moravia (Czech Republic). The study was performed between April and October of 2003 and 2004. Three wooden bat boxes were studied, two of them were placed in trees situated within the forest and its edge, the third was placed on a hide at a forest edge. Altogether four boxes were installed in the study area, but only three of them were occupied periodically. The reproductive season under study was divided into three periods: pregnancy (until 7th June), lactation (8th June till 7th July, 30 days after first parturition observed) and post-lactation (after 8th July — cf. Bartonička and Zukal 2003). The time of first parturition was assessed in a nearby (14 km) nursery colony of *P. pygmaeus* in a building at Vranovice (T. Bartonička *et al.*, unpublished data). There, the bats were captured, examined and released. We also netted bats emerging from all boxes during the years 2001 and 2002, but never captured males. Therefore we suppose the absence of adult males during the study in 2003 and 2004. All captures were performed under the licence No. 922/93-OOP/2884/93 of the Ministry of Environment of the Czech Republic. The authors have also been authorized to handle free-living bats according to the certificate of competency No 104/2002-V4 (§ 17 of the law No. 246/ 1992).

Technical Equipment

The changes in bat activity with respect to the boxes were monitored continually by passive IR Trail Monitors, TM (TM550, TrailMaster, Goodson & Associates, INC., USA — used by Thomas *et al.*, 1995) and active IR gate (Berková and Zukal, 2004). The IR gate consisted of 2 infrared light emitters (diodes) and two receivers (phototransistors). This technique allows discrimination between those bats leaving and those entering the bat box. With the event delay set to 0.1 minutes the TM record 10 events in a single minute. Battery life of both types of recording systems was usually two weeks or more. One bat box was equipped by IR gate and two boxes by TM. The TMs were situated in front of the entrance (see Fig. 1) and the IR gate in the entrance of each bat box. Hobo Data Loggers (Onset Computer Corporation, USA), continuously recording the temperature (internal and external) and the internal humidity, were situated under the roof of each bat box. The sensors measuring external temperature were situated about 50 cm apart from each box. The relative humidity sensor had an accuracy of ± 4% and the temperature sensor ± 0.4°C. The bats flying out and in were also monitored using a digital video recorder HD-2166 and AVC-720 connected with IR diode cameras B/W AVC 307R.

FIG. 1. Wooden bat box. Monochrome (B/W) IR diode camera monitored the entrance of the box and was connected to a battery and digital recorder on the ground. Two grey shapes mark out a space monitored by passive IR Trail Master monitor (TM550) on a bracket in front of the bat box. Internal temperature was measured with help of Hobo Data Loggers (Onset Computer Corporation) (inside box). Sensor measuring external temperature was placed outside roughly half a meter from the bat box

Statistical Analysis

All variables showed a normal distribution after log transformation. Statistica for Windows 7.0 was used for data analyses (ANOVA, *t*-tests, correlations). The level of activity (from TM) in morning hours (1 to 5 a.m.) positively correlated with the number of bats which rested in the box during the same day from IR gate (Pearson's correlation coefficient, $r = 0.61$, $P < 0.05$). Therefore we chose medians of activity from TM only from this period and pooled them with data from IR gate to analyse impact of microclimatic parameters and changes in bat numbers among bat boxes. Generalised additive models (GAMs) were fitted using the R-Gui 2.0.1 software package. Functions for continuous variables were fitted using cubic smoothing splines, initially with four degrees of freedom. At the completion of the selection process the function for each selected continuous variable was reduced if this did not result in a significant ($P < 0.05$) reduction in goodness-of-fit. These models assume that the mean of the dependent variable depends on an additive predictor through a nonlinear link function. Generalized additive models permit the response probability distribution to be any member of the exponential family of distributions (Hastie and Tibshirani, 1990). Hourly values of microclimatic parameters correlated positively ($P < 0.05$) with their means during the light part of a day (sunrise to sunset) (internal temperature, $r = 0.53$; external temperature, $r = 0.69$; humidity, $r = 0.85$; in all cases the number of ligh parts of day $n_1 = 330$ and the number of hourly values $n_2 = 1771$). Therefore the model was fitted by means of microclimatic parameters from the light part of a day and the numbers of bats using a particular bat box as their day roost. This step removed the effect of shifts in temperature and other parameters during the respective day from sunrise until sunset.

Materials

We monitored activity at the three bat boxes occupied by *P. pygmaeus* for 111, 105 and 115 days, respectively. Hourly values were available on internal and external temperatures, and internal humidity. For each day, the number of roosting bats was counted resting in the box via passive TM and/or active IR gate.

RESULTS

Insulating Properties of Bat Boxes

We found that the internal and external temperature neared equilibrium within five hours (median) after all bats left the particular bat box (max. 8 hrs, min. 3 hrs). Although each of the bat boxes under study differed in exposure, no significant difference among the boxes was found in the time needed to balance the level of internal and external temperatures (ANOVA, $F_{2,\ 19} = 0.69$, $P >> 0.05$). When the bats moved to another roost the next day, they left the respective box soon after sunset without returning to it during the night. Therefore, the five-hour period, after emerging, allowed us to exclude nightly observations i.e., data obtained between sunset and sunrise from further statistical analyses.

Impact of Internal Temperature and Humidity

Factors with possible impact on changes in internal temperature were only studied during the periods of pregnancy and lactation. The post-lactation period was omitted because of great fluctuations in numbers of roosting bats due to the weaning of young. The following independent variables were used in the GAM models: external temperature (tex), internal humidity (hum), reproductive stage [i.e., pregnancy versus lactation (sez)], exposure of bat box (box), and the number of bats roosting in the box during next the day [ind]. Normal distribution and link function identity was considered of the dependent variable, which was internal temperature (tin). The dependence of internal temperature was defined as the function of (tin) ≈ s(hum) + s(tex) + s(ind) + (sez) + (box), where s is a specific smoothing spline fit in a GAM. The interactions among variables were found to be insignificant and did not increase the proportion of variability within the sample. The impact of each of the first three independent variables on the dependent variable was significant (humidity, $\chi^2 = 16.08$, $P < 0.01$; external temperature, $\chi^2 = 26.11$, $P < 0.001$; individuals, $\chi^2 = 8.45$, $P < 0.05$; in all cases $n = 27$). Together they described 87.1% of variability of the internal temperature. The impact of reproductive stage and exposure of bat box was insignificant (*t*-test, $t = 4.21$ and $t = 5.23$, respectively; in both cases $P > 0.05$ and $n = 27$). There was an almost linear dependence between the values of internal and external temperature (Fig. 2). Internal temperature also increased with internal humidity (Fig. 2). The number of bats in the box explained only 29% of the residual variability of internal temperature (holding constant external temperature and internal humidity). Nevertheless, we found a negative correlation between the internal temperature and

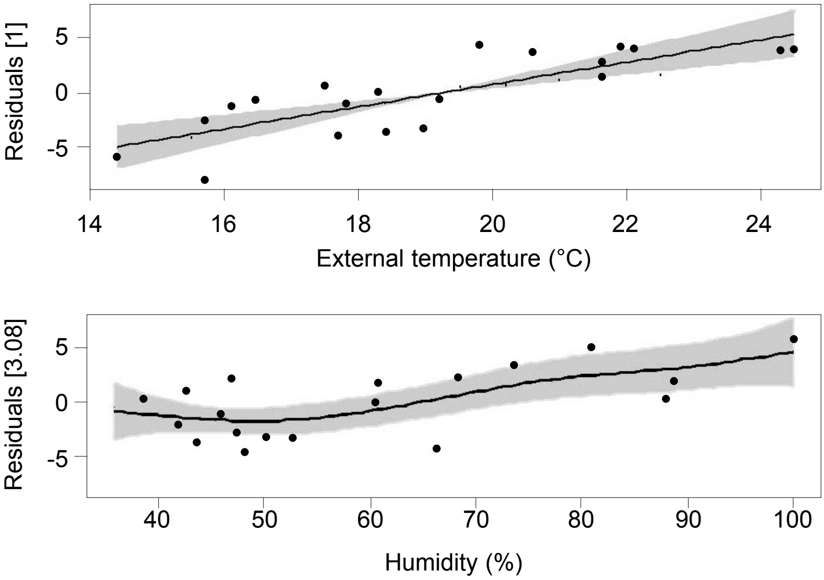

FIG. 2. Graphic output of a generalised additive model for the internal temperature. Both graphs depict the predicted probability of the external temperature (a) and internal humidity (b) holding other predictors constant. 95% confidence limits for the function are also shown as grey zone. Linear dependence between residuals of internal temperature from GAM and external temperature was found. Internal temperature as factor in GAM also increased with internal humidity. Degrees of freedom are in brackets in label of *y*-axes

the number of bats roosting in the box the next day (Fig. 3). The highest median of the number of roosting bats (75 individuals) was found during pregnancy, i.e. at the time when females form nursery colonies. The size of roosting groups was very low during the lactation and post-lactation periods. Cross-correlations were also tested, but the variability of internal temperature was better described by correlations with individual variables than with their combinations.

The dependence of internal humidity on the number of roosting bats was again tested by GAM [only pregnacy and lactation periods, hum ≈ s(ind) + s(tex) + s(tin) + (box)]. It was found that changes in internal humidity correlate with the number of bats much better than do the changes in internal temperature until 7th July (χ^2 = 76.82, *d.f.* = 607, $P < 0.001$, Poisson distribution, link function: log — Fig. 4). The impact of bat box exposure was insignificant again ($t = 6.16$, $P > 0.05$). The number of bats described 52.6% of the variability of internal humidity (holding constant external and internal temperature). The correlation found between the number of bats and humidity in bat boxes was positive (Pearson's correlation coefficient, $r = 0.40$, $n = 330$, $P < 0.05$). There was no correlation between rainy days and presence or absence of bats in bat boxes ($r = 0.03$, $n = 330$, $P >> 0.05$).

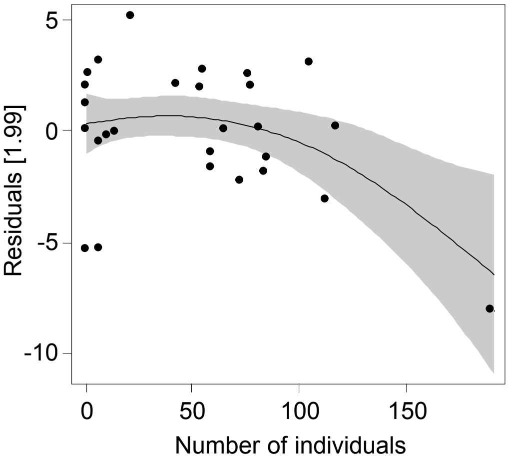

FIG. 3. Graphic output of a generalised additive model for the number of bats in bat boxes, holding all other predictors (external temperature, humidity, localization of bat boxes, seasonal aspect) constant at their mean value. 95% confidence limits for the function are also shown as grey zone. Degrees of freedom are in brackets in label of *y*-axis

Changes in Microclimatic Parameters during The Season

Intraseasonal differences in microclimatic parameters (internal, external temperature, humidity) were evaluated with respect to the reproductive periods. We chose only days with the presence of bats. ANOVA showed significant differences in microclimatic parameters among the periods (pregnancy, lactation, post-lactation — $F_{2,\ 81} = 2.08$, $P < 0.05$). As shown by a *t*-test, the differences arose entirely from the fluctuation in internal temperature on days when bats were present during the periods of pregnancy and lactation ($t = 2.20$, $n_1 = 11$ and $n_2 = 13$, $P < 0.05$). Concerning the internal temperature, it was slightly higher during pregnancy than during lactation (Fig. 5). But the fact that external temperature was significantly higher in lactation than in pregnancy ($t = -3.81$, $n_1 = 11$ and $n_2 = 13$, $P < 0.001$) excludes its direct dependence on internal temperature. This indicates that different internal temperature could reflect differences in thermoregulatory strategies between pregnant and lactating females rather than different external temperature. To verify this result, we analysed the same microclimatic parameters on the days when bats were absent from the boxes. In such cases, insignificant differences were found among periods of the reproductive season (pregnancy, lactation, post-lactation) (ANOVA, $F_{2,\ 249} = 0.49$, $P >> 0.05$). Differences in internal temperatures between boxes occupied and not occupied by bats were insignificant during both pregnancy (*t*-test,

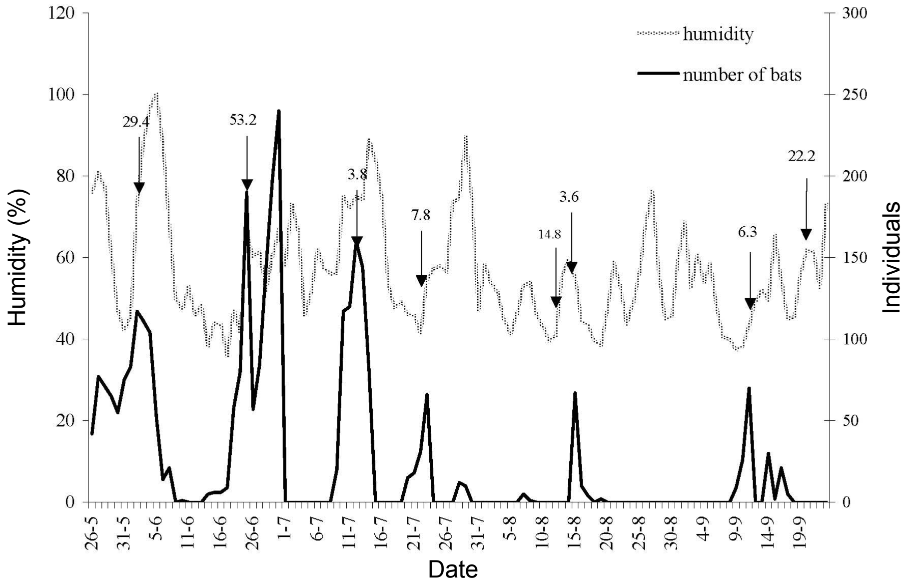

FIG. 4. Changes in internal humidity and number of bats. Pointers show days with daily sum of the precipitations higher than 2 mm. Numbers above pointers are precipitation values in mm. Progress in internal humidity along with bats occurence is noticeable during the pregnancy and lactation periods (until 7th July)

$t = 0.49$, $n_1 = 15$ and $n_2 = 13$, $P > 0.05$) and lactation ($t = 0.34$, $n_1 = 8$ and $n_2 = 19$, $P > 0.05$).

During the pregnancy period, the mean humidity (71.7 ± 19.4%) was higher than during lactation (55.1 ± 11.8%). During the lactation period, humidity was significantly higher in occupied than in not occupied boxes (t-test, $t = 3.49$, $n_1 = 8$ and $n_2 = 19$, $P < 0.05$), but insignificant differences were found during pregnancy ($t = 0.58$, $n_1 = 15$ and $n_2 = 13$, $P >> 0.05$).

Comparison of Microclimatic Parameters and Bat Numbers among Bat Boxes

Analysis of variance showed significant differences in microclimatic parameters and numbers of bats among particular bat boxes (ANOVA, $F_{2, 330} = 159.63$, $P < 0.001$). Students t-tests were used to investigate the impact of individual parameters. Significant differences (in all cases $n = 330$) were found concerning external temperature ($t = 2.10$, $P < 0.05$), humidity ($t = -6.25$, $P < 0.001$) and number of bats ($t = -8.60$, $P < 0.001$); no difference was revealed in internal temperature ($t = -0.45$, $P >> 0.05$).

Throughout the season, the numbers of bats roosting in the boxes differed significantly (ANOVA, $F_{2, 330} = 20.45$, $P < 0.001$). However, insignificant differences were revealed by the paired test used to compare

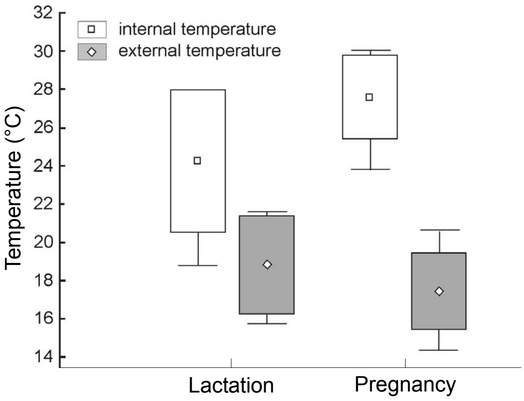

FIG. 5. Differences in external and internal temperatures found between pregnancy and lactation period. Internal temperature, when bats are present, was significantly higher during pregnancy than during lactation. Mean — central tendency, SD — large box, and min–max range as 'whiskers'

the number of bats in a box the day before the bats had left it, with the numbers of bats in the remaining two boxes the following day (Friedman ANOVA, $\chi^2 = 5.57$, $n = 14$, $P > 0.05$). During the lactation period, the largest number of bats was observed in the box showing highest internal temperature (20.5 versus 18.3 and 18.4°C; ANOVA, $F_{2,27} = 8.76$, $P < 0.001$).

Discussion

Changes in Microclimatic Parameters over The Season

Bats of temperate zones, when reproducing, may have strict thermoregulatory requirements on their shelters because low roost temperatures induce torpor, which can delay the development of embryos and offspring (Racey and Swift, 1981; Kunz, 1982; Wilde *et al.*, 1995). For heterothermic bats living under conditions of cool climate, warm roosts are very important and allow them to extend their natural range (Kunz, 1982). Lactating females may sometimes choose warmer roosts to avoid torpor, whereas pregnant females sometimes choose cooler roosts to decrease their thermoregulatory costs (Lewis, 1996; Kerth *et al.*, 2001). In contrast to the actual opinion, we found that the internal temperature of bat boxes with animals inside was higher during pregnancy than during the lactation period. In our study area, females were able to become torpid also during lactation, not only during pregnancy. Our observations come from similar (even lower) altitude (170 m a.s.l.) in central Europe as the study areas of authors who offered similar results (e.g., Kerth *et al.*, 2001; Heise 2005), therefore they are fully comparable. Heise (2005) found lactating females of *P. pygmaeus* to be able to go into torpor, frequently in periods with low temperatures. The negative correlation between low internal temperature and presence of a large number of bats could also be the consequence of their flying off when the bat box was overheated. It seems that high internal temperature is the factor restricting occupation of roosts during pregnancy and lactation periods (Weigold, 1973). We cannot assume that box overheating is a possible risk, because the test of internal temperature between occupied and non-occupied boxes was insignificant. We found empty boxes when internal temperature reached above 40°C at all times (cf. Kerth *et al.*, 2001; Lourenço and Palmeirim, 2004). Box overheating may not be so dangerous if a sufficient supply of alternative roosts, mainly tree cavities, are available in the vicinity. Awareness of natural and/or artificial roosts facilitates the maintenance of thermal comfort due to roost switching which, at the same time, strengthens the social relationships.

Keeping other predictors constant, presence of bats in bat boxes significantly influenced internal temperature and humidity even despite of high variability of microclimatic conditions throughout pregnancy and lactation periods. The comparison of internal temperatures in the days when boxes were and were not occupied shows differences in bat thermopreferences during the season. This fact complicates the use of internal temperature as an indicator of bat presence in the roost. Despite our expectation, internal humidity indicated much better the presence of bats, especially when large numbers of bats were present in a box. Internal humidity positively correlated with the number of bats due to their respiration.

Changes in The Occurrence of Bats

Previous studies have shown that a colony of bats can occupy a complex of tree cavities in one area (e.g., O'Donnell and Sedgeley, 1999; Cryan *et al.*, 2001). Many of the arguments about roost switching in tree dwelling bats are based on predator avoidance, impact of parasites (Wolz, 1986) or finding large numbers of short-lived roosts (Barclay and Brigham, 2001), but also on microclimatic conditions (Kerth *et al.*, 2001).

The data obtained from infrared activity readers show the highest median numbers of bats in boxes during pregnancy, while they were usually lower during the lactation and post-lactation periods. After weaning the numbers of bats fluctuated considerably. The boxes can be used by weaned young either as temporary night roosts or as shelters for several days, due to the increased competition for warmer roosts (Entwistle *et al.*, 1997). At that time, bat boxes were occupied by only few non-reproducing adults during most of the days.

Concerning *P. pygmaeus*, this could be confirmed by records of its high flight activity in close vicinity of a big shelter throughout the season, although the bats did not use the shelter at every recording (Bartonička and Řehák, 2004). Among European bats, the fission-fusion model was shown by *M. bechsteinii* groups (Kerth and König, 1999), but this may not apply to other tree dwelling species (Willis *et al.*, 2003). We found small fluctuations in the numbers of bats in particular bat boxes during pregnancy. In the same period, Willis and Brigham (2004) found a higher roost-sharing index for female *Eptesicus fuscus*. High metabolic activity dependent on high internal temperature positively influences embryo development speed (Racey and Swift, 1981). On the other hand, reproductive stage and information exchange can enhance the sharing of a low number of cooler roosts by females facilitating their torpor during early pregnancy (Kerth and König, 1999).

Numbers of females in colonies determine an effectivity of thermoregulation and affect the level of roost switching (Haddow, 1993; Barlow and Jones, 1999). In the Czech Republic, lower mean numbers of female *P. pygmaeus* in buildings were found (65 individuals, authors' personal observations) than in Britain (200 individuals — Barlow and Jones, 1999). The size of colonies roosting in natural roosts is even lower than in buildings. Therefore smaller colonies roosting partly in hollows may switch roosts more often due to difficult thermoregulation than larger colonies roosting in stone buildings e.g. in Scotland (Haddow, 1993).

Positive impacts of thermally different roosts can have important implications for the management of bat populations via their shelters in forests. Not only must large numbers of natural hollows be maintained, but enough bat boxes also have to be recruited. On the other hand, bat boxes alone can be insufficient for successful reproduction of bats in monoculture forests with little or no natural cavities of adequate space. Our data are consistent with the theory of Willis and Brigham (2004). Bats roosting in caves or big spaces in buildings are in a situation different from tree-roosting bats because the shelters fulfill their microclimatic requirements. Tree-roosting bats are dependent on shelters with different microclimate and must often switch among several cavities or boxes. Pipistrelles (both *P. pipistrellus* and *P. pygmaeus*) are classified as hemi-synanthropic species, the nursery colonies of which usually roost in buildings. But concerning *P. pygmaeus*, it seems that the roosts in buildings do not provide them with microclimatic conditions suitable for the whole reproduction season. They therefore choose the strategy of roost-switching, typical of forest-roosting bats.

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Roost switching and activity patterns in the soprano pipistrelle, *Pipistrellus pygmaeus*, during lactation

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We studied roost switching and habitat selection of 16 *P. pygmaeus* females tagged in two nursery colonies. There were differences in overnight roosting and flying. The highest foraging activity was observed over water bodies, at forest edges and near street lamps. During each night, each female visited at least one night-roost, and five females regularly visited two roosts. On average, the females visited a roost 3.7 times per night. After parturition, the distances between night roosts and day roosts increased. The number of night roosts used declined as pups neared weaning. Five females changed day roosts after two nights. Based on the observation of flightless young in new roosts a conclusion was reached that some females transported their offspring to new roosts at night. After lactation began, some females visited roosts occupied by vocalizing males of *P. pygmaeus* and *P. nathusii*.

Introduction

Differences in peak frequency of echolocation calls (Ahlén 1990, Jones & van Parijs 1993), reproductive isolation and segment differences in the cytochrome *b* gene (Barratt *et al*. 1997) were the main reasons for distinguishing between the two species of common pipistrelle bats, *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus*. Although the discovery of the systematic status of the newly validated species *Pipistrellus pygmaeus* was interesting, little is known about its ecology, in particular its activity patterns and habitat use. *Pipistrellus pipistrellus* can forage in almost all habitat types (Oakeley & Jones 1998, Warren *et al*. 2000, Gaisler *et al*. 2002, Davidson-Watts 2003). By contrast, the composition of prey of *P. pygmaeus* suggests that it is more closely related to aquatic habitats (Barlow 1997). Davidson-Watts (2003) found that *P. pygmaeus* spends a considerable percentage of its foraging time over water. Furthermore, Russo and Jones (2003) showed that river habitats are very important for *P. pygmaeus* in the Mediterranean region. However, Glendell and Vaughan (2002) suggest that *Pipistrellus pygmaeus* selects semi-natural woodland and tree lines more often than water habitats.

Females of *P. pipistrellus sensu lato* often switch roosts during the season (Thompson 1990, 1992). Pregnant females usually inhabit more temporary and cooler roosts, moving to one main parturition roost a few days before parturition (Swift 1980, Webb *et al*. 1996). In England and Scotland, large nursery colonies of *P. pipistrellus sensu lato* rarely switch roosts (Haddow 1993), while individuals from less numerous nursery colonies in central Europe switch their roosts more often, as found in *P. pipistrellus sensu stricto* (Feyerabend & Simon 2000). Our preliminary results suggest that the nursery colonies of *P. pygmaeus* occurring in the floodplain area of southeastern Moravia change roosts during the summer.

Contrary to *P. pipistrellus*, which occupies buildings in almost 95% of cases (Simon *et al*. 2004), *P. pygmaeus* prefers forest and wetland habitats (Barlow & Jones 1999). However, nursery colonies of the latter were usually found in buildings (Park *et al*. 1996, Oakeley & Jones 1998, Sattler *et al*. 2003). In the Czech Republic, most records of *P. pygmaeus* come from low altitudes (Řehák & Bartonička unpubl. data). The main range of this species is in the lowlands of Moravia and central Bohemia and around fishponds in southern Bohemia, where the bat prefers floodplain forests and other wetlands in the alluvial plain of large rivers as well as pond landscapes (Bartonička & Řehák 2004). Regarding the relatively high foraging activity of *P. pygmaeus* in well preserved floodplain forest, we expected that pipistrelles would use natural roosts in hollows or similar types of roosts such as bat boxes, hiding in them more often than in buildings.

