MASARYKOVA UNIVERZITA
PŘÍRODOVĚDECKÁ FAKULTA

Netopýři a úkryty

poznatky ze studia letounů (*Chiroptera*) v oblasti Moravského krasu

Habilitační práce

Brno, 2015 **Mgr. Jan Zukal, Dr. MBA**

Bibliografická identifikace

Jméno a příjmení autora: Jan Zukal

Název disertační práce: Netopýři a jejich úkryty: poznatky ze studia letounů (*Chiroptera*) v oblasti Moravského krasu

Název disertační práce anglicky: Bats and roosts: findings from research of bats (*Chiroptera*) in the Moravian Karst region

Obor: Zoologie

Rok zahájení řízení: 2015

Klíčová slova: netopýři, monitoring, změny početnosti, výběr úkrytu, hibernace, syndrom bílého nosu, letová a lovecká aktivita, anti-predační chování

Keywords: bats, monitoring, changes of abundance, shelter selection, hibernation, white-nose syndrome, flight and foraging activity, anti-predation behavior

Prohlášení

Prohlašuji, že jsem práci napsal sám za použití patřičných literárních zdrojů, které jsou v práci všechny řádně ocitovány.

V Brně, 30.6.2015 Jan Zukal

Poděkování

Svoji práci bych chtěl věnovat památce prof. RNDr. Jiřího Gaislera DrSc., který mě přivedl k výzkumu netopýrů a byl skvělým učitelem i kolegou.

Ekologický výzkum je náročný časově a často i fyzicky, a proto by většina prací nemohla vzniknout bez vydatné pomoci mnoha přátel, kolegů a studentů. Vyzvednout bych však chtěl zejména přínos kolegy a přítele doc. Zdeňka Řeháka, se kterým jsem začínal svoji vědeckou kariéru a strávil dlouhé noci při terénních výzkumech. Z dalších spoluautorů jsem vděčný za stimulující spolupráci svým bývalým studentům H. Berkové, K. Petrželkové a M. Pokornému, a v posledních několika letech také kolegům z Veterinární a farmaceutické univerzity prof. J. Pikulovi a MVDr. H. Banďouchové.

Výzkum v jeskyních Moravského krasu by nebyl možný bez spolupráce s organizacemi, které se o ochranu tohoto významného území starají a zodpovědně jej spravují. Dík tedy patří i pracovníkům Správy CHKO Moravský a Správy jeskyní Moravské krasu, přičemž neocenitelným pomocníkem byl vždy RNDr. M. Kovařík, který je nejen dobrým kolegou, ale i kamarádem.

Nakonec bych rád poděkoval své manželce a dcerám, bez jejichž podpory by moje „bláznivá“ honba za netopýry nebyla možná a tato práce by nikdy nevznikla.

Abstract

This thesis is presented as a set of selected 28 scientific papers with 20 of them published in peer reviewed journals indexed by impact factor. All papers concern various aspects of bat ecology and behavior studied mostly at their roosts located in the Moravian Karst or its surrounding, as the roosts are one of the most important limiting resources for bats, influencing many factors of their life and the Moravian Karst with high number of natural roosts is ideal region for such bat research.

Thesis is divided into five parts (see below) which reflect high variability of bat life. Nevertheless, in bat roosts or their entrances one may obtain with high probability sufficient material for both study of specific parameters of bat life (e.g. hibernation) or testing of general ecological hypothesis such as predator – prey interaction, respectively. Of course, we tried to minimalize disturbance of bats during all our studies

Themes of bat research studied at their roosts in the Moravian Karst and its surrounding:

1. changes of bat abundance and community structure