Our objectives were: (1) to test whether *P. pygmaeus* switches its day roost as often as *P. pipistrellus* throughout the lactation period; (2) to describe the movement patterns and habitat selection of females tagged in two nursery colonies of *P. pygmaeus*; (3) to consider whether the number of night/day roosts and frequency of roost switching are an important factor for the monitoring and conservation of pipistrelle bat populations; and (4) to describe other activities connected with possible energetic costs during lactation.

Material and methods

Study area

Fieldwork was carried out in southeastern Moravia (Czech Republic). One nursery colony (A) roosted under the roof of a pheasantry, a brick building in the vicinity of the village of Vranovice, in an oak wood forest along the Svratka River (48°57´45.4´´N, 16°37´48.2´´E). The surrounding landscape is characterized by patches of woodland, linear vegetation, and fields. The other colony (B) roosted in a guesthouse in the village of Nové Mlýny, situated in the neighbourhood of an old-growth floodplain forest along the Dyje River (48°51´22.2´´N, 16°43´50.8´´E). Each colony comprised ca. 150 adult females.

Equipment, tracking and spatial analyses

Between June and July 2004, lactating females were netted individually when emerging from a colony roost. After capture and tagging, the bats were held in cloth bags. Only visibly lactating females, identified by the presence of bare patches around their nipples and the expression of milk, had transmitters affixed. The lactation period was defined as 6 June–4 July based on the capture of the first lactating female and the first presence of flying young, respectively. Bats were captured and kept in captivity for a short time under licence No. 922/93-OOP/2884/93 granted by the Ministry of the Environment of the Czech Republic. The authors have been authorized to handle free-living bats according to the certificate of competency No. 104/2002-V4 (§17 of law no. 246/1992).

Studies have shown that low ambient temperatures reduce bat activity by reducing the availability of Diptera, the main prey of *P. pygmaeus* (Barlow 1997). Therefore, minimum night air temperatures were recorded on the nights of tracking outside buildings where colonies were roosting.

Fourteen females from colony A and two from colony B were equipped with 0.38 g radiotransmitters (LB-2N, Holohil Systems Inc. Carp, ON, Canada). Transmitter batteries lasted for a minimum of eight days. The transmitters were glued to the back of each bat between their scapulae, after trimming the fur, using liquid cement (Adhesin, Henkel Ltd., Germany). Neubaum *et al*. (2005) found that bats carrying transmitters representing 5% or less of their body mass were reproductively active and did not suffer from major long-term effects. Davidson-Watts and Jones (2006) successfully tracked bats of *P. pygmaeus* with tags representing 7% of body mass. Our transmitter mass amounted to less than 6% of body mass (range 4.8%–5.7%).

The bats were released and then tracked continuously from sunset to sunrise, using AR8000 hand held receivers (AOR UK LTD, Matlock, UK) and five-element Yagi antennas (Wildlife Materials Inc., Illinois, USA). The locations of the tagged bats were recorded throughout the night by (1) triangulation when two mobile workers co-ordinated their movements using hand-held FM radios (Motorola T5522) or cell phones, and (2) "homing-in" on a bat. Locations were assigned into three distance classes, which differed in open and forest habitats because of differences in the diffusion of sound waves. Therefore, we used different bufferings (circles around a location) in open spaces (< 50 m, 50–100 m, > 100 m) and in forests (< 20 m, 20–100 m, > 100 m). The highest accuracy class (< 50 m and < 20 m, respectively) could only be assigned when we were in close proximity, e.g. close to roosts or in small foraging areas when we could unlink the Yagi antenna, use patch wire only and still receive a signal. Classes of confidence in the accuracy of location were tested experimentally for each transmitter prior to attaching it to a bat in the two different habitats (forest and open space). One person positioned a transmitter while other researchers classified its position into the three distance classes mentioned above. Differences between the estimated locations (intersections) and exactly known locations were not significantly different (t-test: $Z = -4.8$, df = 8, $p > 0.1$) and an assignment of distance class between researchers differed by only 2.9% (n = 61, similarly to Zimmerman & Powell 1995). Behaviour of the bats, their location time, the position of the worker and the position's accuracy were immediately recorded on a handheld tape recorder. The location of bats was estimated using a handheld GPS (Garmin 12XL) and later entered into a geographic information system (GIS) ArcView 3.3 (ESRI, USA). Foraging activity was observed directly and vocalizations (foraging, commuting, territorial signals) were recorded with bat detectors (D 240x, Pettersson Elektronik AB, Uppsala, Sweden).

The foraging areas of the bats were determined by the minimum convex polygon method (95% confidence area, Harris *et al.* 1990, Kauhala & Tiilikainen 2002). The accuracy of foraging ranges was estimated using ArcView 3.3 with the Animal Movement extension (Hooge & Eichenlaub 1997). Tracking locations were buffered by circles with classes of accuracy and minimum polygons were separately calculated around these locations for each night and bat. Only bats with more than 30 telemetry locations were used in space and habitat analyses (Seaman *et al.* 1999). Habitats were divided into six categories, i.e. forests, tree-lines, vegetation edges, water bodies, pastures and fields, and street lamps. All habitats in the area were mapped into ArcView using 1:10 000 grids (Zabaged/2). Habitat use for each female was estimated via the kernel estimation method using 95% of point locations with least-square cross-validation (LSCV) as the smoothing parameter, to exclude the effects of random outliers for general home ranges (Worton 1989). Core foraging areas were defined by the 50% contour lines of the fixed kernel estimation in the program Biotas 1.03.1. This type of estimation more accurately encloses the foraging area in use (Schwartz *et al.* 2002). When area of a specific habitat class was negatively correlated with areas of other classes, we used the single animals instead of the locations as a sample unit in order to avoid the constraints of compositional analyses (Aebischer *et al.* 1993, Otis & White 1999). Autocorrelation, resulting from short sampling intervals, was not a problem with the data used in analyses (cf. Swihart & Slade 1985).

Statistical analyses and material

Females that were tracked for less than 60% of the night were excluded from evaluation. The night was divided into three parts, i.e., thirds (1: sunset–23:30; 2: 23:31–02:00; 3: 02:01–sunrise) (cf. McAney & Fairley 1988).

The bats carried active transmitters for an average of 3.1 ± 1.1 (SD) days (range 2–7 days). The two days with values below the 25th and above the 75th percentiles of mean daily air temperature, air temperature at 21 h, cloud cover, mean daily humidity, wind speed and rainy nights were excluded (cf. Roche & Elliott 2000).

We recorded and analysed data for a total of 35 nights for 16 different female bats. Generally, it is recommended to use data from individual animals as sampling units when testing habitat preference hypotheses (Bontadina *et al.* 2002). Habitat selection was investigated only in females from colony A ($n = 11$). All tagged females showed relatively similar patterns of behaviour and no significant differences among the days of study and the females themselves could be detected. Similar sample sizes were used in studies dealing with the same research questions (e.g. Nicholls & Racey 2006, Feyerabend & Simon 2000 in pipistrelles; Bontadina *et al.* 2002 in *R. hipposideros*) and make our data comparable with these studies. One location on a hunting ground or in a roost was considered a unit of the analysed set. The asymptote was attained at about 30 independent locations in each of the females studied. Home range size did not change significantly after adding further locations (> 30) (similarly in Smith & Racey 2005). However, for some of the females we were able to obtain more than 100 locations. In all, 15 roosts were used in the analysis to determine flight distances and types of roosts.

Roosting and foraging activity data were not normally distributed and were analysed using non-parametric tests (Kruskal-Wallis and Mann-Whitney tests). Data of roost visits were transformed successfully to approximate normality using arcsine transformation. ANOVA and logistic regression were used to check changes in roost switching and distances between night or day roosts and foraging sites. The Bonferroni correction was applied if multiple tests were used for the same data set. Differences in habitat use were tested using contingency tables (χ^2-test). We used Statistica for Windows 7.0 to conduct the analyses.

Results

Roosting and flight activity

No significant differences in roosting time (Kruskal-Wallis test: $H_{13} = 11.62$, $n = 61$, $p > 0.05$), commuting time ($H_{13} = 8.91$, $n = 61$, $p > 0.05$), and foraging time ($H_{13} = 17.76$, $n = 61$, $p > 0.05$) were found either among studied females or among successive nights of one female. Therefore, the data from different females and nights were pooled for subsequent analyses.

Overnight changes in activity

Significant differences in roosting (Kruskal-Wallis test: $H_2 = 10.62$, $n = 61$, $p = 0.005$) and foraging activity ($H_2 = 13.27$, $n = 61$, $p = 0.001$) were found among night thirds (early, middle and late). The highest foraging activity was recorded at the beginning of the night (1st third), and decreased during the following thirds. A significant increase in roosting activity was recorded during the 2nd third. Roosting activity was as high in the 3rd as in the 2nd night period. On the other hand, no differences in commuting activity were found (Fig. 1). Foraging activity was spread, on average, across 4.1 ± 1.6 (mean ± SD) foraging periods (range 1–10), i.e. periods when bats left the roost and foraged continuously.

Foraging areas and habitat use

Habitat selection was investigated only in females from colony A ($n = 11$). We found that range sizes depended on the number of telemetry locations. Maximum size of foraging areas was archived with approximately 30 locations. However, we located only five females at more than 30 locations. Therefore, using minimum convex polygons we calculated the absolute foraging area for those five bats and the minimum foraging area for the others (similar to Bontadina *et al.* 2002). The foraging areas of the bats ranged between 1.6 and 117 ha (median 57.5 ha).

Habitat use did not differ significantly among females, nights or thirds of a night (Kruskal-Wallis test: $H_{10} = 10.32$, $n = 111$, $p > 0.05$). The comparison of habitat use in the core foraging areas (50% contour line of the fixed kernel estimation) with colony range showed that bats preferred vegetation edges and water bodies more

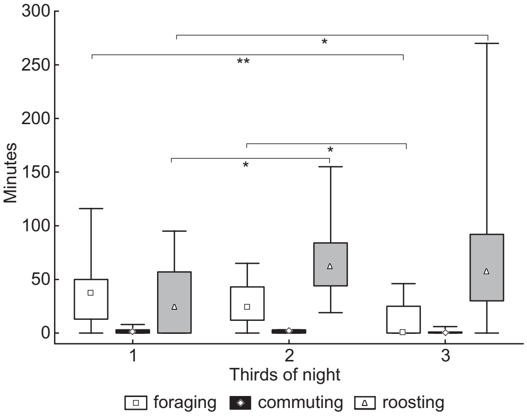

Fig. 1. Foraging, commuting and roosting of *Pipistrellus pygmaeus* (time in min) for the early, middle and late thirds of the night. Mean (square, diamond, tringle); box: 25% and 75% percentiles; whiskers: minimum–maximum. * $p < 0.01$; ** $p < 0.001$.

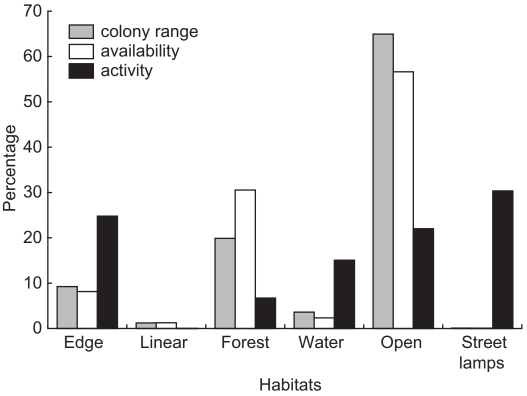

Fig. 2. Habitat use by *Pipistrellus pygmaeus*. Comparisons of habitat availability *vs.* habitat use are based on the minimum convex polygon of all females *vs.* the level of activity (mean percentage) per individual. Habitat selection was investigated only for females from colony A ($n = 11$).

than forests and open fields ($\chi^2 = 118.89$, df = 5, $p < 0.001$). The colony range was predominantly fields and pastures (56.8%) and woodlands (30.7%, Fig. 2). The composition of habitats was not different between colony range and the availability of foraging habitats ($\chi^2 = 0.06$, df = 4, $p > 0.05$). To estimate the availability of foraging habitats we used the area of a circle of 1-km radius drawn around the colony site. Nonsignificant differences in foraging activity were found in open fields, pastures and forests. Often all females foraged at water bodies and vegetation edges, despite their low availability (Fig. 2). High foraging activity found in the vicinity of street lamps was not representative for all tagged females, as only six females foraged in this habitat type.

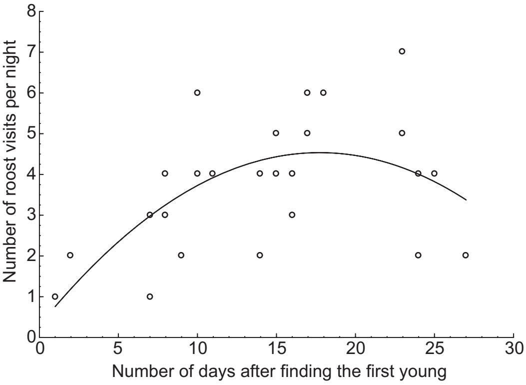

Fig. 3. Number of roost visits per night during the whole lactation period. A quadratic polynomial regression describes, significantly, the increase of roost visits at the beginning of lactation and the decrease at the end of lactation.

Night roosts and roost switching

Each female visited at least one roost per night, but five females visited two roosts during several nights, i.e. on 28% of the nights. Night roosts were visited on average 3.7 times (range 1–7) per night and female. A quadratic polynomial regression significantly described the increase in roost visits at the beginning of lactation as well as the decrease at the end of lactation ($R^2 = 0.45$, $F = 24.13$, $p = 0.008$, Fig. 3). In all, 13 roosts were found in tree cavities and only two in buildings (the main daily roosts of the two colonies). Five females (45%) changed day roosts after the night being tagged (32% of nights in total). For this reason, data from the first night after capture were not used in the roost switching analysis. Nursery colony A used six different day roosts, while nursery colony B only three during the study period. Tagged females from colony A used three roosts simultaneously consisting of ca. 60, 80 and 100 adult females, and three individual roosts.

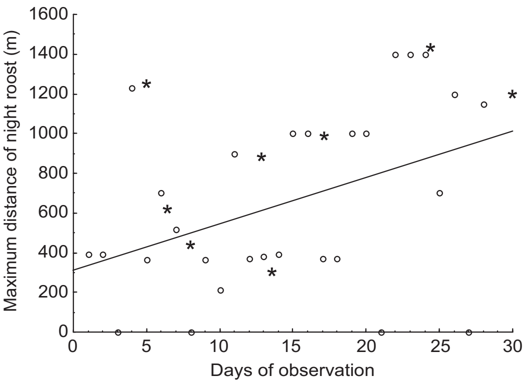

Fig. 4. Linear regression (R^2 = 0.18, F = 5.43, p = 0.027) describing changes in maximum distance between night and day roosts. * indicates nights when females visited a male roost.

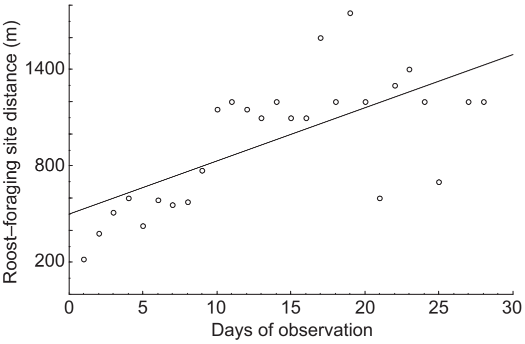

Fig. 5. Linear regression (R^2 = 0.47, F = 20.83, p = 0.001) describing changes of distance between roosts and foraging sites. An increase in distance between the roosts and the foraging sites was found towards the time of weaning.

Distances between roosts and foraging sites

The median distance between night and day roosts was significantly different (ANOVA: F_{26} = 7.79, $p < 0.001$) as were the medians of distances between foraging sites and roosts (F_{26} = 6.27, $p < 0.001$). Foraging sites were over 1 km away from the main roost for 8 of 15 females (maximum 1.75 km). The distances between night roosts were over 1 km in only 36% of all events (maximum 1.4 km). Three trends were obvious at the time of weaning: (1) a decrease in the number of roosts visited per night, (2) an increase in the distance between night and day roosts (Fig. 4), and (3) an increase in the distance between roosts and foraging sites (Fig. 5). Time spent by females in night roosts was constant (ANOVA: F_{78} = 1.17, $p > 0.05$). We tested the distances between day roosts and foraging sites which the females flew directly from their day roost. Five tagged females (during 11 nights) used foraging sites more distant from their original day roost in the building (mean ± SD = 446 ± 276 m). After day roost switching the distance between foraging areas and the new day roost in trees was lower (mean ± SD = 216 ± 84 m) (paired t-test: $t = 2.50$, $n = 7$, $p = 0.04$).

Other general observations

Three females probably transported their young to night roosts, as evidenced by records of the presence of flightless young inside roosts only used at night. Four lactating females visited sites where we recorded song-flight calls of *P. pygmaeus*. The song flights were performed by males near their roost along fixed flight paths. However, we located only one male roost. A tagged female, accompanied by a male, zigzagged for on average 70 minutes (n = 6, mean ± SD = 70 ± 13) among the trees in the vicinity of a male roost. In general, sites where males vocalized were more distant from the day roost than night roosts used only by females (mean ± SD = 989 ± 176 m *vs*. mean ± SD = 646 ± 231 m). A lactating female was even found in a company of one male *P. pygmaeus* and one *P. nathusii* in the same roost during a day. Other females switched their day roosts in the vicinity of male territories. All new female day-roosts within male territories were over 1 km away from their main day roost (range 1.0–1.4 km).

Discussion

Changes in activity

We found differences in some aspects of flying behaviour and roost switching between pipistrelle populations in central Europe and in England (e.g. Bartonička & Řehák 2004, Davidson-Watts *et al*. 2006). Maier (1992) found a bimodal nocturnal activity pattern in *P. pipistrellus* *sensu lato* during the lactation period. During the same period, Davidson-Watts and Jones (2006) observed 1.1 foraging bouts on average in *P. pygmaeus*. However, in our study the number of foraging periods was higher, 4.1 per night. On the other hand, several bouts were shorter, less than 15 minutes. In our study, the average flying time was 76 minutes per night. This value is considerably lower than those found by Davidson-Watts and Jones (2006) for the two pipistrelle species. Longer flying times were also found by Jenkins *et al*. (1998) and Maier (1992) for *P. pipistrellus sensu lato*. The relatively short time spent foraging could be due to the availability of rich food sources in the Czech floodplain forests as compared with those occurring in different habitats in England or Scotland.

Foraging areas and habitat use

Davidson-Watts and Jones (2006) found that *P. pipistrellus* used more habitat types than *P. pygmaeus*. Their results show that *P. pygmaeus* seems to prefer a limited spectrum of habitat types, while *P. pipistrellus* is more opportunist and uses a wider range of habitats. The association of *P. pygmaeus* with water is well known (Vaughan *et al*. 1997, Oakeley & Jones 1998). Rydell *et al*. (1994) found higher activity in pipistrelles above cluttered rather than open water surfaces. Our results support the unambiguous preference of water habitats in core foraging areas as compared with a cluttered forest or open field and meadow, respectively. We confirm that *P. pygmaeus* also prefers vegetation edges characterized by a high density of potential prey, which can also be used more frequently due to their proportionately greater presence in the landscape under study compared to infrequent water surfaces. Our results also agree with those of de Jong and Ahlén (1991) who found that pipistrelles prefer vegetation edges.

Roost switching and distances

Roost switching is often identified in pipistrelles, *Pipistrellus pipistrellus*/*pygmaeus* (e.g. Thompson 1992, Park *et al*. 1996). We chose the lactation period to study roost switching because of high roost fidelity during this period (Racey & Swift 1985). Colony size is important for thermoregulation which affects the frequency of roost switching (Haddow 1993, Barlow & Jones 1999). In England, the mean size of *P. pygmaeus* nursery colonies is about 200 females (Barlow & Jones 1999). We found considerably fewer females in colonies in buildings (median = 65, *n* = 39, author's unpubl. data). The lower number of bats in pipistrelle colonies in central Europe may be due to more frequent roost switching — induced by changes in preferred temperatures during the reproductive season — as compared with large pipistrelle colonies occupying stone houses in Scotland (Haddow 1993). Feyerabend and Simon (2000) observed bats moving even between eight different shelters in a colony consisting of nearly 200 adult females. We found three parallel shelters visited by females coming from the main colony (with 80 bats on average). The whole colony did not move between shelters all at once, as Feyerabend and Simon (2000) found in *P. pipistrellus*. Nevertheless, almost half the females moved between six shelters discovered every other day.

There are no genetic data available to determine kinship among bats within colonies of *P. pygmaeus*, but the simultaneous use of more shelters by females coming from one colony indicates an analogy with the fission–fusion model used in *Myotis bechsteinii* (Kerth & König, 1999) and *Eptesicus fuscus* (Willis & Brigham 2004). Most authors (Racey & Swift 1985, Jenkins *et al*. 1998, Davidson-Watts & Jones 2006) assume that the foraging areas are close to the roosts. Feyerabend and Simon (2000) found the distance between day roosts to be several times longer than the distance between the roost and the foraging site. Although the sample was small (only seven movements between day roosts), our results support the hypothesis that females may change their roosts because they are closer to attractive foraging sites (e.g. Feyerabend & Simon 2000). The distance between the first night foraging site and the previous day roost was always longer than the distance between the new day roost and the foraging site in the subsequent night. However, females can also use different roost types due to different microclimatic conditions inside them (buildings versus natural hollows) as conditions change during the reproductive season (Thompson 1990).

Our data show that it is extremely difficult to determine the actual size of a *P. pygmaeus* colony in central Europe because females use simultaneously more than one roost. By contrast, numerous pipistrelle colonies in England do not switch roosts for most of the reproductive season (e.g. Davidson-Watts & Jones 2006). This observation is very important especially in view of long-term monitoring programmes in which the size of maternal colonies is considered a basic index of population dynamics.

Transport of young

Suckling female *P. pygmaeus,* similarly to *P. pipistrellus*, were found to use several day roosts during the reproductive season (Bartonička & Řehák 2007). In the area exploited by colony B, bat boxes equipped with IR diode cameras were installed for our earlier research. Owing to all-night recordings, we found that one female carried its naked suckling infant into the empty box and returned for it some hours later. A similar behavioural pattern was also observed (by means of radio-tracking) during this study in case of two tagged females from nursery colony A. Juveniles were observed during the night in all roosts. For lactating females, transporting young and depositing them in a temporary roost within the hunting ground can be less costly than flying back and suckling the young in a distant nursery.

Paying visits to males

Four lactating females visited sites where we recorded song-flight calls of *P. pygmaeus*. We concluded that they might have been uttered by a male flying along the same flight route (Barlow & Jones 1997) although male advertisement calls were registered later, after weaning the young. However, Gerell-Lundberg and Gerell (1994) already found the first territorial vocalization during the lactation period at the end of June. Social calls undistinguished from song-flight calls were also emitted during group foraging but no feeding buzz was found during our recordings. Calls were produced at regular intervals indicating their attractive character (Lundberg & Gerell 1986). All four females mentioned above showed signs of nursing but we cannot exclude the possibility that their young died soon before, or during our study. The day after males were visited, three females chose their night roost in a tree hole in the vicinity of the male territory they had visited the previous night. Most observations were carried out for 15 days following the first find of an infant in the main roost. Hughes *et al.* (1989) never found pipistrelle females suckling offspring other than their own, therefore we suppose that the females had to carry their own young. The possibility of the young remaining inside a roost in the absence of its mother during daylight needs to be tested in more controlled experiments. Our results suggest that lactating females must be able to invest some of their stored energy in visits to male territories even during lactation, a period of highest energetic cost.

In summary, our results suggest that females of *P. pygmaeus* from a maternal colony use more than one roost and the movements among these roosts are often. This observation makes colony-counting as a method commonly used to evaluate changes in population dynamics unreliable and should be consulted within the framework of international conservation and management measures. Roost switching and the transport of young by females reduce energy costs related to movements in foraging sites. New findings that lactating females visit territorial males and share their roost suggest that the lactation period is less costly than is generally accepted.

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8.2 Životní cyklus štěnic a přežívání v úkrytech netopýrů

BARTONIČKA T. & GAISLER J., 2007

Seasonal dynamics in the numbers of parasitic bugs (Heteroptera, Cimicidae): a possible cause of roost switching in bats (Chiroptera, Vespertilionidae).

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ORIGINAL PAPER

Seasonal dynamics in the numbers of parasitic bugs (Heteroptera, Cimicidae): a possible cause of roost switching in bats (Chiroptera, Vespertilionidae)

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Abstract The objective of the present paper is to extend the knowledge of roosting strategies of bats and the interaction of bats with their roost ectoparasites, the bat bugs *Cimex pipistrelli*. The project was focussed on the potential causality of bat movements and the variation in bug numbers. For 2 years, three model bat boxes with breeding female *Pipistrellus pygmaeus* were monitored inside floodplain forest. After the arrival of bats in May, adults and first instars of bugs were observed in the boxes. During the lactation period in June, when the bats did not occupy the roosts, the first instar bugs died out followed by the adults. The decrease in bug numbers began only several days after the bats had left the boxes. After a month of the bats' absence, the abundance of adult bugs decreased by half of their number. Only the eggs survived the period when the roosts were unoccupied in summer. In mid-July, after the arrival of lactating females, an increase in the number of bugs was observed. At the beginning of August, however, no new eggs were found. Although adult *C. pipistrelli* are able to survive the winter period in the boxes, the bats, by shifting the roosts within the vegetation season, prevent the massive reproduction of these parasites.

Introduction

Previous studies were mostly focussed on communities of ectoparasites of different bat species with no regard to their life strategies (e.g. Whitaker and Mumford 1978; Whitaker et al. 2000; Ritzi et al. 2001; Ritzi and Whitaker 2003). Few papers described the relationship between ectoparasite density and the reproductive cycle of its bat host (Dietz and Walter 1995; Christe et al. 2000). There are several studies in which the impact of ectoparasites living permanently on the host body (e.g. mites, Spinturnicidae) was analysed (Lewis 1996; Giorgi et al. 2001). Bugs of the family Cimicidae are the most important roost ectoparasites of bats. The family includes 91 species, 61 of which parasitize bats (Ryckman et al. 1981). Great mobility and ability to survive without their hosts for a long time makes the bugs important model ectoparasites, not only of bats (Usinger 1966). Several authors dealt with the taxonomy of bugs parasitizing bats, yet unsolved problems persist, including the status of *Cimex lectularius* and *Cimex pipistrelli* (Wendt 1941; Povolný 1957; Usinger 1966; Péricart 1972; Aukema and Rieger 1995–1996; Péricart 1996). In this paper, both taxa mentioned above are regarded as separate species.

Fast ontogeny enables the bugs to increase their numbers rapidly soon after a roost was occupied by bats. Due to the fact that it is also parasitizing man, the bed bug (*C. lectularius*) is the best-known cimicid species. Female bed bugs usually lay from two to four eggs per day. During its life, a single female can lay as much as 500 eggs (Davis 1964). Bed bugs suck blood in periods of few days and are able to starve for months (maximum 1.5 year). Due to their long starvation capacity, bed bugs survive the winter period or the absence of the host in its roosts (Johnson 1942; Povolný 1957). Usinger (1966) found that the temperature and humidity in the roost influences the survival of all ontogenetic stages and the promptness of adults to copulate. Bugs in general prefer lower roost temperature than other bat parasites do because they stay on bats for a short time to feed only (Usinger 1966). At present, scant information is available on the possible impact of microclimate fluctuation

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and the length of starvation on the survival of particular ontogenetic stages and adults of the two sexes (Rivnay 1932; Johnson 1940; Omori 1941).

Nursery colonies of several bat species dwelling in crevices of trees, hides or buildings switch roosts frequently during the reproduction season (e.g. Thompson 1990 in pipistrelles or Kerth and König 1999 in *Myotis bechsteinii*). In the territory of the present Czech Republic, the bugs (Cimicidae) were found in roosts occupied by *Pipistrellus pipistrellus* s.l. (Gaisler 1966; Hůrka 1988). New observations show that bat bugs *C. pipistrelli* occupy the roosts of two pipistrelle species, *P. pipistrellus* and *Pipistrellus pygmaeus* (Bartonička, unpublished). As in *P. pipistrellus*, females of *P. pygmaeus* switch roosts very often. Typically, groups of females leave their respective roosts in early June and move to the main nursery roost few days before parturition. They move back to their previous roosts with flightworthy juveniles in mid-July (Swift 1980; Webb et al. 1996). The so-called fission–fusion model common in primates, i.e. a temporary splitting of one nursery colony into sub-groups, was described in *M. bechsteinii* (Kerth and König 1999; Willis and Brigham 2004). Similar roost switching strategy was observed in *P. pipistrellus* and *P. pygmaeus* (Feyerabend and Simon 2000, own observation). Sub-groups of genetically close females occupied a complex of tree hollows and bat boxes within a small area and switched roosts every 2 days (Willis and Brigham 2004). Although the fact of roost switching was demonstrated, the cause or causes of this phenomenon were not understood completely till now (e.g. Lewis 1995; Vonhof and Barclay 1996; Brigham et al. 1997). There are several hypotheses explaining why bats switch their roosts. The movements may be caused by sudden changes in microclimatic conditions, restricting the circulation of air inside a crevice type shelter (Whitaker 1998; Lourenço and Palmeirim 2005) or changes in thermoregulatory requirements of bats regarding their reproduction cycle (pregnancy vs lactation) (e.g. Thompson 1990; Hamilton and Barclay 1994; Kerth et al. 2001). In addition, the impact of humans, animal predators or unknown factors can disturb bats (Lewis 1995).

In our study, we focussed on a hypothesis assuming that by switching roosts the bats avoid the increasing invasion by ectoparasites and keep their individual fitness (Wolz 1986; Lewis 1996). The large numbers of ectoparasites observed on adult females and young after parturition show synchronisation of the population cycles of ectoparasite and host. Seasonal changes in the numbers of *C. pipistrelli* in semi-natural roosts were evaluated regarding the presence of bats. We attempted to answer the following two questions: (1) Do temperature and humidity influence the numbers of bat bugs in the roosts? (2) Is there any correlation between the numbers of bats and bat bugs? We think that the data on roost infestation by parasites together with the knowledge of other parameters such as the microclimate of occupied roosts (Lourenço and Palmeirim 2005; Bartonièka and Øehák 2007) can be utilised for the purposes of conservation of bats and their shelters.

Materials and methods

Study area and technical equipment

Nursery and temporary colonies of *P. pygmaeus* with high densities of bat bugs *C. pipistrelli* were monitored in three wooden bat boxes inside the Křivé jezero floodplain forest (S Moravia, Czech Republic) for 2 years. In the first growing season (2004), all bat boxes were equipped with passive IR Trail Monitors, TM (TM550, TrailMaster, Goodson & Associates, USA) (after Thomas 1995) and thermometers (Hobo, Onset Computer Corporation) to record the presence of bats and the temperature in the boxes over the season. TM monitors were situated in front of the entrance to each box (Bartonička and Řehák 2007). Hobo Data Loggers continuously recording internal temperature and internal humidity were situated under the roof of each bat box. The accuracy of the relative humidity sensor was ±4% and that of the temperature sensor ±0.4°C. Monitoring of bat bugs was carried out by manually sampling from inside the box roof and recording the activity of insects by a video system (B/W AVC 307R+12 IR diode). In each of the three boxes, the IR diode camera monitored a particular inside area (3×10 cm) and recorded the relative activity of bugs in 30 min periods. All monitoring was carried out regularly in 10-day periods just before most bats left a particular bat box to limit the effect of human disturbance. Each sample of bat bugs was divided into four groups: (1) adults, males and females together, (2) first to third instars, (3) fourth and fifth instars and (4) eggs. Females prevailed among the adult insects. In each group, the number of bugs was recorded. Bug movements within the experimental inside area scanned by the camera were counted regardless of the groups.

The reproductive season under study was divided into three parts: pregnancy (till 6 June), lactation (7 June till 6 July) and post-lactation (since 7 July). The time of parturition was indicated by the presence of seven newborns in one of the three boxes.

Statistical analysis

No significant differences were found between the two seasons, 2004 and 2005, which allowed to pool the data. All variables showed a normal distribution (Kolmogorov–Smirnov test). Statistics for Windows 7.0 was used for data analyses (PCA, ANOVA, *t* tests, correlations). The level of morning bat activity (returning from foraging flights at 1–5 AM) (Trail Monitors) positively correlated with the number of bats found in a box later during that day when the boxes were checked manually (Pearson's correlation coefficient, $r=0.73$, $p<0.05$). The numbers of bugs, revealed from records of their activity by a video system, positively correlated with that obtained by manual sampling from inside the box roof ($r=0.84$, $p<0.05$). No significant differences were found in the numbers of bugs and presence of bats between particular bat boxes (ANOVA, number of bugs, $F=0.57$, ns; presence of bats, $F=2.10$, ns), therefore the data were pooled. Analysis of variance (ANOVA) was used to check the differences between the parts of season and *t* test as post hoc. Principal component analysis (PCA) was used to check the effect of partly correlated variables (internal temperature, internal humidity, numbers of bugs and bats). The model was fitted by means of microclimatic parameters from the light part of the day and the number of bats using a particular bat box as their day roost. Bonferroni correction was applied if multiple tests were used for the same data set.

Material

During the two growing seasons, the three bat boxes occupied by soprano pipistrelles (*P. pygmaeus*) were monitored for 151, 199 and 115 days, respectively. For each day, hourly values were available for internal temperature, internal humidity and the level of the bats' activity. The samples of bat bugs (*C. pipistrelli*) were taken on 19 occasions.

Results

Seasonal changes in the occupancy of bat boxes

The differences in the numbers of bugs and presence of bats among 19 checks were statistically significant (ANOVA, $F=1.89$, $p<0.01$). Two peaks in the occupancy of boxes by bats were recorded in May and August. Pregnant females used these roosts before parturition and some of them returned with flightworthy young (Fig. 1). During the pregnancy of bats until the parturitions in mid-June, a significant increase in the number of bat bug eggs was recorded (*t* test, $t=-6.69$, Bonferroni correction, $p=0.001$, $n_1=3$, $n_2=3$). A significant decrease was found from early June until the end of July. No bug eggs were found on subsequent checks (Fig. 1). The number of adult female bugs, surviving from previous winter, decreased until mid-June ($t=-83.59$, Bonferroni correction, $p=0.001$, $n_1=3$, $n_2=3$) when they died out. Only nymphal stages were present in the boxes during the first half of August. A rapid increase in the number of adult bugs was registered after the bats, mainly young ones, returned to the boxes in mid-August ($t=-3.34$, Bonferroni correction, $p=0.01$, $n_1=3$, $n_2=3$). After mid-September, when the metamorphosis of the last (fifth instar) nymphae was completed, the number of adults stagnated. Only adults survived the winter period. Two peaks in the presence of bats were distinct throughout the season. The first peak during pregnancy, however, was insignificant (*t* test, $t=2.03$, Bonferroni correction, ns, $n_1=3$, $n_2=3$). During the lactation period, the number of bats in the boxes was very low. A significant increase was found from the beginning of July until the second half of August ($t=-1.54$, Bonferroni correction, $p=0.001$, $n_1=3$, $n_2=3$) (Fig. 1). During the period in which the bats were absent (lactation, June), a rapid dying of the early stages of bugs (first to third instars) was recorded. After all the bats had left, the abundance of this bug group decreased to a half in 9 days. A similar decrease in the number of fourth and fifth instar bugs was observed in 16 days. Likewise, the number of adults decreased to less than a half during the absence of bats. In general, the population of bat bugs survived the summer absence of bats in the egg and nymphal stages.

Relationship among particular variables

Internal temperature, relative humidity, number of bugs (adults, first to third instar, fourth and fifth instar), number of eggs and number of bats were included in the principal component analysis. The first two factors accounted for 78.8% of variability. Table 1 shows the relations of individual components and variables. The cluster of days during bat pregnancy differed due to the positive correlation of component 1 with the number of adults and first to third instar bugs and due to the negative correlation with the number of eggs, which increased rapidly in these days. A large number of eggs were also observed during lactation, but nymphal and adult bugs and bats were found in very low numbers. The cluster of days in the post-lactation period was characterized by positive correlation of numbers of adult bugs and bats at the absence of eggs (Fig. 2). Furthermore, significant differences using discrimination analysis were found related to the reproduction period (Wilks' $\lambda=0.34$, $F=2.91$, $p<0.002$).

A positive correlation was found between the number of adult and fourth and fifth instar bugs (Pearson's correlation coefficient, $r=0.52$, $p=0.005$) and between the number of eggs and number of individuals of first to third instar ($r=0.33$, $p=0.001$). The number of eggs and relative humidity correlated negatively ($r=-0.37$, $p=0.01$). The presence of bats positively correlated with the number of first to third instar bugs ($r=0.39$, $p=0.001$). The possible impact of temperature on the number of bugs was tested. A positive correlation was found only between the internal tempera-

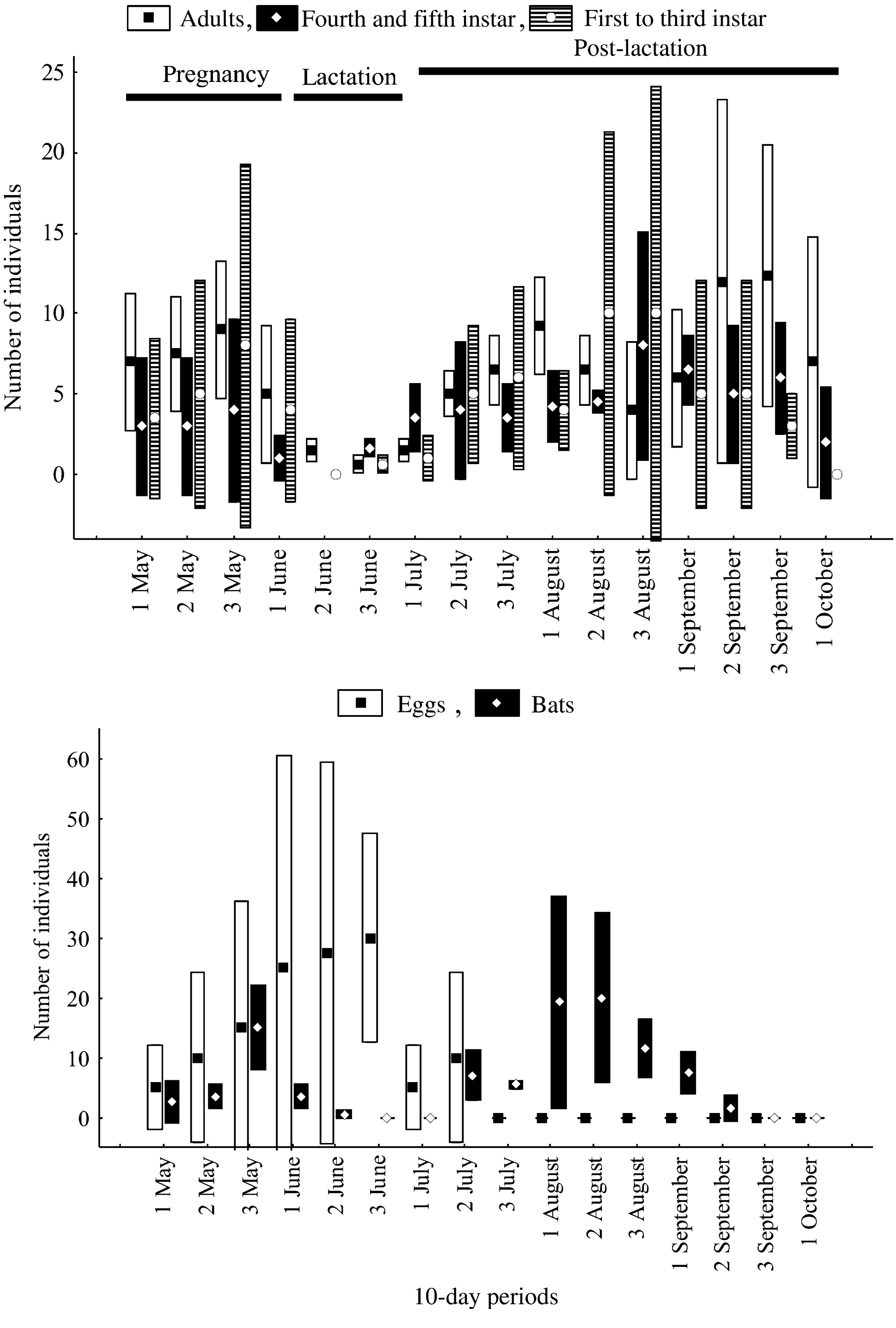

Fig. 1 Changes in numbers of bat bug adults, all instars and eggs, and numbers of bats; mean—central tendency, SD—large box

Table 1 Correlations (Pearson's correlation coefficient) between variables and components of PCA

	A	B	C	D	Bats	Tin	Hum
Component 1	0.68*	0.47*	0.39*	−0.69*	0.46*	0.04	0.34*
Component 2	0.23	−0.27	0.25	0.21	0.05	−0.62*	0.05

A: adult bed bugs, *B*: fourth and fifth instars, *C*: first to third instars, *D*: eggs, *Tin*: internal temperature, *Hum*: relative humidity.
*$p<0.05$, all values are significant.

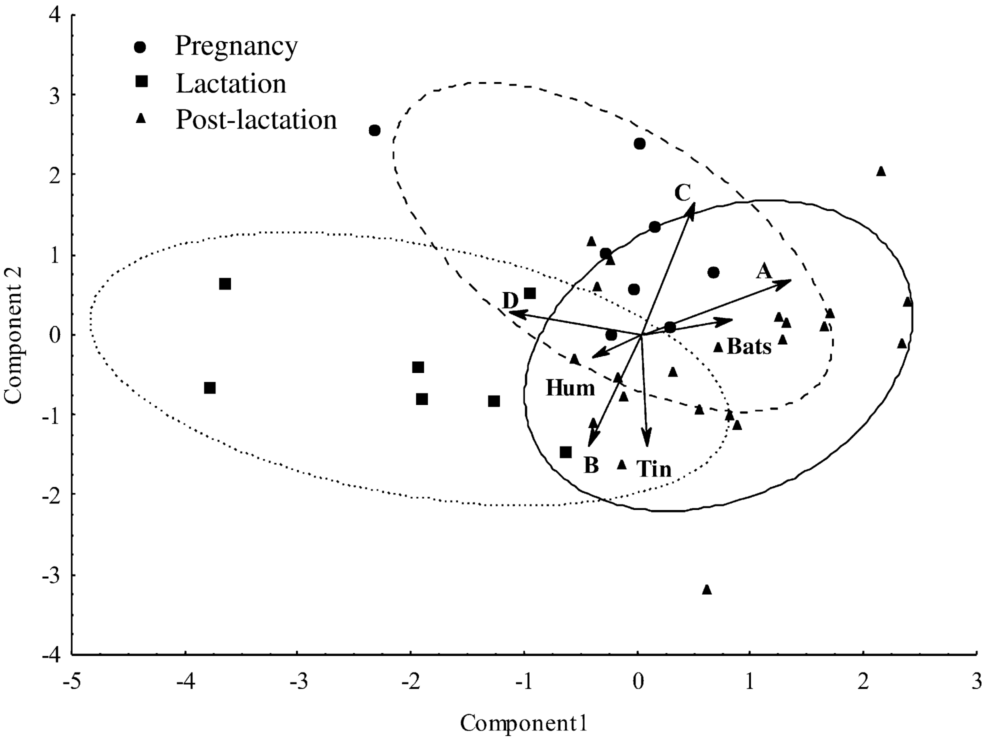

Fig. 2 Principal component analysis (PCA): on the axes are values of the two first factors (describing 78.8% of inner variability). *Ellipses* represent the 75% confidence interval for the days of the reproduction periods—pregnancy (*dashed line*), lactation (*dotted line*) and post-lactation (*solid line*). *A*: Adult bat bugs, *B*: fourth and fifth instars, *C*: first to third instars, *D*: eggs, *Tin*: internal temperature, *Hum*: relative humidity

ture 10 days before the checks and the number of first to third instar bugs (Pearson's correlation coefficient, $r=0.41$, $n=38$, $p<0.005$).

Discussion

Microclimatic conditions

From among various environmental factors, temperature seems to be the most important because it influences the development and activity of bugs. In most roosts, temperature varies significantly during both the season and the day, being rather constant only in large caves and similar shelters (Usinger 1966). In this paper, however, nearly no correlation between the temperature and the number of bat bugs was revealed. The only exception was a positive correlation between the temperature in the boxes and the number of early developmental stages of bugs found 10 days later. This may have been due to the accelerated embryonic and nymphal development at optimum temperature. In contrast to *C. lectularius*, no information is available concerning the response of *C. pipistrelli* to various microclimatic conditions (e.g. Rivnay 1932; Johnson 1940; Omori 1941) but the main aspects may be similar in the two species. In *C. lectularius*, the embryonic development is accelerated by temperature increasing up to 30°C, when it is the shortest (3.7 days, Omori 1941). The upper temperature limit, blocking the hatching, nymphal development and adult activity, is 37°C in *C. lectularius* (Omori 1941). Mortality sets in at 44–45°C (Hase 1917) but for a short time, in the order of hours, bed bugs are able to survive even such high temperatures. During our study, the temperature in the boxes reached values of this kind on 13 days yet the daily mean temperature never exceeded 30°C. Laboratory testing is required to reveal the impact of temperature on the timing of development in bat bugs.

Relative humidity of air is another factor that possibly influences the seasonal dynamics of bat bugs. The experiments with *C. lectularius* suggested that in some instars their development was shortened at 32°C when the humidity was low; in contrast, at 22°C the development was shortened when the humidity was high (Rivnay 1932). According to Kemper (1936), extremely low relative humidity (0–20%) often causes the death of nymphae in the course of ecdysis. The negative impact of low humidity on the first instar may even be greater. At relative humidity <60%, its survival is reduced to a fifth (Jones 1930). Mortality is caused by dehydration (Mellanby 1932). In the bed bug, both high and low temperatures when combined with high humidity cause up to 90% mortality of eggs (Johnson 1940). Correspondingly, the results of our study on the bat bug show negative correlation of the number of eggs with relative humidity. The combination of low temperature and high humidity was typical for the post-lactation period when no new eggs were recorded. As indicated by the results of laboratory experiments with *C. lectularius*, the survival of eggs of *C. pipistrelli* during the humid autumn season is reduced if not impossible. The hibernation of adults and not of eggs, therefore, could be an adaptation to the cold and humid climate of the temperate zone.

Availability of food

In the bed bug, the availability of food affects oviposition, duration of individual instars and total length of life (Usinger 1966). The frequency of bloodsucking is positively correlated with temperature, e.g. every third day at 27°C in adults (Kemper 1936). The nymphae are able to feed 24 h after ecdysis but they suck in shorter intervals than the adults do. Concerning various developmental stages, early instars are the most vulnerable if they do not have enough food. Food deficiency has a negative impact mainly when ambient temperatures are high. Such temperatures, though not yet lethal, force the bugs to suck more often because they prevent bug torpidity and increase the danger of dehydration. The availability of food affects the weight of a particular adult female, which is positively correlated with the number of eggs laid. When feeding regularly twice a week, the females produce 2.76–8.26 (average 5) eggs per week for about 18 weeks (Johnson 1942). The adult life span may encompass 12–18 months and after harvesting, they usually die (Usinger 1966). The results of this study correspond to these data in that the early instar bugs will die soon after their hosts have left the boxes and no eggs were found at the end of July. The presence of pregnant female bats after the end of April is essential to start the process of gradation of the bug numbers and assure the production of sufficient number of eggs that will survive the absence of bats during lactation.

Impact of bugs on bats

Abundant bat bugs may induce heavy stress to their hosts. The trouble they cause may lead to disquiet of bats resulting in their abnormally frequent scratching, cleaning of fur and wing membranes and efforts to catch the parasites on their body. Several authors argue that the increased number of roost parasites forces the bats to leave the roost and find another one (e.g. Wolz 1986; Lewis 1996; Walter 1996). Roost switching seems to be a good anti-parasitizing strategy of bats with respect to *C. pipistrelli*. This bug species was by far not recorded in all maternity colonies of bat species known as its potential hosts. According to Reinhardt and Siva-Jothy (2007), only 12% of *Myotis myotis* colonies were parasitized by *C. pipistrelli*. In shelters of bat species preferring tree hollows and various fissures in summer, such as *P. pipistrellus* and *Nyctalus noctula*, the presence of bat bugs was recorded more often (K. Hůrka, unpublished). However, it is more difficult to check fissure-like roosts than spacious shelters such as lofts of buildings for the presence of bugs. At present it seems that in the territory of the Czech Republic, *C. pipistrelli* is common in “fissure” bat species that often change their roosts, while *C. lectularius* occurs rarely and only in “space” bat species with a high degree of philopatry to their summer shelters (O. Balvín, unpublished). Further research is needed to specify the differences in seasonal dynamics between the two species of bugs.

In Europe and North America, the highest abundance of bat ectoparasites has usually been recorded in the second half of May when maternity colonies of bats are well-established (e.g. mites, Estrada-Peña et al. 1991; Lučan 2006). The simultaneous use of more shelters by females coming from one colony was observed in certain bat species during pregnancy (e.g. Kerth and König 1999; Willis and Brigham 2004). Shortly before parturition, heavily pregnant females return to their original large maternity shelter. They give births there and suckle their young. Among other initially tree bats, this is also the case of pipistrelles (Swift 1980). During the lactation period, most satellite roosts are empty (bat-free). The present study shows that the absence of hosts, coupled with high temperatures and natural mortality of parasites reduces the number of bat bugs in bat boxes to less than a half of the number recorded before the bats left. Immature bugs die almost totally. However, why do female bats return to the satellite roosts after lactation and perhaps show them to their young in spite of the previous bad experience with bat bugs? The answer may be that the roost selection of female bats is governed by their thermopreferendum: they seek thermally optimum shelters that differ within their reproduction cycle (Thompson 1990).

The bugs are not the only ectoparasites of temperate bat species. Very likely, bat anti-parasitizing strategies against mites, fleas, nycteribids and bugs are mutually different. In bat species parasitized mainly by spinturnicid mites, e.g. *Miniopterus schreibersii* and *M. daubentonii*, the course of clustering and shift of shelters of reproducing females during the season is different from that in bats parasitized mainly by bugs (Estrada-Peña and Serra-Cobo 1991; Dietz and Walter 1995). To conclude, the study shows that pipistrelle bats are able to prevent the massive reproduction of bat bugs by temporarily abandoning particular roosts. Nevertheless, they are unable to rid their roosts of the ectoparasites completely, which is the obvious consequence of the co-evolution of *Pipistrellus* bats and *Cimex* bugs under the conditions of the temperate climate.

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ORIGINAL PAPER

Survival rate of bat bugs (*Cimex pipistrelli,* Heteroptera) under different microclimatic conditions

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Abstract Survival of facultative ectoparasites, e.g. bed bugs (*Cimex* spp.), is more intensely affected by climatic factors, namely temperature, than that of permanent ectoparasites. The ontogenetic time of the bat bug (*Cimex pipistrelli*) in bat roosts is limited by different survival rates under different temperatures in particular nymphal stages. This limitation could affect bug densities and cause asynchrony between the ectoparasite and bat reproductive cycle. Therefore, bug survival under different temperatures was tested in the laboratory. Survival success was evaluated by three types of survival analyses: Kaplan–Meier estimation, the Cox proportional hazards model and Weibull parametric regression. The bugs survived for only a few hours at 45°C; however, such a high temperature was never found in natural roosts. Different survival probability among different ontogenetic stages was found at the temperatures of 5–35°C, and it was the highest in adult females and nymphs of fourth and fifth instar. Early instars first to third were found to be the most sensitive with the highest mortality of all stages studied and having their best survival at 5°C. The hazard rate ratio of Weibull regression shows the low daily failure rate of 2.23–4.34% within the span of 5–35°C. *C. pipistrelli* had the shorter life cycle and the better survival at higher temperature (35°C) than *C. lectularius*. The ability of the former to survive high temperatures could be the consequence of its long-term coexistence with bats preferring crevice-like roosts or attics which become overheated during the summer months.

Introduction

Fast ontogenesis, high reproductive potential, mobility and, at the same time, a hidden way of life make bugs (*Cimicidae*) important ectoparasites. All species of this family inhabit the shelters of warm-blooded animals, but mostly without physical contact with their hosts. They fully engorge in 10 to 20 min, after which they return to their refugium (Usinger 1966). Up to the present, studies concerning the life history of bugs have dealt mainly with species which are usually human parasites and vectors of more than 40 human diseases (*Cimex lectularius* and *Cimex hemipterus*, Hwang et al. 2005; Goddard and deShazo 2009). Neglected, though more abundant in central Europe, are bugs from the species group *Cimex pipistrelli*, obligatory ectoparasites, wholly associated with bats or birds (Reinhardt and Siva-Jothy 2007). A high bat bug population density in a roost soon after its settlement by bats is caused by synchronisation of the ectoparasite density with the reproductive cycle of bats (Bartonička 2008). Roost switching in bats during one season is well-known and seems to be an anti-parasite strategy with respect to bat bugs (Bartonička and Gaisler 2007). The authors showed that the absence of bats in satellite roosts during the lactation period, coupled with high temperatures and natural mortality of parasites, reduced the number of bat bugs and their ontogenetic stages in bat boxes to less than half the initial number. According to the present state of knowledge, two climatic factors, temperature and humidity seem to have the largest impact on bugs survival (Usinger 1966). The effect of humidity, however, is quite incalculable and could only have been discovered by investigations in the laboratory (Rivnay 1932). Usinger (1966) demonstrated that temperature and humidity significantly affect survival of all ontogenetic stages and also the relative

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promptness to copulation in bed bugs (*C. lectularius*). However, it is generally stated that bed bugs are very little affected by different degrees of humidity (Kemper 1936). Rivnay (1932) tested the effect of humidity (10–70%) on the ontogeny of *C. lectularius* nymphs. He found only small insignificant differences in life cycles and assumed the humidity effect was negligible. Nevertheless, direct correlation between temperature and relative humidity is well-known. However, up to the present, only few surveys have dealt with ecological relations between temperature and survival of particular instars after starvation periods of various lengths (cf. Funakoshi 1977; Overal 1980). Previous studies showed high tolerance towards dehydration (up to 40% loss in body water) and a desiccation hardiness against high temperatures (35–40°C; Dubinin 1947; Southwood 1954). Early instars with higher body water content could have worse survival under high temperatures in climatically extreme conditions of natural bat roosts (Jones 1930; Johnson 1960; Usinger 1966). In general, bugs prefer lower temperatures than do other bat ectoparasites which live on their hosts' bodies permanently (Usinger 1966). Reinhardt et al. (2008) compared the temperature in a roost of the fruit bat (*Rousettus aegyptiacus*) and in the refugia of its ectoparasite, the cimicid bug *Afrocimex constrictus*. They found that bugs preferred moving several metres to staying together with fruit bats and risked suboptimal conditions in which they were unlikely to reproduce. Even relatively small temperature differences can be translated into ectoparasite fecundity.

Surviving water and temperature stress considerably prolonged periods of inactivity, hence the readiness for sucking could be different within particular ontogenetic stages. We examined all stages of *C. pipistrelli* to illustrate their survival shift under a wide range of temperature conditions, to pinpoint the stage which is most or least vulnerable.

Material and methods

Bat roosts and sampling of bat bugs

Bat bugs (*C. pipistrelli*) were sampled from two nursery colonies of mouse-eared bats (*Myotis myotis*) roosted under roofs of the castles in Jevišovice and Luhačovice (south Moravia, Czech Republic). Both populations of bat bugs belong to the haplotype B group that is very homogenous throughout central Europe (Balvín 2008). Bugs were collected with soft forceps and exhaustors into small plastic boxes (10×10×5 cm) lined with soft paper. A Hobo Data Logger (Onset Computer Corporation, USA), continuously recording the temperature and relative humidity, was situated at the site of the highest density of bat bugs under the roof of the Jevišovice castle during the growing season 2007 (April–October). Microclimatic data were used to design experiments in the laboratory.

Equipment and experimental settings

The sampled bat bugs were manually divided by instars and sex and placed in laboratory containers using a binocular following the method by Stutt and Siva-Jothy (2001). To sort the samples according to developmental stages and sex safely and reliably using tweezers and to transfer the insects into experimental tubes, individual bat bugs were immobilised by sudden "freezing" at 0°C for 10 min. Particular instars would be very difficult to breed separately because of their metamorphosis over several days. Therefore, they were divided into four groups as follows: mixed age, viz, first–third instar, fourth–fifth instar, adult males and adult females. They were kept in glass tubes, ten bugs in each. The tubes were plugged with mineral cotton-wool swabs to enable air circulation between the tube and thermostat atmosphere. All bugs were fed at the beginning of experiments with blood of a specific host (*M. myotis*). All groups of bat bugs were exposed to typical temperatures that occur during the growing season in their natural shelters (5, 20, 25, 30, 35, 40 and 45±0.5°C). Kemper (1936) showed that dry conditions (relative humidity 0–20%) during ecdysis often leads to high mortality of nymphs. A somewhat high and stable relative humidity of 65–75%, typical for natural bat roosts, was therefore fixed for all experimental bug groups (see Fig. 1). Numbers of dead bugs were monitored each day. To test the promptness to suck blood after a period of starvation, when mortality exceeded 50% in each bug group, the box with one adult non-reproducing female *M. myotis* and a sample of the remaining bugs were placed together in a thermostat (30°C) for 20 min (cf. Giorgi et al. 2004). After the feeding period, all bugs were removed from the box and collected from the bat. Their numbers and feeding status were determined. Collecting five bugs took about 30 s as they were clearly visible on the wings and tail membrane of the bat. In total, five non-reproducing females were used to test the promptness to suck and bats were changed after feeding each two bug groups. Bats were fed ad libitum with a mixed diet consisting of crickets (*Acheta* sp.) and mealworms (*Tenebrio molitor*) and after experiments returned back to the colony. The bats were captured, handled and temporarily kept in captivity under the licence no. 922/93-OOP/2884/93 and 137/06/38/MK/E/07 of the Ministry of Environment of the Czech Republic. The author has been authorised to manipulate with free-living bats according to the certificate of competency no. 104/2002-V4 (§ 17 of the law no. 246/1992). Experiments were carried out between April and August in 2007 and 2008.

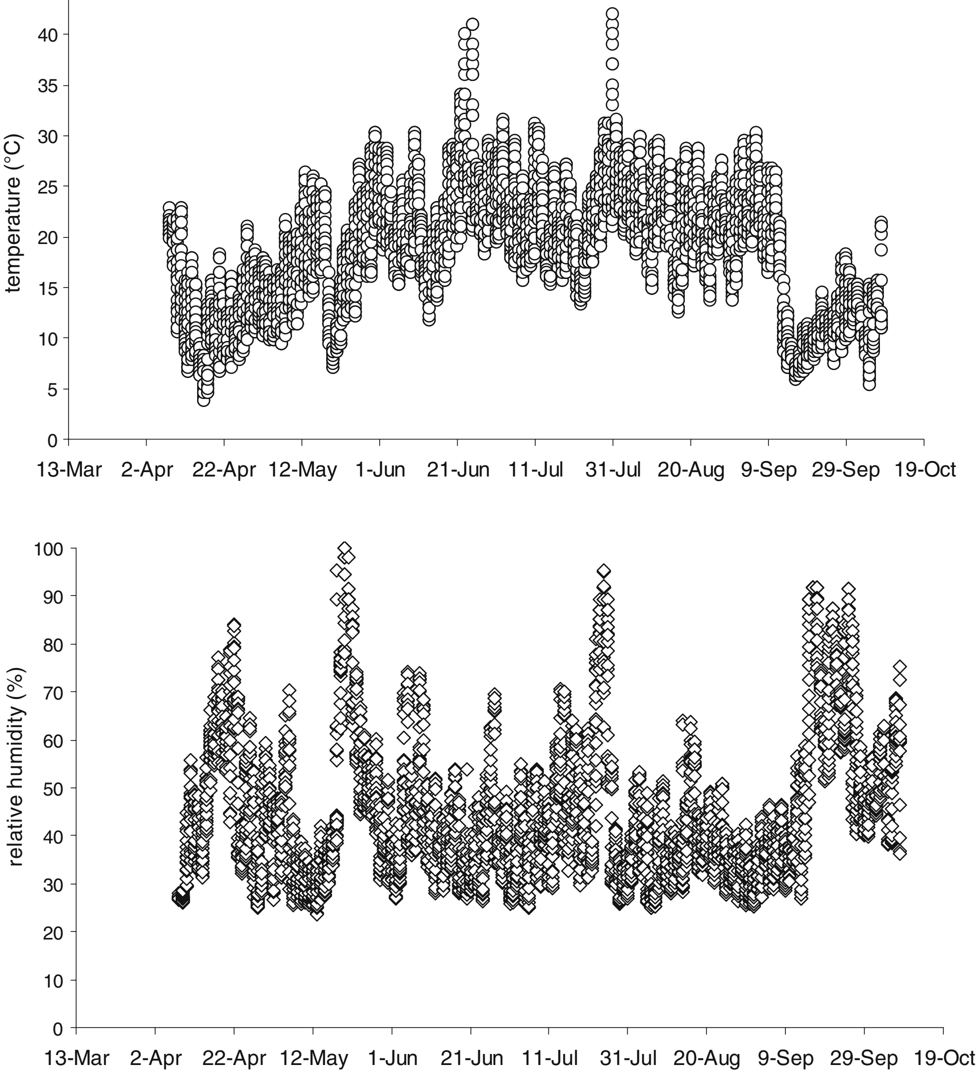

Fig. 1 Temperature (°C) and relative humidity (%) measured at sites with high bat bug density under the roof of Castle Jevišovice

Statistical analysis

All variables showed a normal distribution after log transformation. Statistica for Windows 8.0 was used for data analyses. Survival analyses and GLM were used to compare the changes in survival among particular age classes and temperature. Survival was evaluated during 1 month as this corresponds to the period of bat absence from the crevice-like roosts (cf. Bartonička and Gaisler 2007) and/or using other ectoparasite free sites within large attics (own unpubl. data). A mortality of 50% in a sample was considered significant to finish the experiment, and such a session was marked as complete in the database. Other groups were evaluated as censored. The differences among temperatures and age groups were tested using the Kaplan–Meier survival functions. For the Cox proportional hazards model and Weibull regression, the Chi-square value was estimated as a function of the log-likelihood for the model with all covariates. It was suspected that the effect of the treatment (exposure to different temperatures) on the underlying hazard was not constant, that is, that the proportionality assumption may be violated. To test whether this assumption was tenable, a model was fitted to the data that included the fixed covariate "temperature". We tested only data concerning the first–third instar because of their high influence by temperature (see Fig. 2, Kaplan–Meier tests).

Material

During two growing seasons, a total of 840 bat bugs were collected in bat roosts. Each experiment (at one temperature) was repeated three times (3×10 bat bugs). For experiments with seven different temperatures, 210 individual bat bugs in each age group were used, viz, first–third instar, fourth–fifth instar, adult males and adult females, as mentioned above.

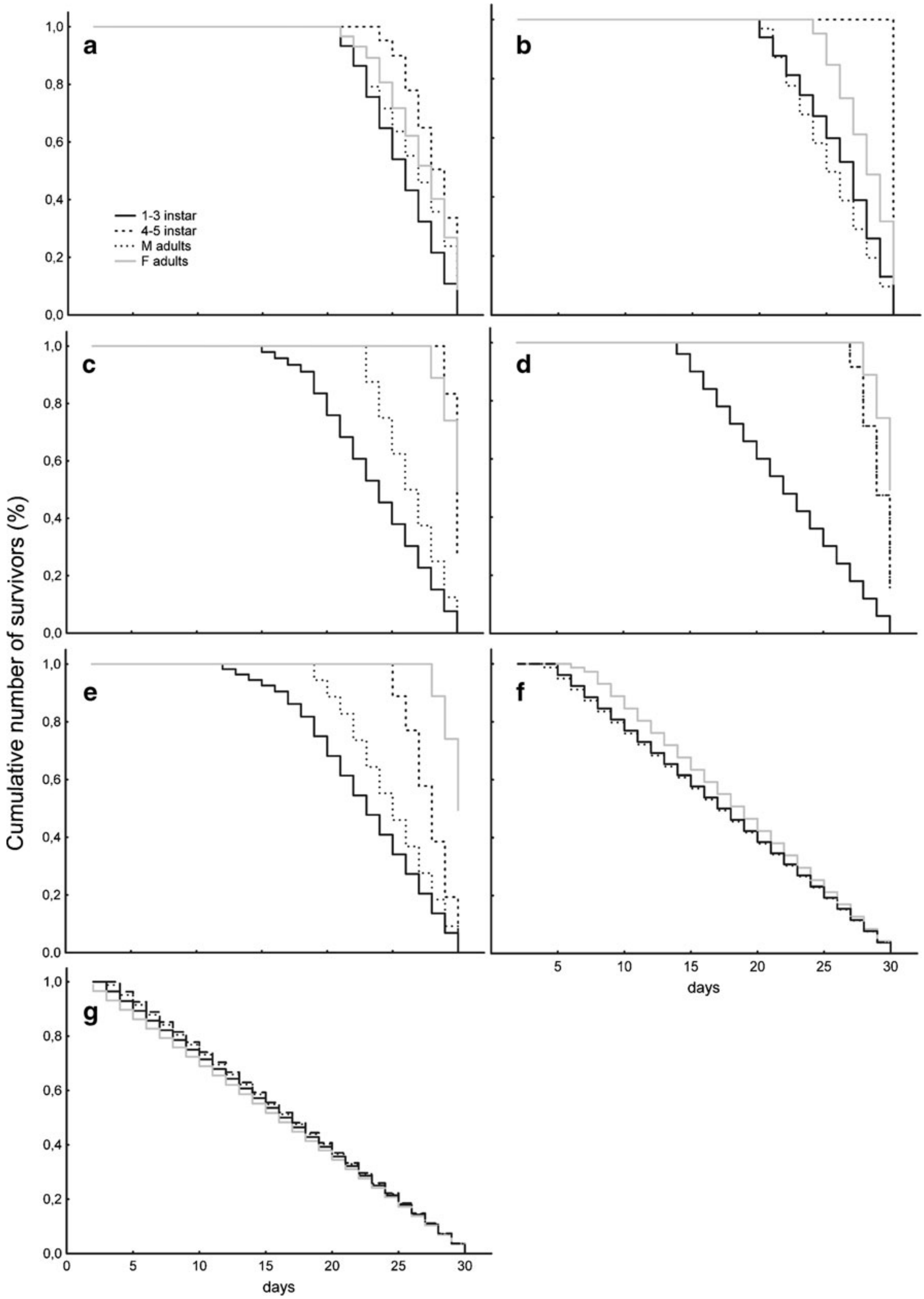

Fig. 2 Cumulative survival functions for age groups of bat bugs, showing the fraction surviving in relation to the different temperature. Kaplan–Meier survival functions for T 5°C, χ^2=10.15, n.s. (**a**); T 20°C, χ^2=39.19, p<0.001 (**b**); T 25°C, χ^2=66.99, p<0.001 (**c**); T 30°C, χ^2=124.77, p<0.001, however, M adults and fourth to fifth instar had same dynamic (**d**); T 35°C, χ^2=58.14, p<0.001 (**e**); T 40°C, χ^2=1.56, n.s. (**f**); and T 45°C, χ^2=0.77, n.s. (**g**). "Complete" means at least 50% mortality in the particular bug group

Results

Changes in the microclimate of bat roosts

In bat roosts, the internal temperature was usually <30°C and, only in two cases, the maximum internal temperature reached 41–42°C. Very low temperatures (5°C) were found during April and September. The relative humidity varied between 25% and 75%. High humidities correlated with rainy days during the second half of May and in July and September (Fig. 1).

Survival at different temperatures

Survival among particular age groups at different temperatures was tested using GLM. Different changes were found only in temperatures between 20°C and 35°C, i.e. T 20°C (GLM, F=10.68, p<0.002), T 25°C (F=166.83, p<0.001), T 30°C (F=352.92, p<0.001) and T 35°C (F=126.42, p< 0.001). Apparent Kaplan–Meier estimates are presented for each temperature and age group. Survival probabilities among different ages were highest in adult females and groups of fourth and fifth instar (Fig. 2). At T 5°C and 20°C, the survival probability of fourth and fifth instar was even higher than in adult bugs. However, survival under high temperatures, i.e. 40°C and 45°C, was the lowest in all age groups (5±1 days at 40°C and 3.7±0.2 days at 45°C for 50% of mortality). The most vulnerable stage was the age group first–third instar, which displayed the highest mortality in general. Their best survival was found at T 5°C (50% mortality within 21 days). The difference between the temperature survival of early instars (first–third) is due to the assumption in the Cox model that the ratio of the hazard rates is not constant over time and temperature (χ^2=256.66, df=6, p<0.001).

The daily mortality rate estimated hazard rate functions as predicted by the Weibull regression model for all tested temperatures. The Weibull model showed a similar hazard rate ratio for the first–third instars in temperature ranges of 5–35°C while higher temperatures caused the hazard rate to increase (Fig. 3). The hazard rate ratio in relation to date (treated as a linear variable for temperatures of 5°C to 35°C), increased the daily failure rate by 2.23–4.34% (95% CI, p< 0.001) per day, but at T 40°C and 45°C, an increase was found with mean daily failure of 12.10% and 22.06% (treated as a logarithmic) per day.

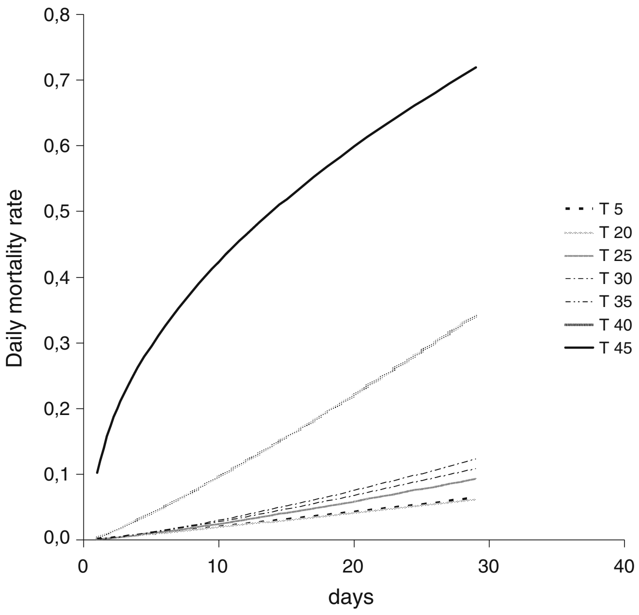

Fig. 3 Daily mortality rate of early instars predicted by the Weibull regression model for all tested temperatures, treated as a linear variable for temperatures between 5°C and 35°C and as a logarithmic model for temperatures 40°C and 45°C

Concerning the relative promptness to suck blood in bat bugs of different age and sex after starvation periods, significant differences were found in first–third instars (ANOVA, F=5.77, df=24, p=0.003), in fourth–fifth instars (F=8.95, df=24, p=0.001) and adult males (F=5.13, df= 24, p=0.006). In these age groups, temperature negatively correlated with relative promptness to suck blood (Pearson correlation coefficient; first–third instars, r=−0.81, p<0.05; fourth–fifth instars, r=−0.78, p<0.05; adult males, r=−0.56, p<0.05). Adult females had high promptness to suck even at high temperatures.

Developmental changes

Changes in nymphal development at various constant temperatures were tested. On temperature gradient plate of 5–40°C, it was found that *C. pipistrelli* had the shortest development at 35°C (mean 21.1 days). In general, the third and fourth instars needed the shortest time period between ecdyses at all constant temperatures (Table 1). Low temperatures (5°C, 20°C) prolonged the time between two ecdyses and increased the survival probability.

Discussion

Ambient temperature is well-known to be by far the most important environmental factor affecting all aspects of the activities of these insects, e.g. nymphal development, timing of ecdysis or feeding. There are only few bat roosts, e.g. warm caves in the tropics, where the fluctuation of temperature during day or year is so uniform that it does not affect the rate of vital activities of bugs (Usinger 1966). Results of experiments showed that bug survival, especially of adults, is highest at low temperatures (5°C, 20°C). This is an important point contributing to survival over the

Table 1 Total period of mean development of *C. pipistrelli* at various constant temperatures

T (°C)/stage	1st	2nd	3rd	4th	5th	Total days
5	33.2	29.3	26.7	24.8	41.9	155.9
20	21.0	19.0	16.0	20.4	28.9	105.3
25	8.5	7.6	6.1	5.6	9.4	37.2
30	6.1	4.2	3.1	4.7	6.3	24.4
35	5.1	3.2	3.1	4.5	5.3	21.2
40	5.0	6.2	unfinished [5]	unfinished [4]	5.2	24.4

T 45°C was excluded because of the high mortality of all instars. At T 40°C, the number of days in brackets are estimates, as most nymphs died before metamorphosis

winter; it is also found in other insect groups (e.g. Lee 1991). Hase (1930) noted the threshold for cessation of all bug metabolic activities to be between 13°C and 15°C, though they can tolerate even lower temperatures for brief periods. In general, adult females had the best survival, and early instars were the most vulnerable to temperature. Although bugs are well-known to have high tolerance of drying, they died of water deficiency associated with lower relative humidities (0–60%). In particular, survival of early instars rapidly decreases with decreasing humidity (Mellanby 1935). The low survival rate of adult males is similar to that of early instars, in contrast to the best survival of adult females. This might correlate with an unknown sex-biased factor (e.g. immunodepression after mating, female guarding, etc.).

The mere ability to survive extreme life conditions such as high temperature or long periods without feeding does not evidence completely the viability of a starving bug population. To safely approach the host and maintain readiness to suck blood are no less important. Success in blood sucking may not be trivial because of the high mobility of bats in roosts, their antiparasitic behaviour or host unavailability during its day torpor (Bartonička 2008). Experiments showed that early nymphs and probably adult males, which survive longer without a host, are often unable to find it and suck. Adult females were more efficient, and a high percentage of the survivors sucked. This ability of adult females explains their position as the most often recorded bug stage on netted bats. For the same reason, adult females are the optimum vector and founder of a bug colony in a new still uninfested bat roost (Reinhardt and Siva-Jothy 2007).

In bat boxes, bugs survive the temporal host absence as eggs (Bartonička and Gaisler 2007), and this period is, at the same time, usually the hottest. If the first instars hatched, e.g. 1 week before the return of bats to the roost and under high temperatures (40°C), their mortality would be 50% during the next 5 days. Development timing, i.e. keeping synchronisation of the bug density with the reproductive cycle of bats, is a very important ectoparasite life strategy. This strategy, therefore, could represent a mechanism facilitating early development from eggs, as described in flies (Reckardt and Kerth 2007).

The thermal death point was found between 44°C and 45°C (Omori 1941), but our present results showed that development of all stages of *C. pipistrelli* ceased at 40°C. Such a high temperature (40°C) was observed in bat roosts only rarely, but no simultaneous decrease in bug numbers was found (cf. Bartonička and Gaisler 2007). Therefore, bat bugs are probably able to roost in thermally more favourable sites, usually cooler, with temperatures different among ontogenetic stages. Different survival rates with respect to the temperature gradient could lead to an age-related microhabitat segregation (O. Balvín unpubl. data). Temperature-sensitive sensors present on the bug antenae, and mobility of bugs very likely enable them to determine thermally favourable sites and move there (Sioli 1937).

The less specific requirements for roost conditions found in bed bugs *C. lectularius* led us to anticipate a similar dependency of survival on temperature gradient in *C. pipistrelli* (Usinger 1966). Both species are frequent roost ectoparasites of bats. *C. pipistrelli* parasites mainly on crevice-dwelling bats *Pipistrellus pipistrellus* and *Nyctalus noctula* switching their roosts several times per growing season. In contrast, *C. lectularius* parasites usually on *M. myotis*, which is more faithful to one roost (Audet 1990). Sun-exposed roosts in buildings are well-known for extreme temperature fluctuations (Kunz and Fenton 2003). Therefore, bat bugs could be more tolerant to the high temperatures. The results of this study showed that *C. pipistrelli* has the shortest development at higher constant temperature (ca. 35°C), while *C. lectularius* matures quicker at 30°C (Usinger 1966). At temperatures >30°C, its development slowed and ceased completely at 36°C (Omori 1941). The efficiency of *C. pipistrelli* to tolerate high temperatures could be the consequence of its long-term coexistence with bats preferring crevice-like roosts in both trees and buildings but also to more spacious attics becoming overheated during the summer months. Direct observations of bat bugs in reproduction colonies of *M. myotis* show that, as long as the temperature does not exceed the thermal death point, the cause of the decrease in population density is not only temperature, but the absence of bat hosts at the same time. Usually, the bats left their roosting places and moved either to cooler sites or those with less or no bug infestation, often in the same building.

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BARTONIČKA T. & RŮŽIČKOVÁ L., 2013

Recolonization of bat roost by bat bugs (*Cimex pipistrelli*): could parasite load be a cause of bat roost switching?

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ORIGINAL PAPER

Recolonization of bat roost by bat bugs (*Cimex pipistrelli*): could parasite load be a cause of bat roost switching?

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Abstract Roost ectoparasites are believed to have a negative impact on fitness of their hosts as birds or mammals. Previous studies were mostly focussed on the synchronization between reproduction cycles of ectoparasites and hosts living in infested roosts. However, to date, it has not been examined how fast ectoparasites colonize new, non-infested roosts and thus increasing the impact on the local populations of hosts. The parasite–host model was studied, including bat bugs *Cimex pipistrelli* and soprano pipistrelles *Pipistrellus pygmaeus*, where bat behaviour was observed which tended to reduce the parasite load in bat roosts. We investigated (1) whether bats change their roosting behaviour when we discontinued synchronization of their reproduction and the life cycle of the bat bugs and (2) how fast and which stages of bat bugs reoccupy cleaned roosts. In a 3-year field experiment, we removed all bat bugs from six bat boxes in each spring. Pipistrelles bred young in all non-infested boxes during these 3 years. In addition, 8 years of regular observations before this experiment indicate that bats avoided breeding in the same bat boxes at all. Bat bugs were found again in clean boxes in mid-May. However, their densities did not maximise before the beginning of June, before parturition. A re-appearance of bugs was observed after 21–56 days after the first bat visit. Adult bugs, mainly females, colonised cleaned boxes first though at the same time there were a lot of younger and smaller instars in non-manipulated roosts in the vicinity.

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Introduction

Roosts and their availability play a crucial role in bat ecology (e.g. Russo et al. 2004). Females have evolved to spend at least part of their lives roosting together in nursery colonies. Regarding the different requirements during reproduction, there is growing interest in identifying roosting strategies (Kunz and Lumsden 2003). A number of studies have provided critical insights into roost switching and have suggested several reasons for why bats change roosts, i.e. to minimize the costs of thermoregulation (e.g. Kerth et al. 2001, Willis and Brigham 2005), to reduce the risk of predation (Vonhof and Barclay 1996) and to allow for an optimal group size and increased survival (e.g. Barclay and Kurta 2007). Roost switching in bats is often phenomenon, is known as fission-fusion (Kummer 1971), where a social unit of roosting bats may split into several subunits when the bats select their diurnal roosts (e.g. O'Donnell 2000). Under this scenario, roost sharing and switching between roosts within a local area could serve to increase the knowledge of potential trees for roosting and/or maintain cohesiveness of the colony (e.g. Willis and Brigham 2004).

However, pipistrelles do not switch roosts as many times as typical forest species, e.g. *Myotis bechsteinii* or *Eptesicus fuscus*, where the causes of roost switching most probably relate to the fission-fusion social organization (Kerth and König 1999; Willis and Brigham 2004), although some roost selection to avoid ectoparasites was observed in tree-dwelling species like *M. bechsteinii* (Reckardt and Kerth 2007). Pipistrelles usually only switch roosts two to three times in one season, according to the reproduction phase. Typically, groups of females leave their respective roosts in early June and move to the main nursery roost a few days before parturition. They move back to their previous roosts with flightworthy juveniles in mid-July (Webb et al. 1996). Therefore, we think that there may be a cause other than social demands for this behaviour, which is probably related to a reduction in the parasite load. When leaving the shelter should lead to a reduction of parasite load, it is advisable to find out how long it will take for bugs to occupy new or abandoned roosts, and when they reach there the maximum population.

Avoiding infested habitats is known to be the most effective behavioural parasite defence strategy in order to reduce costs associated with infestation (e.g. Christe et al. 1994; Moore 2002). Most studies on bat ectoparasites have dealt with different species and their ontogenetic stages that permanently live on their hosts' bodies (e.g. Gannon and Willig 1995; Giorgi et al. 2001; Dick et al. 2009). These studies are not sufficient to elucidate the correlation between parasite load and roosting strategies related to movements within and outside the shelters. Probably only such strategies could be effective when avoiding pressure from ectoparasites that spend on the body of their host a short time only and have low ability to colonize new host roosts. Therefore, as an improved model, we chose bat bugs (*Cimex pipistrelli*) which, except for the time they spend engorging, mostly co-habit without physical contact with their hosts and are rarely found on bats netted out of roosts (Reinhart and Siva-Jothy 2007).

Currently, it is unknown whether roost switching is an adaptation for reducing parasite load or whether the observed decrease in the number of parasites (e.g. Bartonička and Gaisler 2007) is only a side effect of switching. Prior to this field experiment, there were no bat boxes and no non-fledged young bats. Pipistrelles leave the roosts just before parturition because of a growing parasite load or different microclimatic demands during pregnancy and lactation (Bartonička and Řehák 2007). Bats can monitor the level of roost infestation during their repeated visits during pregnancy and choose a roost with a relatively low parasite load or even with none at all (Reckardt and Kerth 2007). The odour produced by bat bugs is very intense and is probably easily distinguishable by bats even outside the roost (Usinger 1966). Females become torpid more often during pregnancy than during lactation, and therefore are less available for bat bugs (Montes et al. 2002). Populations of bat bugs usually reach the first gradation at the end of May (Bartonička and Gaisler 2007). Laboratory experiments showed higher levels of infestation in young bats than in adults. The movement of females to non-infested roosts just before parturition could have an important role in the post-natal growth of the young and could be seen as a kind of maternal effect (Kunz and Stern 1995).

We present a field study in which a roost manipulation experiment was combined with a long-term study on the roosting strategies of pipistrelles (*Pipistrellus pygmaeus*). The aim of our study was to determine whether or not the removal of bat bugs from infested roosts would lead to a change in the roost selection pattern and whether or not the females would bear and wean young in roosts with no bat bugs. The goal of this paper was to reveal, by a simple field experiment, what is the speed of (re)colonization of new roosts and whether or not the parasite load is a possible cause of roost switching in this particular bat species.

Methods

Study area and technical equipment

Nursery and temporary colonies of *P. pygmaeus* with high densities of *C. pipistrelli* bat bugs were monitored in eight wooden bat boxes inside the Křivé jezero floodplain forest and the Bulhary game-hunting ground. Both of these localities belong to one woodland complex near Milovice village (Milovice Wood), in southern Moravia, Czech Republic. Milovice Wood is one of the largest remaining oakwoods in this part of the north-western fringe of the Pannonian Basin. All of the bat boxes were regularly observed from the year 2000: at least once at month. At the beginning of the season in 2003, all of the bat boxes were equipped with active infrared gates (IRG, Litschmann and Suchý, AMET, Czech Republic) or passive IR Trail Monitors, TM (TM550, TrailMaster, Goodson and Associates, INC., Kansas, USA) and thermometers (Hobo, Onset Computer Corporation, Southern MA, USA) to record the presence and/or numbers of bats and the internal and external temperatures inside/outside the boxes throughout the seasons. Monitoring of the bat bugs was carried out by manually sampling from inside the box roof. In each spring of 2008, 2009 and 2010, we removed all bat bugs from six bat boxes (three boxes in the Křivé jezero and three in the Bulhary game-hunting ground). Only IRGs were used to monitor the bats during these three seasons. One box in each locality was left alone; therefore, the normal life cycle of the bugs could proceed. In January, the manipulated bat boxes were inserted into plastic bags, to which several drops of benzene were added. The next day, we returned the bat boxes to their original places in the field. Hobo Data Loggers, which continuously recorded the temperature (internal and external) and the internal relative humidity, were situated under the roof of each bat box. The sensors that measured the external temperature were situated about 50 cm apart on each box. The relative humidity sensor had an accuracy of ±4 %, and the temperature sensor had an accuracy of ±0.4 °C. All bat boxes were regularly monitored over 10-day periods just before the end of July to limit the effect of human disturbance. Each sample of bat bugs was divided into four groups: (1) adults, males and females, (2) first to third instars, (3) fourth and fifth instars, and (4) eggs (cf. Bartonička and Gaisler 2007). The number of bugs in each group was recorded. The number of young present was evaluated. The reproductive season studied was divided into three parts: pregnancy (up until June), lactation (7 June to 6 July) and post-lactation (from 7 July). In each season, the time of parturition (8 June 2008, 3 June 2009 and 15 June 2010) was indicated by the presence of the first newborns.

Statistical analysis

All variables showed a normal distribution after log transformation (Kolmogorov–Smirnov test). Statistics for Windows 9.0 was used for the data analyses (GLM, ANOVA, logistic regression). The level of returning and emerging bat activity (returning from foraging flights at 1:00–5:00 a.m.; Trail Monitors) positively correlated with the number of bats (IRG) found in the boxes (Pearson's correlation coefficient, $r=0.81$, $P<0.05$); therefore, the number of bats roosted in boxes during the day could be estimated. Analysis of variance (repeated measures ANOVA) was used to check for differences between the levels of bat activity, and logistic regression was used to test for changes in occupancy in the manipulated and non-manipulated bat boxes. Logistic regression was also used to check for differences between the internal temperature and bat numbers in different bat boxes.

Material

During the three seasons of 2008–2010, the six manipulated bat boxes occupied by soprano pipistrelles (*P. pygmaeus*) were monitored for 660 (2008, 110 days/box), 732 (2009, 122 days/box) and 780 (2010, 130 days/box) days, and two non-manipulated bat boxes for 216 (2008, 108 days/box), 194 (2009, 97 days/box) and 238 (2010, 119 days/box) days, respectively. On each day, hourly values of internal and external temperature, internal humidity and the level of the bat activity (presence/absence of bats, number of bats throughout the day, night exploring activity) were recorded. Samples of bat bugs (*C. pipistrelli*) were taken on 11 (2008), 9 (2009) and 13 (2010) occasions in each bat box. In addition, monthly observations of the numbers of bats and bat bugs were available for the 8 years preceding the present experimental study.

Results

Seasonal changes in occupancy of the bat boxes

No significant differences in number of bats were found between two localities (Křivé jezero and Bulhary), the seasons when the bat bugs were removed (GLM, $F=0.871$, NS, $df=2{,}154$; effect of bat box, Tukey test, $F=0.827$, NS; effect of season, $F=0.329$, NS) and among years when boxes were not manipulated (GLM, $F=0.331$, NS, $df=642$; effect of bat box, Tukey test, $F=0.016$, NS; effect of season, $F=0.771$, NS), thus allowing us to pool the data to the two groups, i.e. manipulated and non-manipulated. Logistic regression showed significant differences in the patterns of bat box occupancy between the manipulated and non-manipulated boxes ($F_{(1,\ 2{,}171)}=30.48$, $p<0.001$; Fig. 1).

Exploration activity of the bats and bug transport

The use of infrared gates allowed us to evaluate the numbers of bats that roosted in the bat boxes during the daytime, when the bugs could suck onto the bats, but also the level of bat exploration activity overnight, when the bat bugs could be transported to clean boxes. Although the bats were not observed in manipulated bat boxes during the lactation period, a high exploration activity was noted during most of the nights. When we tested the exploration activity between the manipulated and non-manipulated bat boxes, significant differences were found (logistic regression; $F_{(1,\ 2{,}171)}=4.62$, $p=0.032$; Fig. 2).

In the non-manipulated boxes, from which bat bugs were not removed, two peaks in bat numbers were observed—at the end of May and at the end of July (Fig. 1). Although there were many overnight visits by the bats (Fig. 2b), no young were found in these boxes in any of the three experimental years. The manipulated bat boxes were re-occupied by adult bugs, mainly females, whereas many first to third instars were observed in the non-manipulated boxes. The first bat bugs were usually found in manipulated boxes from mid-May to mid-June (21 May in 2008, 13 May in 2009 and 16 June in 2010) 21–56 days after the first bats had flown. Other than adult bugs, we found several first instar individuals, but always later on in each year. Although bat bugs were observed early in the growing season in 2008, and even in mid-May, no first stages of gradation were found, and only the second gradation stage appeared at the end of July (only in 2008) (Fig. 3).

Microclimatic conditions

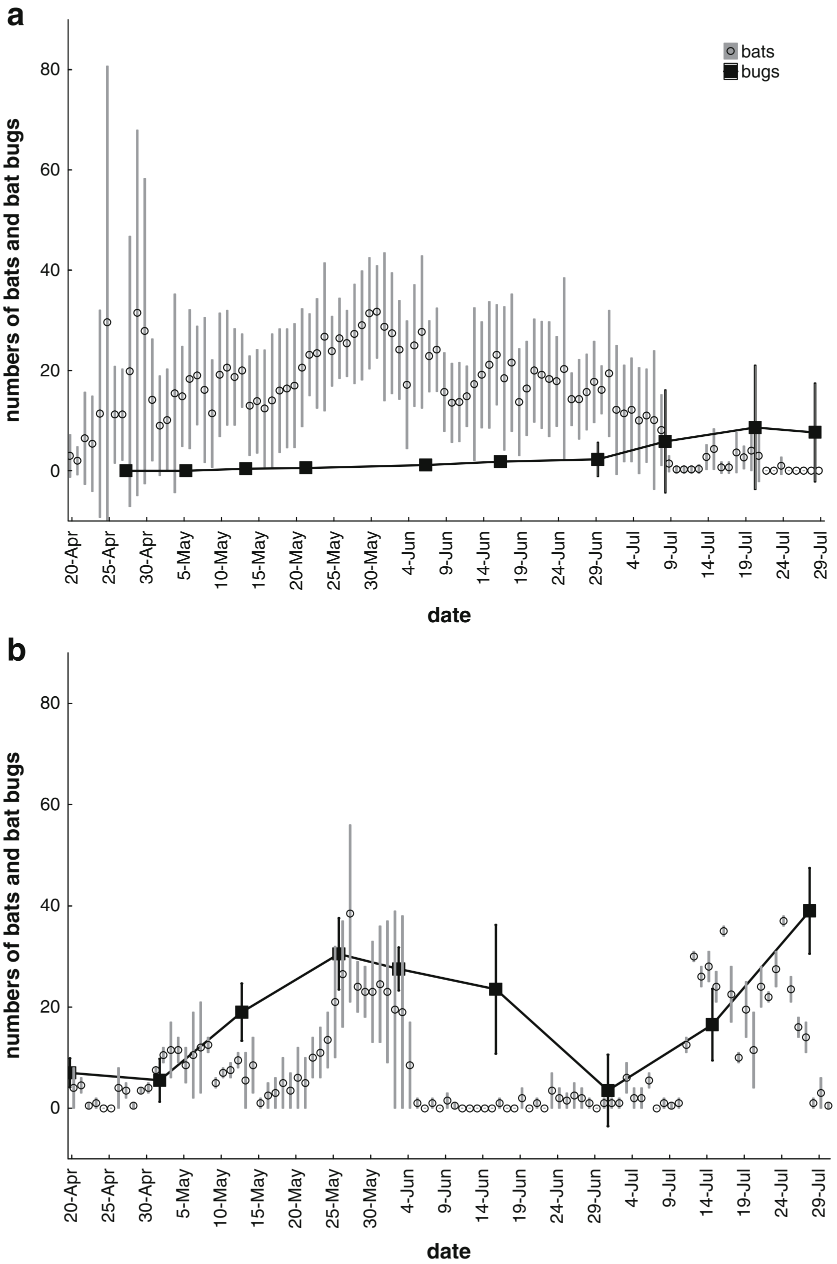
Regarding the positions of the non-manipulated bat boxes and the manipulated boxes in the two forest locations, we determined how they differed in internal temperature throughout the daylight hours. Although each of the bat boxes studied differed in the degree of exposure, no significant difference was found among the boxes in the daily mean internal, external temperatures and humidity (ANOVA, $F_{(7,\ 2{,}819)}=0.69$, NS).

Discussion

Different factors lead to roost switching

Day roosts in trees or artificial bat boxes are essential for tree-dwelling microbats, providing shelter, protection from

Fig. 1 Changes in the numbers of bats and patterns in the numbers of all bat bug stages **a** in boxes from which the bat bugs were removed and **b** in control boxes from which the bugs were not removed during the same seasons; mean—central tendency, standard deviation—large box



predators and an appropriate microclimate for energy balancing and reproduction. Bats often make use of multiple roosting sites, frequently shifting between roosts. Previous experimental studies of bat behavioural responses to ectoparasites indicated that the costs to bats differed, with the costs caused by ectoparasites found in the roost eliciting a stronger response than those remaining permanently attached to the hosts (Côte and Poulin 1995). Therefore, long-term monitoring data, along with field-based experiments, were used to examine the influence of roost ectoparasites on roost selection patterns and roost fidelity. There are at least three commonly cited causes of roost switching in vespertilionid bats, i.e. (1) different microclimatic demands during reproduction, (2) high ectoparasite loads and (3) social organization, for example the fission-fusion model (Lewis 1996). However, the pipistrelles left their respective roosts in early June, a few days before parturition, and moved back to their previous roosts with flightworthy juveniles (e.g. Swift 1980; Webb et al. 1996). This behaviour is different from that found in *E. fuscus* bats, which were found to switch roosts every 1.7±0.7 days (Willis and Brigham 2004). Therefore, the behaviour of the pipistrelles could not be explained by the fission-fusion model based on social interactions alone. More probable are movements related to different microclimatic demands between pregnancy and the lactation period or parasite load. Willis and Brigham (2007) calculated that individuals would save 53 % of their daily energy budget by roosting in a group (45 bats).

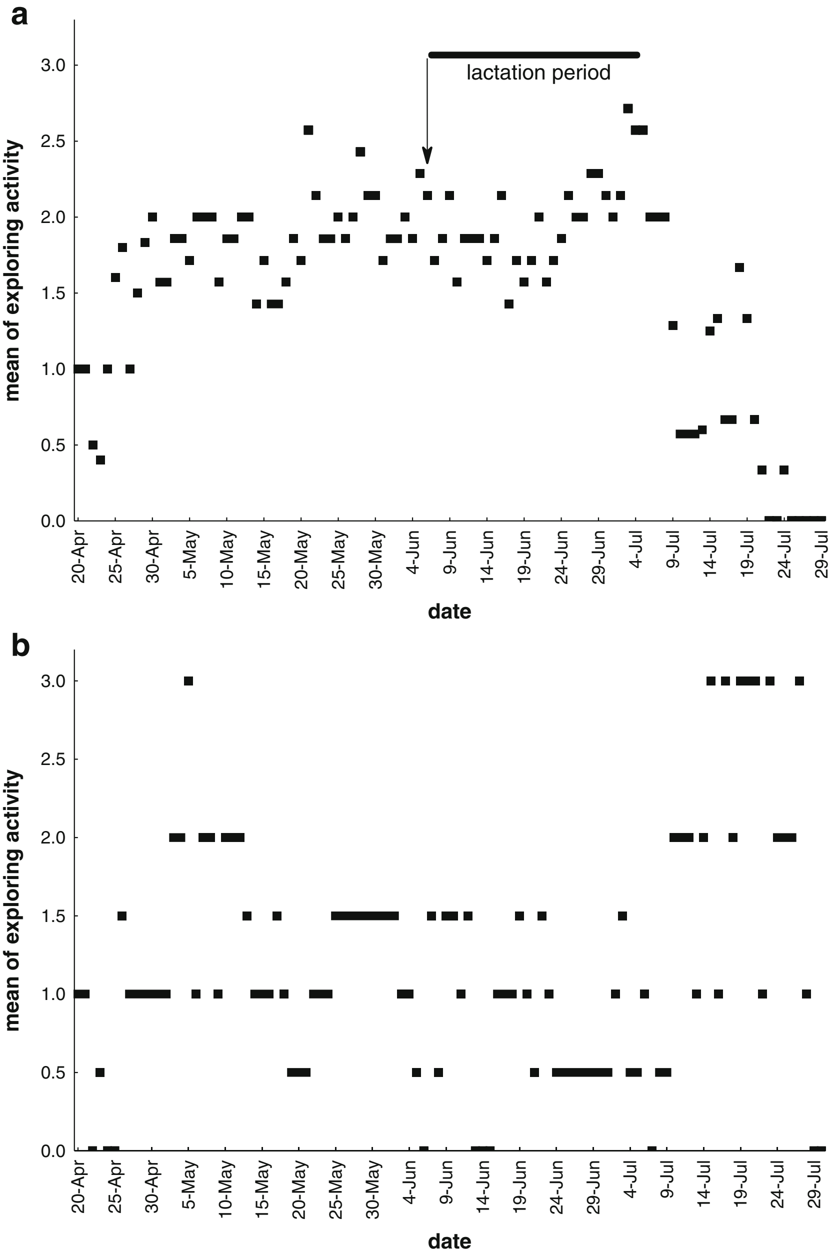


Fig. 2 Changes in exploration activity (1–4, where 1 is the lowest activity) in **a** boxes from which the bugs were removed and **b** control boxes where the bat bugs were present; the *arrow* shows when the first bat young was observed. The *arrow* shows when the first instance of bat suckling was observed, and the line *shows* the duration of the lactation period

Bartonička and Řehák (2007) tested the microclimatic theory of pipistrelle movement because the demands between the pregnancy and lactation periods are completely different. They assumed that the role of the bat boxes studied was as “satellite” roosts, separate from a larger communal roost, that were only occupied during pregnancy and the post-lactation period. They also assumed that the bat boxes were microclimatically suboptimal roosts because for a few days in each season the internal temperature exceeded 40 °C, and the bats left the overheated boxes. Unfortunately, they did not consider ectoparasite load. We cannot confirm that the bats could survive in overheated boxes because internal temperature in the present study (at least during three manipulated years) did not exceed 40 °C. Humidity and evaporative water loss may also be important and could influence the bats to select roosts close to sources of water (Jenkins et al. 1998). However, all of the bat boxes studied were equidistant from the nearest calm water source. In this study, we did not evaluated prey availability, but Bartonička et al.

Fig. 3 Bat bugs (*C. pipistrelli*), adults and early instars have successfully colonized the new roost (photo by O. Balvín)

(2008) showed similar distances of foraging sites of bats coming from non-manipulated or in future manipulated bat boxes.

Switching the roost may be related to the decrease of food supply in foraging area and moving to other foraging sites (e.g. Feyerabend and Simon 2000). Bat boxes in the Křivé jezero have been monitored since 2000 and within the 8 years, pipistrelles occupied boxes only during pregnancy and then in the postlactating period. Between June and July 2004 during radiotracking research on pipistrelles, lactating females roosted in a guest house in the village of Nové Mlýny, situated 1.5 km far from Křivé jezero (Bartonička et al. 2008). However, all tagged females foraged in the old-growth floodplain forest Křivé jezero. Therefore, it is unlikely that change in the roost occupation after reducing parasite load could be explained by change of the foraging sites due to e.g. decreased prey availability there.

Pre-natal maternal effect

Unfortunately, we could not determine the primary mechanisms that enabled the bats to distinguish between infested and non-infested roosts. Bartonička (2008) assumed that the bats left their roosts after bug bites. It is improbable that all bats visiting the bat boxes during the night were attacked by bat bugs; however, if only one bat was bitten, the other bats would have realized that bat bugs were present from the behaviour of the bitten bat (Bartonička 2008). In addition, bats might be able to recognize an infested roost by other signs, i.e. the smell of fresh bug faeces, which is very intense (Usinger 1966). The fact that females leave an infested roost just before parturition could be an interesting example of pre-natal maternal behaviour similar to the maternal effect of androgens or milk composition, which might also influence offspring competitiveness (Pontier et al. 1993; East et al. 2009). However, there are also bat species that roost in one infested shelter throughout the entire reproduction season, but no switching strategies are currently known in such nursery colonies (e.g. *Myotis myotis*). Only a few life history analyses of bats have included representatives of such behaviour (e.g. Read and Harvey 1989), and further examinations of the main factors that influence post-natal growth are desired.

Colonization of new roosts by bat bugs

Reckardt and Kerth (2007) found that *M. bechsteinii* reoccupied bat boxes just 1 month after their first occupation, when they were infested by the bat fly (*Basilia nana*). During this period, the bats were safe in terms of bat fly infestation because fly puparia only become contagious later on. Such an adaptation in bats occupying bug-infested roosts is inconvenient because adult bugs are always prepared to suck. Whenever infested bats roosted in a bat box in spring during the daytime, a bug outbreak was always observed at the end of May. We found that non-manipulated bat boxes were repeatedly visited during the lactation period even though bats did not roost here during the daytime. However, commuting bats transported bat bugs to different roosts. The probability of bug transport is very low, as shown by the low number of bat bugs found on commuting or foraging bats (e.g. Heise 1988). Since only a few early instars, better transported because of their size, were found in the manipulated bat boxes, we believe that they are not able to stay in the fur of a flying bat or they cannot survive the microclimate changes in the new roost. A re-appearance of bugs in the manipulated boxes was observed during the period when a high number of early stages were observed in non-manipulated boxes (May and June), when adult females were rapidly dying off (Bartonička and Gaisler 2007). It seems that the transport of bat bugs by their hosts to new roosts is not entirely random (cf. Balvín et al. 2012). Heise (1988) supposed that the bugs may travel on the body of their hosts on the purpose of dispersal, not only because they did not manage to escape when the bats emerged from roost to forage. Pfiester et al. (2009) found that the females actively disperse earlier than males when bedbug abundance is increasing. Unfortunately, it was not shown if the dispersing females were mated or virgin. However, in almost all manipulated boxes we found a few new eggs and early instars after the appearance of first adult bug. This fact showed that at least some females were mated before the transport.

The period of high fluctuation in the numbers of roosted bats during pregnancy is very convenient for the colonization of new roosts. But the time for transport to the other roosts is limited. At about 27 °C, recently emerged adult bed bugs (*Cimex lectularius*), when fed and mated, will start ovipositing about 3 days later. When bugs are fed weekly but not mated again, which is most probable when they are moved to different roosts separately, three eggs per day are produced over a period of 5 weeks. Oviposition ceases after 11 days if the bugs are not fed again (Davis 1966). The limited time available for the successful transport and settling of a new population could be optimized by asynchronization in the timing of oviposition between different bug females. However, this theory needs to be tested further.

A second gradation stage in the bug populations was only observed in 2008, probably because of the early transport of bat bugs to the manipulated bat boxes, when bugs were found in boxes 21 days after the first appearance of bats. No other gradation stages were observed in 2009 or 2010, when the re-appearance of bugs occurred later in the vegetation season. In 2008, fed and mated females were transported. Therefore, the bug population grew and reached the original numbers at the end of July. Bat bugs transported in

2009 and 2010 were probably not fed or mated (cf. Davis 1966). The fact that bat bugs are able to move to a different roost and reach a high population density all in one season correlates well with the very fast ontogenesis that is reportedly even faster than in the bed bug (*C. lectularius*; Bartonička 2010). A fast ontogenesis could be a consequence of the coevolution between bugs and roost-switching bats.

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8.3 Vliv štěnic na chování netopýrů

BARTONIČKA T., 2008

Cimex pipistrelli (Heteroptera, Cimicidae) and the dispersal propensity of bats: an experimental study.

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ORIGINAL PAPER

Cimex pipistrelli (Heteroptera, Cimicidae) and the dispersal propensity of bats: an experimental study

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Abstract Previous results have shown frequent movements of crevice-dwelling bats between different shelters. Low roost fidelity of some dwelling bat species reduces the reproductive success of ectoparasites. The question of whether high densities of bat bugs (*Cimex pipistrelli*) represent a cost for crevice-dwelling bats (*Pipistrellus pygmaeus*), resulting in roost switching, has been examined. Sessions in a volary equipped with two bat boxes were carried out. One of the boxes was loaded with ectoparasites (low and high densities), the other served as a control and new roost for bats, which left the loaded box. Differences in the level of bat self-grooming, movements inside experimental boxes, and leaving the boxes between experiments with bat bugs and controls were significant. Allogrooming was observed only in few cases; therefore, the hypothesis of cooperation among individual bats in defense against bat bugs was rejected. Experiments with artificial parasitation, when bugs were added to a bat roost, showed that leaving a confined roost infested by bat bugs, i.e., roost switching, is a natural reaction of crevice-dwelling bat species, which reduces parasite load.

Introduction

Behavioral responses of hosts during their exposure to parasites usually reduce the effects of costly parasitism (Moore 2002). Field and experimental research has demonstrated that nest ectoparasites can reduce reproductive success of their hosts (Loye and Carroll 1991; de Lope and Moller 1993; Richner et al. 1993; Christe et al. 1996). Some birds have shown sensitivity to costs associated with parasites and the ability to discriminate levels of possible infestation and to choose less soiled nests (Barclay 1988; Opplinger et al. 1994; Rendell and Verbeek 1996). Low roost fidelity observed in many dwelling bat species significantly reduces the reproductive success of ectoparasites such as bat flies (Reckardt and Kerth 2006) or bat bugs (Bartonička and Gaisler 2007). However, up to the present, studies on the life history of bat ectoparasites have mainly dealt with species and their ontogenetic stages, which live on their hosts' bodies permanently (mites, e.g., Giorgi et al. 2001; nycteribiids, e.g., Reckardt and Kerth 2006, 2007; streblids, Gannon and Willig 1995).

As a result, the relations between bat species roosting in crevices and their ectoparasites living in the same shelters but mostly without physical contact with their hosts are unclear and often based only on speculation. Reckardt and Kerth (2007) showed that roost switching of *Myotis bechsteinii* between seasons can be explained as bat adaptive behavior to the life cycle of bat flies, i.e., their emergence from puparia. Roost switching during one season is well-known in pipistrelles, and seems to be an anti-parasite strategy with respect to roost parasites such as bat bugs (Bartonička 2007). Bartonička and Gaisler (2007) showed that the absence of bats in satellite roosts during the lactation period, coupled with high temperatures and natural mortality of parasites, reduced the number of bat bugs in bat boxes to less than half the initial number. Whereas bat bugs are common not only in fissure-like roosts but also in spacious shelters such as attics of building, we observed that some bat species (e.g., *Myotis myotis*) can reduce the load of parasitation by movements within such large roosts (Bartonička and Gaisler, unpublished observation).

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Little is known about parasite-induced behavior of bats within the roost, e.g., grooming. In this paper, I tested whether self-grooming or allogrooming can serve to reduce the parasite load. An experimental test of the hypothesis that high parasite load increases the probability of roost switching in crevice-dwelling bats is presented. In particular, the level and type of grooming and movement behavior with respect to different levels of parasitation, survival rate, and sucking success of bat bugs during laboratory infestation of bats are reported. Based on this new data, I discuss possible co-evolutionary responses between bat bugs and parasite-induced bat behavior, sucking success of bugs, and energy loss caused by roost switching.

Materials and methods

Volary sessions and equipment

Only adult female bugs of the *Cimex pipistrelli* Jenyns 1839 group were selected for the experiments. Until the beginning of experimental sessions, bat bugs were kept in darkness at low temperature (15°C, humidity 70%) when their survival without food was longest (Jones 1930; Johnson 1941 observed in *Cimex lectularius* Linnaeus 1758). Sessions were held in a volary (3×3×2.5 m) equipped with two bat boxes and under standard microclimatic conditions (temperature 25°C, humidity 70%). The soprano pipistrelles (*Pipistrellus pygmaeus* Leach 1825) used in the experiment were netted in the roof of a brick building housing a pheasantry at the village of Vranovice (48°57′50″ N; 16°37′51″ E), Czech Republic where a large colony of this species was situated. Only 20 females were kept in captivity simultaneously. The bats were fed each day after a session and had access to water enriched with vitamins. All bats were returned to their original colony after the sessions. During captivity, the light regime was natural and the air condition is stable. The boxes were equipped with thermometers and hygrometers (HOBO, Onset Computer Corporation, software BoxCar 3.7); they provided the only roosting space to bats in the volary. Both bat boxes were equipped with a camera (SONY DCR SR 52E) to monitor the bat behavior; another camera was installed on a tripod in the middle of the volary. Bat bugs were last fed 1 week prior to the beginning of the experiments (first release of the bugs into the appropriate box) (Hase 1917; Adkins and Arant 1959).

Other ectoparasites were removed from all bats before the first session. Bats inhabited the volary 1 week before the first session. Each session started 2 h before sunset, when bats were still in torpor, thus avoiding mutual disturbance. Only infrared light was applied during the observations. *C. pipistrelli* sample was divided into two experimental groups with different numbers (20, 50) of bat bugs. Only one bat box was infested by bat bugs, the other was bug-free unless the bats themselves carried the parasites into it.

At the beginning of each session, bugs were applied directly into the box via a small hole using a glass tube. Video sequences were recorded during 1 h after application of the bugs. One day after a session with addition of bugs to the roost, a control was carried out with an identical bat group and started 2 h before sunset. In control sessions, video recordings were made 120–60 min before sunset. After each session, all bugs were removed from the boxes, and their numbers, feeding status, and visible injuries were reported.

The bats were captured, handled, and temporarily kept in captivity under license numbers 922/93-OOP/2884/93 and 137/06/38/MK/E/07 of the Ministry of Environment of the Czech Republic. The author has been authorized to manipulate with free-living bats according to the certificate of competency number 104/2002-V4 (section 17 of the law number 246/1992).

Experimental groups and observed behavior

Video sequences lasting 1 h recorded the level of allogrooming and self-grooming, the number of bat movements within the box, and the number of bats leaving the box. The experiments were carried out with early pregnant, post-lactating, lactating females, and newly fledged young. Video sequences were recorded of two different bat assemblages: (1) early pregnant or post-lactating females (ten individuals in each session, 20 session pairs—experiment and control) and (2) age-mixed groups (five lactating females and their five young, ten session pairs).

Statistical analysis

All variables showed a normal distribution (Kolmogorov–Smirnov test). Statistica for Windows 7.0 (StatSoft, Tulsa, OK, USA) was used for data analyses. Paired *t* tests were used to check the differences between the level of grooming and movements of bats under experimental and control conditions. *T* tests were used to test differences in the numbers of sucked (clearly visible blood in the abdomen)/unsucked (no blood mark in the abdomen) bat bugs found on bats, which stayed inside/left. The Bonferroni correction was applied if multiple tests were used for the same data set.

Materials

In all, I carried out 20 experiments (half with 20 and half with 50 bugs) and 20 controls without bugs. In the volary, I also tested ten age-mixed (five adult and five newly fledged young) bat groups and ten controls without bugs. To quantify the level of grooming, the number of movements and the condition of bat bugs (unsucked/sucked, lost, dead, on/off the bat body), 3,600 min of video records were examined.

Results

Level of grooming

All variables showed a normal distribution (Kolmogorov–Smirnov test); therefore, mean values were used. Paired t tests were used to check the differences between the level of self-grooming and allogrooming and the movements of bats in the experiments (with bugs) and in the controls (no bugs). The differences in the level of self-grooming ($t=7.65$, $p<0.001$, $n=20$), movements inside experimental boxes ($t=5.40$, $p<0.001$, $n=20$), and bats leaving between experiments ($t=5.98$, $p<0.001$, $n=20$) and controls were statistically significant (Fig. 1). Allogrooming was observed only in a few cases and differences were not significant ($t=2.02$, ns, $n=20$).

When comparing the session with 20 and 50 bugs, the differences were found in the level of self-grooming (t test, $t=-7.66$, $p<0.001$, $n_1=10$, $n_2=10$), movements ($t=-5.74$, $p<0.001$, $n_1=10$, $n_2=10$), and the number of bats leaving the boxes ($t=-4.04$, $p<0.001$, $n_1=10$, $n_2=10$), and no differences were found in the levels of allogrooming ($t=-0.16$, ns, $n_1=10$, $n_2=10$) (Fig. 1).

Bat movements and bugs

Bats with bugs stuck on their bodies were significantly more frequent among those that left a box than among those that stayed inside ($t=3.96$, $p<0.001$, $n_1=10$, $n_2=10$) (Fig. 2). Because bats started to move shortly after being bitten by a bug, the number of sucked bugs on bats that left the box did not differ even when different numbers of bugs were used in the session ($t=-1.80$, ns, $n_1=10$, $n_2=10$), whereas a higher number of bats left the more infested roost at the same time. The time of the last bat emerging from the box was longer at the higher level of parasitation ($t=-3.81$, $p<0.001$, $n_1=10$, $n_2=10$). On average, 1.3 (range 0–3) sucked bugs were found per bat leaving the box. Furthermore, a higher level of parasitation of juveniles than adult females was observed ($t=-4.70$, $p<0.001$, $n_1=5$, $n_2=5$) in both levels of parasitation (Fig. 3). The majority (89.9%) of bugs stuck on bats that left a box was recorded on juveniles, with a mean of 1.6 (1–3) bugs per bat. In all sessions (mixed and non-mixed-age bat groups together), only 0.43 (0–1) bugs per bat were recorded on adult females that left a box, resulting in 51% of the total number of bugs. Female bats were not stressed by the presence of bugs but were stressed by the bite of bat bugs after which they usually left the roost. Bat bugs were not able to suck on bats during their daily torpor because of the low body temperature, when bat body temperatures were around 24.1°C (±4.3°C).

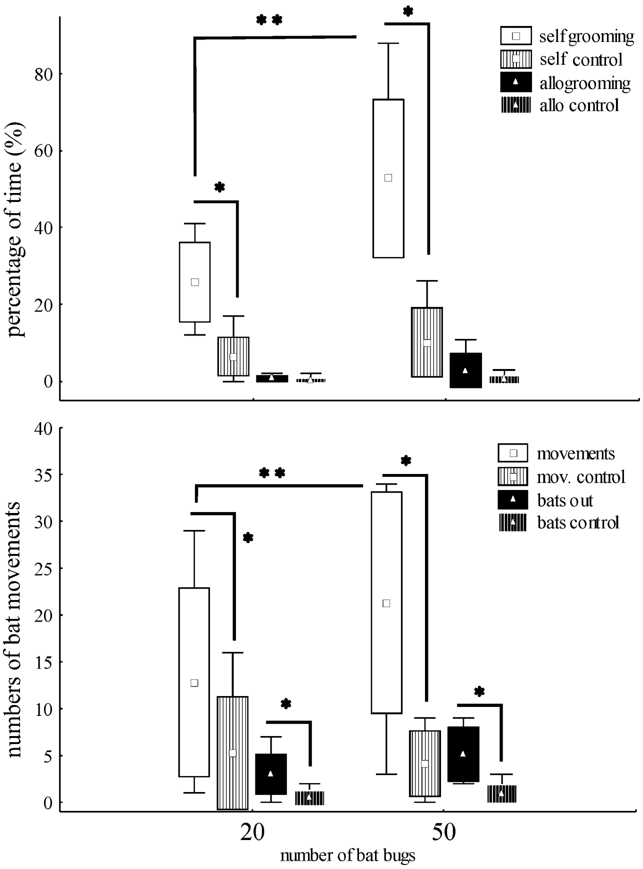

Fig. 1 Level of self-grooming, allogrooming, number of bat movements, and bats leaving bat box under different number of bat bugs. *One asterisk* (*) shows significant paired t tests ($p<0.001$) between the control (*self*, *allo*, *mov.*, and *bats control*) and experiment and *double asterisks* (**) significant t tests between level of grooming and number of bat movements under different number of bat bugs. Box–whisker plots (mean—central tendency, the standard deviation—*large box* and min–max range as *whiskers*). Bonferroni correction was applied ($p<0.012$)

Discussion

Fast ontogenesis of the roost ectoparasites allows them to increase their numbers rapidly soon after the roost occupation by bats (e.g., Usinger 1966). Brown and Brown (1986) described the impact of ectoparasites on hosts' fitness and the negative influence on the coloniality of hosts. Bugs of the family Cimicidae are important roost ectoparasites of bats. An increase in parasite density is usually caused by high starting parasite abundance in the

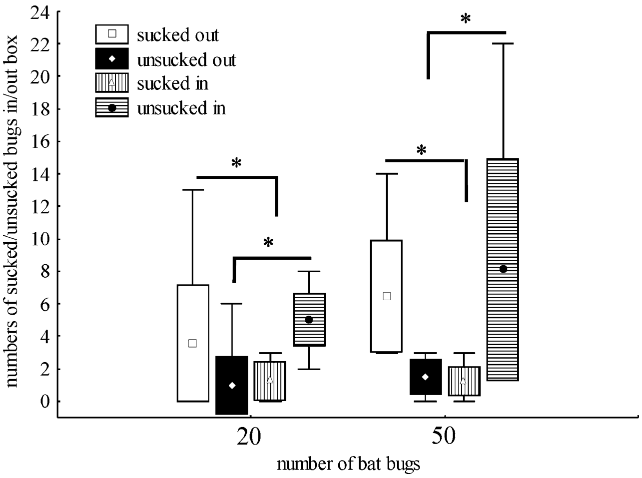

Fig. 2 Numbers of sucked/unsucked bat bugs found on bats that left or remained in the box during the sessions. *One asterisk* (*) shows significant *t* tests ($p<0.001$) among different bug groups. Box–whisker plots (mean—central tendency, the standard deviation—*large box* and min–max range as *whiskers*). Bonferroni correction was applied ($p<0.006$)

host's roost, low antiparasitic behavior and/or immunity reaction, optimal microclimate—microhabitats that are favorable for the parasite, decreased natal dispersal, and occupancy of the same roost for a long time (uninterrupted occupying of roosts by bats) (Brown and Brown 1986; Zahn and Rupp 2004). Resistance of bats against parasites can be influenced by sufficient nourishment; therefore, there are different numbers of parasites during the same time periods and in the same roosts (Christe et al. 2000). Frequent roost switching reduces the numbers of roost ectoparasites, namely, bugs in the abandoned roosts, which can be re-occupied later during the same or in the next season (Bartonička and Gaisler 2007). Although roost switching has been demonstrated as antiparasitic behavior, the phenomenon was not completely understood until now (cf. Lewis 1995; Vonhof and Barclay 1996; Brigham et al. 1997). It seems that parasitation is sufficiently important to be a cause of roost switching.

Ability to suck blood

It is well-known that the bugs spend only the time necessary for feeding on their host's body (Usinger 1966). At the beginning of each session, bat bugs orientated themselves in the experimental boxes quickly and they moved to the cluster of bats in daily torpor immediately. Marx (1955) suggests that the bed bug *C. lectularius* detects human hosts from as far as 1.5 m away through the use of heat cues, host kairomone(s), and/or CO_2. Temperature sensors on the antennae are capable of resolving differences of 1–2°C (Sioli 1937). Rivnay (1932) carried out an experimental study on host's preference of *C. lectularius* and found that a hungry bug can feed on all vertebrates whose body temperature exceeds ambient temperature by at least 3°C. The optimal blood temperature with respect to sucking in *C. lectularius* was found to be 37°C (Montes et al. 2002).

In this study, although bugs were unable to suck on bats during the daily torpor because of low blood temperature, they were able to localize them and reach their bodies. This statement is illustrated by the observation of high locomotor activity of bat bugs on the bats' bodies without host reaction during the first 20 min of most experimental sessions. However, bug activity can cause the activation of the bats, which consequently leads to an increase of their body temperature and allows the bugs to suck. Differences in the availability of feeding are very important with respect to the timing of parasite pressure during the day. If bugs can feed on bats only when they are active, the parasite load of bat bugs will be highest during the short activity period before leaving and after returning to the roost when bats are not torpid. Also when bugs bite bats, the latter can leave the roost and transport bat bugs to a non-infested roost at a distance.

Antiparasitic behavior

Bats, when in torpor, were not stressed by the presence of the bugs themselves, but when woken up and stressed by the bug bites, they normally left the roost. The host's reactions to bug bites are probably caused by substances in the saliva (Valenzuela et al. 1995). Also, allergic response can often be present (Hecht 1930). Adaptive parasite-induced behavior (an effort to kill or eat ectoparasite, or to groom, fidget, and scratch) can decrease the level of successful sucking (Moore 2002). However, results from

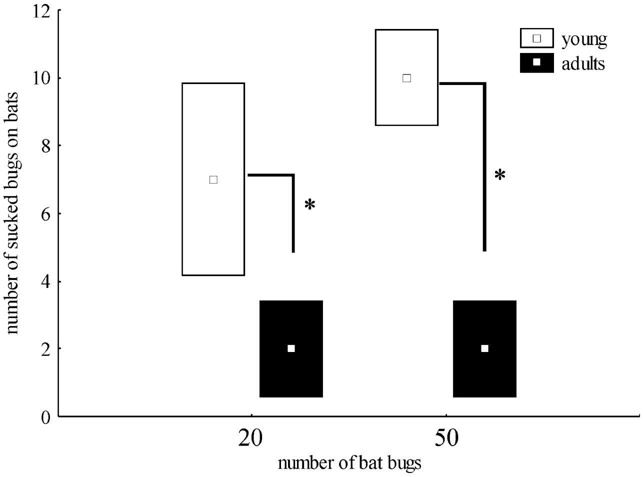

Fig. 3 Different levels of parasitation of juveniles and adult females. Bonferroni correction was applied ($p<0.012$)

the current volary experiments did not show any behavior, which might reduce the pressure of roost ectoparasites before the blood loss. Usinger (1966) notes that bats avoid eating bat bugs because of their intensive smell (an alarm pheromone); therefore, it can be expected that bats would not bite bugs during experimental sessions (cf. Overal and Wingate 1976). However, bat bugs were served periodically together with the common food (mealworm larvae, *Tenebrio molitor*; and crickets, *Gryllus assimilis*) to bats kept in the volary. Bats ate bugs without restraint and even during several days. Analyses of prey of *P. pygmaeus* also showed the presence of *Cimex* spp. body fragments in pellets (Bartonička et al. 2008). Despite the bats' ability to feed or to bite bat bugs, they refrain from doing so during experiments although it could prevent their blood loss.

Cooperation in grooming

Allogrooming (grooming of conspecifics) is one form of social behavior that can be observed in many social mammals (O'Brien 1993; Hart 1994; Mooring and Hart 1995; Gompper et al. 1997). It is suggested that allogrooming has both social and hygienic function. Bats groomed their colony mates mainly on the body parts that are difficult to reach. The hypothesis of cooperation among individual bats in defense against bat bugs has not been confirmed so far, though allogrooming was observed in other bat species, mainly between parents and offspring (Wilkinson 1986; Kerth et al. 2003; Willis and Brigham 2004). The same authors (i.e., Wilkinson 1986; Kerth et al. 2003) did not find significant positive correlation between the time a bat was groomed and the time it groomed itself, and allogrooming was very rare compared to self-grooming in their study. Also in my sessions, only a few allogrooming events were observed. Adult bats did not cooperate in defending each other against bat bugs; neither did the females defend their offspring, despite the higher level of parasitation of juveniles. A higher number of sucked bugs found on the young were also reported by Christe et al. (2000). On the other hand, there is no direct correlation between the number of bugs in the roost during lactation (non-fledging period of young) and first-year survival probability. Therefore, the breeding lifespan, which is usually used as the major indicator of fitness (in birds, e.g., Brown and Brown 1998), should be determined between infested and non-infested roosts. Finally, it can be assumed that the two observed behavior modes, allogrooming and self-grooming, do not serve exactly the same purpose. I suggest that in pipistrelles, self-grooming is used to remove ectoparasites (bat bugs), whereas allogrooming serves mainly for social functions.

Movement of bats and number of bugs

The emergence time of the last bat from the experimental box was shorter when a lower number of bat bugs were applied. A comparison of the number of bugs adhering to bats that left the bat box under low and high parasite load shows that bats react to one of the first bug bites and, when changing position and self-grooming is unsuccessful, they leave the box. Thus, the emergence time can correlate with changing (increasing) the success of bugs to find bats with body temperatures suitable for sucking and, at the same time, with a higher level of self-grooming. Consequently, the bats disturb each other and indirectly induce an increase in the body temperature of adjacent bats. As a result, these bats can also be attacked by bat bugs because of their higher body temperature. Experiments with artificial parasitation, when bugs were added to the roost manually, show that leaving confined roost occupied by a high number of ectoparasites is a natural reaction of crevice-dwelling bat species. However, vacation of the roost is not always needed; bats prefer changing location within the roost in case this strategy helps to reduce the parasite load. Therefore, further studies are needed in species occupying more spacious roosts, which allow effective changes of position.

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Bat bugs (*Cimex pipistrelli*) and their impact on non-dwelling bats

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ORIGINAL PAPER

Bat bugs (*Cimex pipistrelli*) and their impact on non-dwelling bats

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Abstract Bat bugs are often roost ectoparasites of bats. Previous studies have shown that bats shifting roosts within the growing season prevent the massive reproduction of these parasites. We postulated that there could be other antiparasitic strategies of philopatric bats roosting in non-dwelling spacious roosts. Unfortunately, there are no studies devoted to such a topic. For 3 years, two attics highly and less infested by bat bugs (*Cimex pipistrelli*) with breeding females of *Myotis myotis* were monitored. From April, after the arrival of the bats, to November, abundance of all instars and adult bugs was sampled in the attics by adhesive traps. We found different patterns in the bug abundances and dynamics in the two attics. In highly infested attic, bat bugs induced pregnant females to move from the infested site of the attic to the non-infested one. Internal temperature and relative humidity were similar in both infested and non-infested sites. Females roosted in the infested site till time before parturition and then moved to the non-infested site within attic. When bats were absent in their old site, the abundance of nymphal instars of bugs decreased by half. Although adult bats can survive under high parasite loads of bat bugs, reproducing females prevent parasite reproduction and simultaneously reduce parasite load in the young by shifting inside spacious roosts.

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Introduction

Bat bugs (*Cimex pipistrelli*, Cimicidae, Heteroptera) are often roost ectoparasites of bats, which, except for the time spent engorging (Walter 1996), mostly co-habit without physical contact with their hosts (Reinhardt and Siva-Jothy 2007). Fast ontogeny enables the bugs to increase their numbers rapidly soon after a roost has been occupied by the hosts. Bugs, in general, prefer lower roost temperatures than permanent bat parasites, because they associate with bats for a short time only to feed (Usinger 1966). Therefore, they inhabit crevices, restricting the circulation of air inside (Lourenço and Palmeirim 2004; Whitaker 1998), but in close vicinity of bats. Although previous studies have shown high tolerance towards dehydration and a desiccation hardiness against high temperatures in adult bugs (Dubinin 1947; Southwood 1954), early instars with higher body water content have worse survival rates under high temperatures of the climatically extreme conditions in natural bat roosts (Johnson 1960; Jones 1930). In such cases, even short absences of hosts in infested roosts can induce a sharp decrease in bug numbers (Bartonička and Gaisler 2007).

High densities of haematophagous ectoparasites composed of *Cimex* entail a health risk associated with blood loss, unease, stress and changes in grooming behaviour after the bug bites (Giorgi et al. 2001; Walter 1996). Experiments with artificial parasitation, with bugs added to the roost manually, show that leaving a confined roost occupied by a high number of ectoparasites is a natural reaction of crevice-dwelling bat species (Bartonička 2008). Bats left highly infested roosts even when other conditions such as temperature, humidity or air circulation were optimal (Evans 2009). Roost switching during one season is well known in pipistrelles and seems to be partly an anti-parasite strategy with respect to roost parasites such as bat bugs (Bartonička and Gaisler 2007). Pipistrelles do not switch roosts as many times as typical forest-dwelling species, e.g. *Myotis bechsteinii* or *Eptesicus fuscus*, where the causes of roost switching most probably relate to the fission–fusion social organisation or changing requirements connected with reproduction (Kerth et al. 2001; Lourenço and Palmeirim 2004; Willis and Brigham 2004). However, some forms of roost selection to avoid ectoparasites have been observed in tree-dwelling species (Reckardt and Kerth 2007).

The supply of artificial roosts is more limited in bat species that have roosted for a long time (several decades) in summer shelters (associated with roosts in buildings) than that for species that use roosts of short lifespan like pieces of detached bark (Willis et al. 2003). In the case of *Myotis myotis*, females of this species, despite knowing other roosts of nursery colonies in the vicinity, switch rarely, usually as a post-disruption movement (Schneider and Hammer 2006). We postulate that philopatric species roosting in spacious shelters have other anti-parasitic strategies. Therefore, we present a field study in which the roosting strategy of greater mouse-eared bats (*M. myotis*) was carried out. We think that vacation of the roost is not always needed. Bats might prefer changing location within the spacious roost in case this movement strategy helps to reduce the parasite load. The aim of our study was to determine (1) if bats moved inside the large spacious roosts, and (2) if yes, whether the cause of movements was high parasite densities in the previous sites so that the females wanted to bear and wean their young within sites where there were no or low numbers of bat bugs.

Material and methods

Study area and technical equipment

Nursery colonies of *M. myotis* were monitored in two church attics: in Blansko (49 ° 21′ 36″ N, 16 ° 38′ 19″ E) where 200 females roosted and Klentnice (48 ° 50′ 37″ N, 16 ° 38′ 40″ E) with 150 females, in southern Moravia (Czech Republic). Both roosts were regularly (once every 10 days) observed in 2007, 2008 and 2009. Roost checks occurred more often during lactation. Attics were equipped with thermometers (Hobo; Onset Computer Corporation, Southern MA, USA) to record temperatures and relative humidities inside the roosts throughout the seasons. Thermometers were placed in the sites occupied by bats and in new sites where the bats moved to. Monitoring of the bat bugs was carried out by manual sampling from inside the roosts.

Adhesive belts (width 5 cm) for immobilisation of crawling insects (Bio Plantella) were placed on binding joists, poles and rafters. Bat bugs were caught when they tried to cross the belts towards the bats. Ground traps made from plastic bowls turned against each other with longitudinal crevices at the bottom to trap bugs on an adhesive plate (Bio Plantella adhesive paper, 10×10 cm). Traps were taken on the ground next to the piles of bat guano. In each locality, we placed three ground traps and six 50-cm-long belts. Adhesive belts were covered by a steel grill to prevent bats from sticking to it, but allowing bat bugs to crawl through. The positions of the belts were switched at each check. The same number of belts and traps were placed in areas where bats moved during the study.

The sampled bat bugs were manually divided by instars and sex and placed in laboratory containers using a binocular (Olympus SZX 9), following the method of Stutt and Siva-Jothy (2001) to reliably sort the samples according to their developmental stages and sex using tweezers. Bugs were divided into four groups as follows: first to second instar, third to fifth instar, adult males and adult females.

Statistical analysis

All variables showed a normal distribution after log transformation. Statistica for Windows 9.1 was used for data analyses. Analysis of variance (ANOVA) and (paired) *t* test were used to check for differences between the temperature and humidity in two colony roosts. ANOVA was used also to check the differences between parts of the season and *t* test as post hoc. Logistic regression was used to test for changes in the numbers of bat bugs, maximum daytime temperatures and minimum relative humidity in new and old colony sites and between the two different attics.

Material

During the three growing seasons of 2007 to 2009, the two attics were monitored from the beginning of April to mid-October. Samples of bat bugs (*C. pipistrelli*) and number of bats throughout the day were taken on 19 (2007), 20 (2008) and 20 (2009) occasions in each attic. On each day, hourly values of internal temperature and humidity were recorded.

Results

Seasonal dynamic in bug numbers

No significant differences were found among the seasons in Blansko (ANOVA, $F_{(2,\ 59)}=0.961$, NS) when the bat bugs were sampled. However, in Klentnice, we found different numbers of bat bugs ($F_{(2,\ 59)}=1.196$, $p=0.01$). Differences in the numbers of bugs among the checks in 1 year were statistically significant (ANOVA, Klentnice, adults $F_{(19,\ 59)}=14.19$, $p<0.001$, third to fifth instars $F_{(19,\ 59)}=6.44$, $p<0.001$, first to second instars $F_{(19,\ 59)}=9.80$, $p<0.001$; Blansko, adults $F_{(19,\ 59)}=3.10$, $p=0.004$, third to fifth instars $F_{(19,\ 59)}=0.94$, NS, first to second instars $F_{(19,\ 59)}=1.93$, NS). In 2007, we found only few bugs in Klentnice; therefore, we pooled only years with high bug density into the two datasets (Klentnice, 2008 and 2009; Blansko, all 3 years). However, there were differences in seasonal dynamics of bat bugs between the two roosts Klentnice and Blansko (paired *t* test, $t=-4.62$, $p<0.001$, $n_1=40$, $n_2=40$, only 2008 and 2009). Two peaks in the densities of bugs were recorded in Klentnice at the end of May (adults) and mid-July (early instars) (Fig. 1). Comparing high density of bat bugs in Klentnice, only low numbers of bugs were recorded throughout the year in Blansko (Fig. 2).

In Klentnice, from the beginning of the season (mid-May), when the bats were pregnant, until the parturitions in mid-June, a significant decrease in the number of adult bugs were recorded (*t* test, $t=2.86$, Bonferroni correction, $p=0.035$, $n_1=3$, $n_2=4$). A rapid increase in the number of first to second instars ($t=-6.88$, $p=0.01$, $n_1=4$, $n_2=4$) and third to fifth instars ($t=-3.10$, $p=0.03$, $n_1=4$, $n_2=4$) was recorded during late pregnancy at the end of June. A significant decrease in all instars was found from mid-July until the end of August ($t=4.70$, $p=0.003$, $n_1=6$, $n_2=6$).

Although we found changes in the numbers of nymphal instars in the highly infested roost in Klentnice, there was a significant change only in the numbers of adult bugs in the low infested roost in Blansko. The number of adult bugs surviving from the previous winter decreased until the end of May, when they practically died out ($t=4.36$, $p=0.02$, $n_1=3$, $n_2=2$). At the beginning of July, a new generation of adult bugs were observed.

Site switching

A group of females left their respective roosts in early June (2008 and 2009, Klentnice) and moved to the southern part of the attic, 20 m from the old site, in the days when the first young were observed. During the period in which the bats were absent from their old site (17 days in 2008 and 21 days in 2009), a rapid dying of nymphal stages (first to fifth instars) of bugs was recorded (linear regression model, $F=14.66$, $p=0.005$, both years). An obvious, but not significant increase in the number of bat bugs at the new site was also observed ($F=2.40$, NS). After all bats had moved, the abundance of bugs decreased at the old site to less than half during 17 to 21 days.

Microclimatic conditions

Although the studied roosts were 56 km apart and, most probably, differed in the degree of exposure, no significant difference was found in the internal temperatures and humidity (ANOVA, internal temperature $F_{(1,\ 1{,}036)}=2.11$, NS; humidity $F(_{1,\ 1{,}036})=1.43$, NS). Moreover, there were no significant differences in the maximum internal temperature (ANOVA, $F_{(2,\ 538)}=0.83$, NS) and minimum relative humidity ($F_{(2,\ 538)}=1.02$, NS) among the years. In the bat roosts, the internal temperature was usually below 30°C, and in only two cases (Klentnice), the maximum internal temperature reached 37°C. Very low relative humidity, 25 to 40 %, was found in Blansko, while the relative humidity in Klentnice varied usually between 25 and 60 %. The maximum temperature (paired *t* test, 2008, $t=0.13$; NS, $n_1=17$, $n_2=17$; 2009, $t=1.89$; NS, $n_1=21$, $n_2=21$) and relative humidity (2008, $t=0.73$; NS, $n_1=14$, $n_2=14$; 2009, $t=1.13$; NS, $n_1=21$, $n_2=21$) did not differ between the old site, occupied by bats in pregnancy and postlactation, and the new site in Klentnice, occupied during the lactation period (Fig. 3).

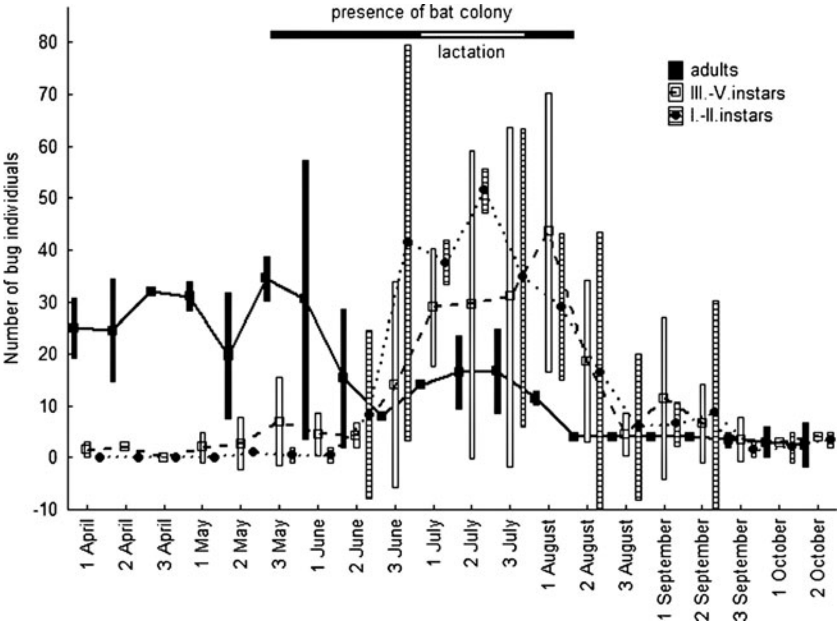

Fig. 1 Changes in the numbers of adult bat bugs and nymphal stages in Klentnice. *Black line on top of the graph* shows the period of bat presence in the roost, while the *white one* shows the lactation period. Mean, central tendency; standard deviation, whiskers

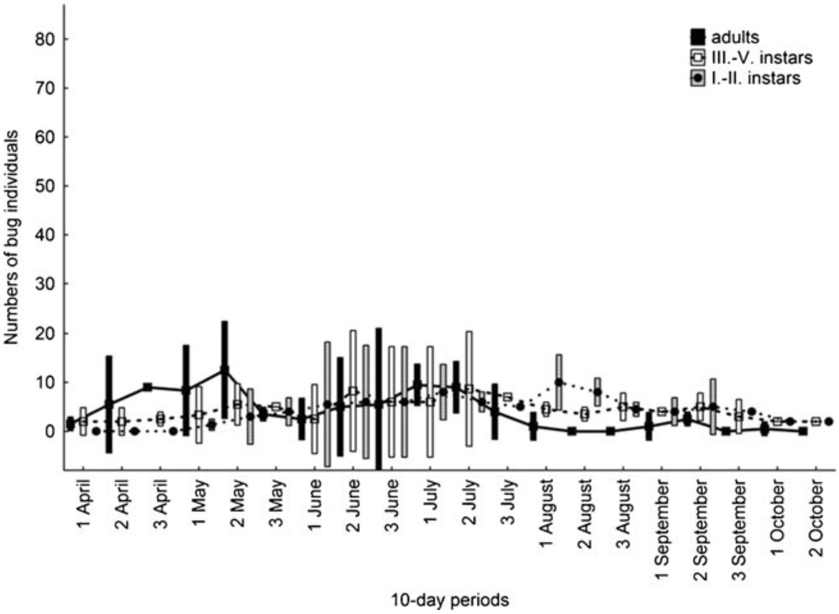

Fig. 2 Changes in the numbers of adult bat bugs and nymphal stages in Blansko. Mean, central tendency; standard deviation, *large box*. Bats occupied the roost during the whole study period. Mean, central tendency; standard deviation, whiskers

Discussion

From among various environmental factors, temperature seems to be the most important, since it influences the development and activity of ectothermic animals like roost ectoparasites and, subsequently, their endothermic hosts, the bats. Many roost activities, including metabolism, are affected by the thermal environment of the roost (Kunz and Fenton 2003). Many studies suggest that females at summer colonies agglomerate in cold days and conversely disperse during hot days (e.g. Kolb 1950; Mislin 1942). Average or extreme temperatures have been reported as the most important factor for bat movements in roosts. On very hot days, bats usually abandon positions near the ridge of the roof and seek refuge in cooler parts of the loft (e.g. Licht and Leitner 1967). Moreover, bats begin to move when the ambient temperature approaches 40°C (Lourenço and Palmeirim 2008). Movements of *Tadarida* sp. in laboratory thermal gradients suggest that these bats do not voluntarily expose themselves to an ambient temperature above 36°C, and movements within or between roosts are used to avoid high temperatures (Henshaw 1960). Therefore, we postulate that if the temperature is the only determinant of bat roosting behaviour, we expect bats to roost at different sites with varying temperatures in the attic to avoid areas that overheat during the day. However, in our study, bats moved to a different part of the attic and occupied the ridge as in the case of the old site where maximum day temperatures did not differ from those of the old site. We suppose the cause of bat movements within the attics must have been due to a different reason other than very high temperatures.

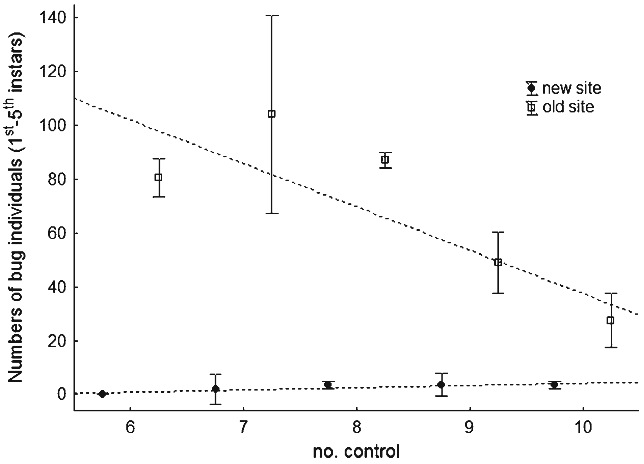

Fig. 3 Changes in the numbers of nymphal stages at the site usually occupied by bats (old) and at the site (new) occupied during lactation in Klentnice. Mean, central tendency; standard deviation, whiskers

Bat bugs formed stable populations (eggs and early instars observed) in both the studied roosts; however, their densities at the sites occupied by the bats and between years differed significantly. In Blansko, we found only 25 % of total bug numbers in Klentnice during the seasonal graduation in 2008 and 2009. The low relative humidity (<30 %) there could often be linked to low bug densities (Rivnay 1932). Bat movements were observed only in Klentnice and only in years with high bug densities. Therefore, we assume that bats always occupied the same site in the large space roof every year, switching their positions in the attics only in the years when bug densities were very high. Roost manipulation experiments, such as removing bat bugs completely from spacious roosts, might be few successful, regarding many crevices and cracks in wood constructions where bugs hide. However, such removal experiments in bat boxes, where are not too much bug shelters, by Bartonička and Růžičková (under review), have already confirmed that roost switching in bats could be the outcome of a high roost parasite load.

At the old site, when the bats were absent (during lactation in July), there was a rapid decline in most of the nymphal stages of the bugs. Although bugs are known to have high tolerance of drying, they died of water deficiency associated with lower relative humidities (0 to 60 %). According to Kemper (1936), extremely low relative humidity (0 to 20 %) often causes the death of nymphs in the course of ecdysis. We did not find differences in the minimum relative humidity between the old and new sites in the attic of Klentnice, relative humidity during lactation being very low (37.3±10.5 %) at both sites. The negative impact of low humidity on early instars may even be greater when combined with food inaccessibility (Bartonička and Gaisler 2007). Therefore, bats left the old site in the attic because of the increase in the number of parasites, mainly early instars. They could do it just before parturition because reproducing females and their hairless offsprings usually have the highest level of parasitation (Zahn and Rupp 2004; Lučan 2006). Therefore, the fact that females leave an infested roost just before parturition could be an interesting example of prenatal maternal behaviour similar to other kinds of maternal effects already published (e.g. East et al. 2009). Very important seems to be absence of bugs in sites where females leave young while forage.

Only few individuals of nymphal stages and adults of bat bugs were sampled by adhesive belts at the new site. It seems that bat bugs cannot move to host that are far away or to the new host site. The means of finding a host is the most controversial subject in the studies of bat/bed bug behaviour. Rivnay (1932) claimed that the bugs searched entirely randomly until they were 3 or 4 cm from the host, when they recognised a temperature differential of 2°C. However, Marx (1955) found that bugs could perceive a human body from a distance of 1.5 m. Nymphal stages of *Cimex lectularius* can travel at a speed of 13 to 28 cm/min, and adults, 126 cm/min (Hase 1917). Therefore, they should be able to reach a distance of 20 m very quickly. In addition, Kemper (1936) found that bugs successfully travelling to very distant hosts followed paths that involved considerable turning from a direct line. They most probably follow faecal spots, food prints or pheromone trails by dragging the engorged abdomen (Hase 1930; Aldana et al. 2008). Unfortunately, there is no detailed study explaining how long the smell of trails lasts. The fact that bats occupy different sites within an attic was observed for the first time in this study, even though a revision nursery colony in Klentnice, evaluated several times a year since 2000, has not confirmed that bat bugs have to regularly find moving hosts. Moreover, only a few faecal spots and exuviae were found at the new site because of short bat stays and very few bugs found. In general, no such high bug densities occur in all infested bat roosts every year (see Klentnice 2007 and Blansko all the time) because the between-year levels of parasitation depend highly on the number of successfully overwintering adults (Bartonička and Gaisler 2007). Therefore, we expect that bats move only occasionally between infested and non-infested sites within the attic, and trapped bugs could be transported to the new site by commuting bats.

Our results show that roost/site switching could be a suitable strategy to prevent the massive reproduction of bat bugs. Bartonička and Gaisler (2007) presented evidence to support the theory that some crevice-dwelling bats switch roosts of nursery colonies due to bat bug infestation. This study documents for the first time that not only did bats prefer fissure-like roosts, but also occupied non-dwelling spacious roosts, with the strategy of temporarily abandoning particular roosts/sites to decrease parasite load.

Mark-recapture and molecular genetic studies have recently documented that female bats exhibit natal philopatry and roost fidelity in temperate regions (e.g. Petri et al. 1997). In many species, such as *M. myotis*, maternity colonies are persistently used for decades, suggesting that natal philopatry leads to long-term association among individuals in a colony (Entwistle et al. 2000). Long-term use of one roost provides optimal conditions for parasites to synchronise their life cycle as closely as possible to that of its host. On the other hand, the possibility of finding adequate microclimates within a roost during pregnancy and lactation is probably thought to favour roost philopatry (Harbusch and Racey 2006). Site-switching behaviour within one large roof appears to be a convenient trade-off, enabling high survival rates of the young and avoiding high parasite loads.

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8.4 Hostitelská specifita štěnic

WAWROCKA K. & BARTONIČKA T., 2013

Two different lineages of bedbug (*Cimex lectularius*) reflected in host specificity.

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ORIGINAL PAPER

Two different lineages of bedbug (*Cimex lectularius*) reflected in host specificity

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Abstract Co-speciation between host–parasite species is generally thought to result in mirror-image congruent phylogenies. For the last several centuries, many bat species have been turning synanthropic, especially those that are hosted by bedbugs in Europe. There is evidence of only limited gene flow from the population of people to the population of bats. This study was focused on comparison of survival, development, and the reproduction rate based on cross-feeding experiments. In our research, we used two bedbugs groups of *Cimex lectularius*—bat- and human-associated and respectively as specific/non-specific host bat and commercial human blood. Both lineages show different behavior according to their host preferences. During the bat blood experiment, we found significant differences between both human- and bat-associated bedbugs (Log rank test fourth χ^2=9.93, p>0.05; fifth χ^2=11.33, p<0.05), while no differences occurred with the human blood experiment between the survival levels. In molting, differences between both groups were significant particularly in the case of the bat blood experiment (fourth χ^2=5.91, p< 0.05). In the case of the bat blood experiment, we found a higher probability of molting in bat-associated groups than in human-associated groups. In the case of the human blood experiment, molting probability was stable in both specific and non-specific, showing similar pattern in both cases for all stages. Our results indicate an occurrence of two ecotypes within the one species *C. lectularius*. These findings support earlier data about morphological and mitochondrial DNA differences. The differentiation of both lineages fits the concept of specific host choice.

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Introduction

Host specificity and anatomical and morphological adaptations are essential for understanding the variability of life strategies and the evolution of parasitic species. There is a wide list of parasites that are connected with a host via their life cycle and that fact, next to dispersal limitations, decrease potential host switching and limit host's range. Most parasites occur on a restricted number of hosts and show some evidence of specificity. Specialization for particular hosts may result by fidelity to different hosts and sympatric occurrence at the same time, host-associated genetic differentiation, and/or restricted but appreciable mutual gene flow (Dres and Mallet 2002). Under such conditions, closely related parasites may be specialized in a particular host. Poulin (2007) described two host choices by parasite: (1) encounter filter, when parasite excludes the host which he can not colonize and feed on because of behavioral or ecological reasons and (2) compatibility filter, excluding all host individuals on which parasite can not feed because of morphological, physiological, and immunological reasons. Host specificity was defined by Dick and Patterson (2007) as a degree to which a parasite species occurs in association with host species. Host-specific parasites generally have a major-primary host (5 % or more host individuals are infested) and a few less frequently used hosts (Tripet et al. 2002). Even generalists show a preference for some species above others (Tripet and Richner 1997; Johnson et al. 2002).

The bedbug, *Cimex lectularius* Linnaeus, 1758, is likely the most widely known cimicid species. Fast ontogenesis, high reproductive potential, mobility, and at the same time, a hidden way of life make bugs (Cimicidae) important ectoparasites. Despite the fact that bedbugs were found on 8 species of birds, 10 rodents and mustellids, 10 bat species, and humans, we recognized only two primary hosts—human and the greater mouse-eared bat (*Myotis myotis*) (Povolný 1957). Since other hosts have different in ecology and behavior, their infestation is probably relatively recent (Povolný and Usinger

1966; Usinger 1966); the bedbug represents an optimal model for a study on initial stages of host specialization. Since the last decade, many studies of re-emerging bedbugs in USA and Europe were published (Romero et al. 2007; Reinhardt et al. 2008).

In some cases, individuals from the Cimicidae family, during the absence of the natural host, are able to switch on non-specific host (*Cimex pipistrelli*, Whyte et al. 2001; *Cimex dissimilis*, Smaha 1976). But to make the substitute host proper for parasites, it has to fulfill certain conditions, especially morphological, physiological and behavioral. As Reinhardt and Siva-Jothy (2007) proven, such limitation could be in some cases the size of the red cells. Erythrocytes diameter can influence sucking ability (e.g., chicken erythrocytes are almost twice as large as those in humans, 11 to 6 μm). Moreover, as was shown before, also sex, age, and reproductive status has an influence on the reproduction of parasites (Lourenço and Palmeirim 2008).

Recent studies suggest that Central European populations of some bat species have shifted their roosting strategy in the last decade, with prefab houses becoming places of their most frequent occurrence. Large-scale renovations of those objects, currently performed in Central Europe, pose a serious threat to populations of several bat species. In cases when the bats switch their roosts, or they are to dislodge from shelters by renovations of houses, people complain repeatedly about the presence of cimicids in their flats. These bugs intensively feed on people (T. Bartonička, P. Schnitzerová personal observation). These records not only come from *C. pipistrelli*, which is more often found on bat hosts, but also from *C. lectularius*. The ability of *C. pipistrelli* in attacking people and sucking on them was confirmed, but they are not able to develop and undergo a full cycle. Adults bugs that were fed on human blood and laid eggs were successful but hatched larvas did not want to suck human blood (Southwood 1953). According to previous papers, it seems that blood meal temperature, not its specificity, play a crucial role in blood feeding experiments (Moloo 1971).

Balvín et al. (2012) suggests that switches between the human- and bat-associated groups of *C. lectularius* are only occasional since their split and that the bedbugs mostly switched from humans to bats. Beside the host choice or less fitness of hybrids, this could be also a reason for the degree and shape of the mutual gene flow; nevertheless, the limited evidence of the contact of the two populations (bat- and human-associated bugs), shows that bats could serve as reservoirs, covers the temporary absence of the primary host, and can contribute to the current dramatic spread of the bedbug among humans.

Moreover, some previous studies (e.g., Usinger 1966; Balvín et al. 2012) show that bedbugs are also adapted to their host morphologically. Balvín et al. (2012) shows differences in relative leg lengths, i.e., longer legs in human-associated bedbugs and shorter, stronger legs and more hair in bat-associated bedbugs. It also shows differences within widths and lengths of rostral segments and dimension of antennal segments or eyes. If these differences occur, we can expect differences as well on an ecological and developmental level (Usinger 1966).

To find out the existence of host specificity within bedbug *C. lectularius*, we carried out an experiment which is supposed to show not only the ability to suck on different hosts but also to illustrate different survival between two lineages of bedbugs (bat and human associated) under specific and non-specific host feeding. The aim of the study was to check what and if there are some reasons for host choice as specific ones and how the host choice influence on bugs life expectancy, speed of molting, or mortality.

Material and methods

Sampling of bedbugs

Bedbugs (*C. lectularius*) associated with bats were sampled from a nursery colony of greater mouse-eared bats (*M. myotis*) roosted under roofs of the church in Hanušovice (north Moravia, Czech Republic). Other samples of bedbugs associated with human host were sampled from hostels in the cities of Ostrava and Bohumín (north Moravia, Czech Republic). Bugs were collected with soft forceps and exhausters into small plastic boxes (10×10×5 cm) lined with soft paper. Together, we had 27 samples (one female and a few males) for each host lineage i.e., human- and bat-associated bedbugs in each experiment. In each tube appeared different stages (from egg–adult).

Equipment and experimental settings

During segregation and forming groups, we avoided infestation of samples with eggs or instars from delivered tubes and chose only adult individuals. To sort the samples, individual bedbugs were immobilized by sudden "freezing" at 0 °C for 10 min. Experimental groups (one female and one or more males) were stored in separate plastic pellucid tubes (6×1 cm). Each tube was equipped with a piece of paper 4×1 cm to let the bedbugs move, defecate, and lay eggs on it. All tubes were numbered and tightly closed with a piece of cotton. Afterwards, they were positioned in a thermostatically controlled apparatus (ST2, POL-EKO Aparatura, Poland) under stable temperature (27±0.1 °C) and humidity (75±10 %) which according to Omori (1941) and Usinger (1966) was best for the development of *C. lectularius*. Humidity was controlled using water in a Petri dish on the bottom of the glass jar with samples and damp cotton. Bugs were fed every 4 days. To facilitate observations during everyday checks and feeding, up-to-date dead individuals and rest from after molting were taken out and stored separately according to particular instars. All experiments were carried out between April and October 2012.

Bat blood feeding

Special cylinder tube with one bat and a sample of the bedbugs were placed together in a dark thermostatically controlled unit (30 °C) for 15 min (cf. Usinger 1966 or Giorgi et al. 2004). To reduce stress for animal and to avoid antiparasitic behavior including eating bugs, the bat was covered with dressing gauze and situated in a feeding tube. Before putting bugs into the feeding tube, they were counted to be sure that afterwards, all of them were collected back. After feeding period, the bat was then taken out from the tube, carefully revised, especially wing membrane, ears, and uropatagium, and bugs that still were attached into the bat's body were delicately removed with entomological soft tweezers to counteract crushing, especially very soft instars. The number of bugs and their feeding status (fed, unfed) were determined. In total, two non-reproducing females of *Vespertilio murinus* and two males of *M. myotis* were used and the bats were changed after feeding each two bug groups. Bats were fed ad libitum with a mixed diet consisting of crickets (*Acheta* sp.) and mealworms (*Tenebrio molitor*) and after experiments, returned back to the colony. The bats were captured, handled, and temporarily kept in captivity under the license no. 922/93-OOP/2884/93 and 137/06/38/MK/E/07 of the Ministry of Environment of the Czech Republic. The author has been authorized to operate with free-living bats according to the certificate of competency no. 104/2002-V4 (§ 17 of the law No. 246/1992).

Human blood feeding

Human blood for feeding (laboratory commercial blood, Japan Medical Supply, B+) was stored in a fridge. Durex condoms were tightly fitted on the plastic tube, which perfectly fit over bug-breeding tubes. During artificial feeding, blood was warmed up to 45 °C in the cylinder and 5 ml of blood was added to each roller. It was crucial to give bugs access to the blood meal, thus, the situate roller was low enough and covered all surface of the condom membrane with blood (cf. Montes et al. 2002). Feeding tubes were closed and thermostatically controlled under stable temperature 27 °C. The most suitable blood temperature was experimentally established on 37–38 °C. Too high or too low (<35 and >38 °C) didn't attract bugs (K. Wawrocka, personal observation). Feeding was continued till the moment when ca 80 % of individuals had taken the blood.

Statistical analysis

All variables showed a normal distribution after log transformation. SPSS for Windows 7.0 (IBM Statistics 19) was used for all statistic analysis. A mortality (molting) of 50 % in a sample was considered significant to finish the experiment and such a session was marked as complete in the database. Other groups were evaluated as censored. The differences in survival rate and molting speed among age groups were tested using the Kaplan–Meier survival functions. For the Cox proportional hazards model, the chi-square value was estimated as a function of the log-likelihood for the model with all covariates. It was suspected that the effect of the treatment (exposure to different host) on the underlying hazard was not constant; that is, that the proportionality assumption may be violated. To check the differences in molting and survival rate among age groups models, we used the Log rank test. We also used the Wilcoxon test to compare survival distribution among groups. Weibull regression was used to estimate molting probability for certain instars in both human and bat-associated bedbugs. Differences between specific and non-specific hosts in amount of the days after which they are able to undergo molting were tested using Mann–Whitney *U* test.

Results

Survival at different host-associated bedbugs

During analysis, we excluded individuals who undergo molting or were alive. No different changes were found in the survival rate within instars in the case of bat blood in bat-associated bedbugs, specific host (Kaplan–Meier survival test, $\chi^2=7.56$, $df=4$, n.s.) neither in the case of human-associated, non-specific host ($\chi^2=4.61$, $df=4$, n.s.) (Fig. 1). While we compared both lineages fed on bat blood, we found statistically significant differences in the fourth and fifth instars at survival rate (Log rank test, fourth instars $\chi^2=9.93$, $p<0.05$ and fifth instars $\chi^2=11.33$, $p<0.05$). Cox proportional hazards model for bat blood experiment showed that the differences between bat and human-associated bugs are significant ($\chi^2=22.51$, $df=9$, $p=0.007$).

Same tests were made also for human blood experiment, where no statistical differences were found among instars in human-associated bedbugs and specific host (Kaplan–Meier survival test, $\chi^2=7.56$, $df=4$, n.s.), neither in bat-associated bedbugs and non-specific host ($\chi^2=2.08$, $df=4$ n.s.). While comparing both associated groups fed on human blood, no statistically significant differences between instars were found. Cox model for human blood experiment compared bat- and human-associated bugs and did not show statistically significant differences ($\chi^2=15.28$, $df=9$, n.s.).

Different molting in host lineages

Evaluating the different molting rate among instars and host lineages we conducted by Kaplan–Meier survival test where

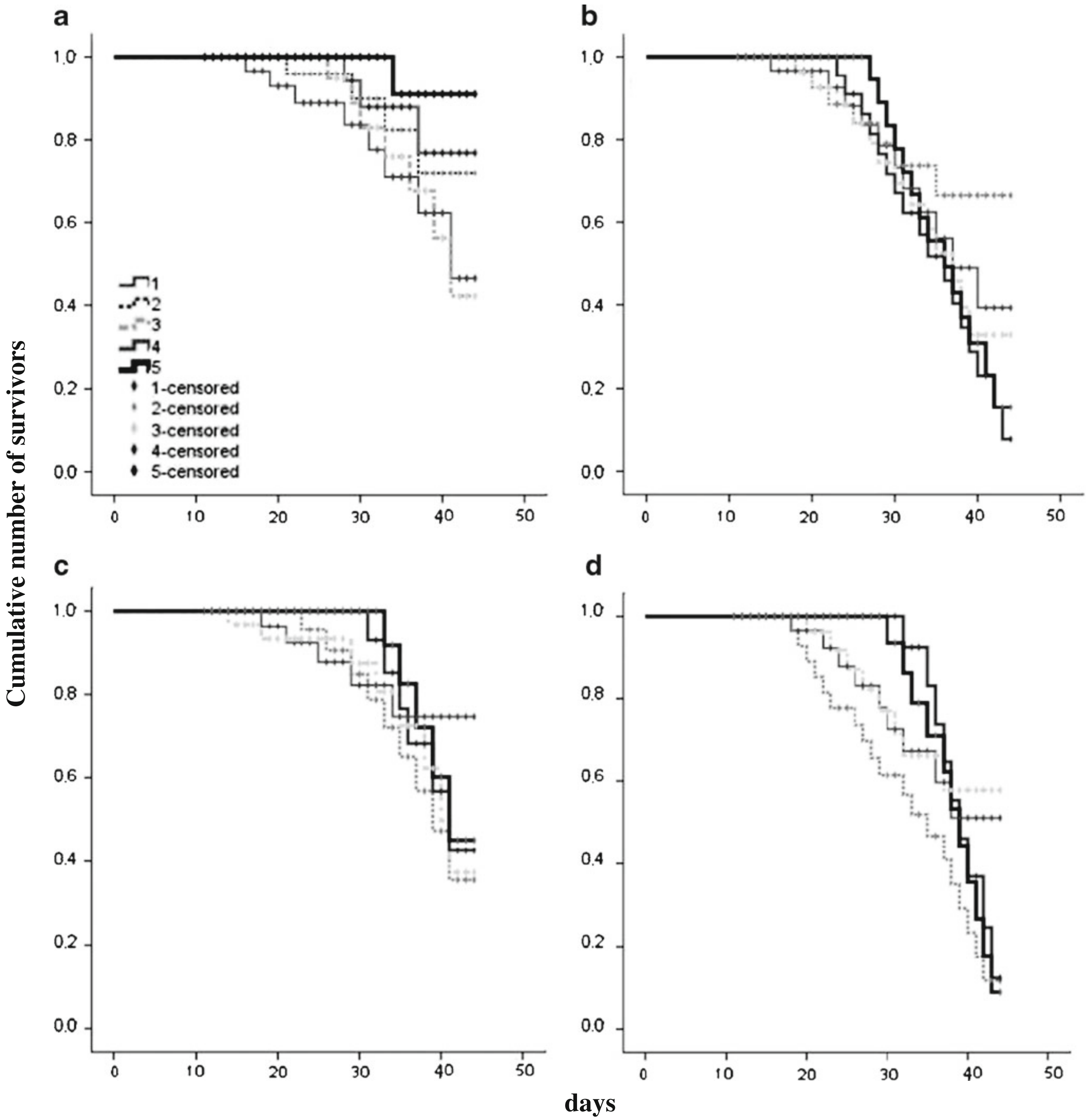

Fig. 1 Cumulative survival function for first–fifth instars, showing the fraction surviving according to different blood meal (specific—**a**, **c**/non-specific—**b**, **d**). Kaplan–Meier survival functions of instars in case of bat blood in bat-associated bedbugs (**a**); in human-associated (**b**); survival functions of instars in case of human blood in human-associated (**c**) and bat-associated bedbugs (**d**). “Complete” means at least 50 % mortality in particular instars

we tested the level of molting. We excluded dead individuals and those who didn’t undergo molting, but survived.

In the case of bat blood experiment, we found no statistically significant differences among all instars of bat and human-associated bugs (Kaplan–Meier survival test, χ^2=5.023, *df*=4, n.s.; χ^2=5.242, *df*=4, n.s.). When we compared both host lineages between each other, we found that a statistically significant difference exists in the molting of fourth instars (Log rank test, fourth instars χ^2=5.91, $p<0.05$). Nevertheless, the Cox model did not show any statistically significant differences (χ^2=14.61, *df*=9, n.s.).

We tested in the same way human blood experiments and found no significant differences among instars of human-associated bedbugs (χ^2=1.16, *df*=4, n.s.) but we found differences in the case of bat-associated bugs (χ^2=11.66, *df*=4, *p*=0.02). Comparisons of both human- and bat-associated bug lineages showed that there is no significant difference between them. Cox model did not show significant differences between molting in both lineages (χ^2=10.59, *df*=9, n.s.).

Probability of molting

Different probabilities of undergoing molting in time (days) for certain instars is described by Weibull regression. We found significant differences between bat- and human-associated bedbugs in the case of bat blood experiment (χ^2=75.18, *df*=9, $p<0.001$) as well in the case of human blood experiment (χ^2=54.04, *df*=4, $p<0.001$). Bat-associated bug shows low molting in earlier instars (first–third) and higher in later instars (fourth, fifth) and in same case, human-associated bugs had the highest molting probability of early instars (second) and the lowest in later instars (fourth). In the case of human blood experiment, molting probability was stable in both specific and non-specific showing similar pattern in both cases for all instars (Fig. 2).

Time period between ecdyses

We found the differences between specific and non-specific hosts in the amount of days after which they are able to undergo molting. Analyzed data starts from the first instar till becoming adults. Mann–Whitney *U* test showed in both cases that ecdyses time differs significantly only in bat blood experiment (*U*=5.0, n.s.) as in human blood experiment (*U*=11.5, n.s.) (Table 1).

Discussion

Although in the course of research in the last decades there have been found numerous occasional hosts of bedbugs (*Cimex lectularius*), the primary hosts are only bats and humans (Usinger 1966). Previous studies have suggested that

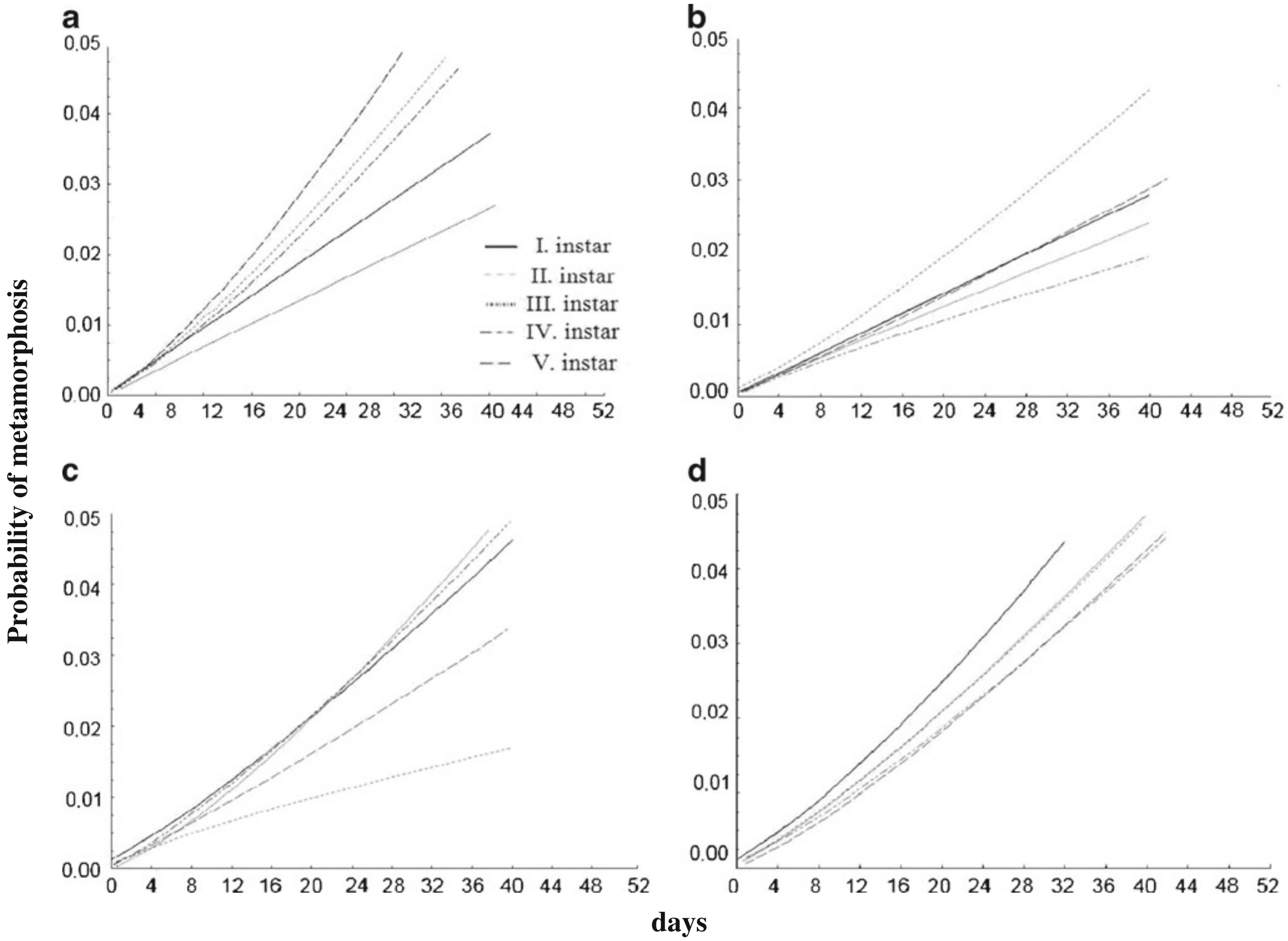

Fig. 2 Probability of molting. Weibull regression for first–fifth instar showing probability of metamorphosis according to different blood meal (specific **a**, **c**/non-specific **b**, **d**). Molting probability of instars in case of bat blood in bat-associated bedbugs (**a**), human-associated bedbugs (**b**), regression model for human blood experiment in human-associated bedbugs (**c**), and bat-associated bedbugs (**d**)

Table 1 Ecdyses between undergoing molting in certain stages (first— adult) for specific and non-specific host in bat and human blood experiment (days ± SD)

Experiment/age stages	1st (Specific/ non-specific)	2nd (Specific/ non-specific)	3rd (Specific/ non-specific)	4th (Specific/ non-specific)	5th (Specific/ non-specific)	Adults (both sexes) (specific/non-specific)
Bat blood	5±0.81/6±0.74	4±0.89/5±0.74	3±0.64/6±1.12	4±0.67/5±0.48	4±0.90/6±0.95	7±0.32/9±0.46
Human blood	4±0.96/6±0.87	3±0.68/5±0.79	7±0.87/10±1.09	8±0.46/15±1.47	4±0.64/2±0.44	9±0.93/7±1.10

bedbugs' primary host were bats (Povolný and Usinger 1966) and from them, they were transferred on humans. Recent studies (Balvín et al. 2012a) showed an interesting host-associated differentiation of the population of the bedbug on both morphological and molecular data.

We are seeing probably a gradual ecological niche diversification and perhaps the origin of reproductive isolating barriers. From this perspective, the study of the host in *C. lectularius* specificity seems to be a good model for the study of microevolutionary mechanisms connected with speciation and adaptive radiation within the group (Fig. 3).

In our study, we used two host lineages of *C. lectularius*—bat- and human-associated bedbugs. In cross-feeding experiment type of the host, specific/non-specific, seems to have an impact on survival, molting, and development rate in both cross-feedings conducted in vitro. The most common bat host of the bedbug, *M. myotis*, is originally the cave-roosting bat. It is perhaps a coincidence that this species began to inhabit buildings all over Europe only several centuries ago. Moreover *C. lectularius* is not found in their cave roosts in Europe (Simov et al. 2006), likely because of the climatic conditions in caves. The only exception in central Europe is the Hranická abyss, which roost a large nursery colony of *M. myotis*, but the bedbugs were never found on emerging bats despite repeated nettings (Z. Řehák in litt.). So, while *M. myotis* and human very often inhabit buildings, populations of bat and human-associated bedbugs show a minimum exchange of individuals between them (Balvín et al. 2012). Balvín et al. (2012) found that nursery colonies of greater mouse-eared bat are more often parasite by *C. pipistrelli* than *C. lectularius* and moreover, both bug species have never been found together in one bat roost. However, on human host, *C. pipistrelli* is found very rare always in anecdotic observations, especially if an infested apartment is near to temporary abandoned bat roost and hungry bugs are looking for food. It has long been known, that *C. pipistrelli* can feed on human host, but it has a significantly lower survival rate than on a specific host bat (Usinger 1966), and at the same time, there is a notable reproductive barrier between *C. lectularius* and *C. pipistrelli*. This isolation of particular bug populations shows a very limited capacity to transport and dispersion to another or even new host (cf. Giorgi et al. 2004).

Fig. 3 Bed bug (*Cimex lectularius*) bat-associated. Visible main characteristic differing them from human-associated bugs: stronger and shorter legs, more hair, difference in antennal segments dimension and eye which is connected with adaptations to certain host (by O. Balvin)

Host switching is usually complicated, especially when the primary host has a very different life strategy than the potential new host. Bat-associated bugs usually inhabit only these parts of buildings attics which are suitable for bats, but from there, they have only a limited ability to move (Bartonička and Růžičková 2012). The frequency of movements between bugs and man is hard to monitor and on this issue, we have only limited information (Balvín et al. 2012). Adult bedbugs can travel at a speed of 126 cm per minute (Hase 1917) and therefore, they should be able to reach large distances very quickly. When bugs can follow fecal spots, food prints, or pheromone trails by dragging the engorged bugs, (Hase 1917; Aldana et al. 2008) they can successfully travel to very distant hosts (Kemper 1936). In cases when the host is available, bat-associated bugs have no reason to find a new one. The probability of sucking on humans is therefore not reduced by the distance of infested attics from the people occupied parts of buildings, but also by human effort to eliminate new bug population in the case of successful transmission. Moreover, no cases of movement of *C. lectularius* from bat host to people in the same building have yet been published. Bugs are adapted to long starving periods. It might confirm Romero et al.'s (2007) experiment, where individuals starved for a shorter time showed much higher activity and movement level than those who were starving for a few weeks. This might help them to limit energy loss connected with movements and decrease metabolism. It appears, therefore, that the bugs are adapted to wait rather than to actively search the host. Such strategy could lead between the two host-associated bug groups to higher degree of mutual isolation.

Blood sucking parasites may favor and choose higher quality hosts who can give them a better blood meal. On the other hand, such host might be hard to feed on (Møller 2000; Khokhlova et al. 2007). Our experiments clearly show the importance of the specific host. However, it can not be assumed that the quality of the blood of a bat and human should be fundamentally different, because other studies showing the gradual isolation of both bedbug populations associated with different host lineages (Povolný and Usinger 1966). As suggested before (Hase 1926; Tawfik 1968), we did not find any significant differences between bat and human red cells diameter (Wawrocka in prep.). The cause of low survival rate on non-specific host might be in immunocompetence (T cell response) and the condition of the host, as evidenced by the numerous proofs has been "well-fed host strategy" (Christe et al. 2000). Previous research showed already differences at morphological level in case of bedbug according to the host type—human or bat (Balvín et al. 2012). Host specificity has impact not only on the parasite himself but also on his host, including the human. Preferences to some hosts can, on one hand, decrease feeding efficiency but on the other hand, make it easier for parasite to feed on host, whose immune system and ecology is already well known to him. As Dick et al. (2009) suggested that lack of some barriers that occur in nature can break down host specificity.

Bats, as partly synanthropic species, adapted to new conditions and also treat connections with human population expansion that impact their natural landscape, food decrease by habitat adaptations, and degradation, forcing bats to use alternative roosts and foraging sites and move to cities. They start to share shelters with humans (attics, churches, basements, vacant buildings, etc.), what provided them cozy, warm, and safe places that they would not find in the wild. Bat–human conflict is a well-known phenomenon. Bat noises, droppings, and presence itself is a big problem for bat conservation. What more is that together with bats appear also their parasites as cimicids. It was suspected that bats are the one of the main reservoirs of global recuperation of bedbug populations (Szalanski et al. 2008) thus they do not possess other than roosts switching (Bartonička and Gaisler 2007) the ability to reduce infestation. That fact could unfortunately deepen antipathy to bats. Nevertheless, there is no evidence at the genetical level that bats could be responsible for their expansion (Balvín et al. 2012) which is much more wider in the case of, for example, poultry (Szalanski et al. 2008). Spreading this information and knowledge among society is crucial in bat conservation.

This study showed the significant limitations in the ability of survival and molting in confusion of the two primary bug hosts and it is likely to fit the concept of host races according to Dres and Mallet (2002). The next step to elucidate the taxonomical status of both bedbug lineages could be to verify whether between the two ecotypes, are there any reproductive mechanisms limiting the free hybridization of bedbugs.

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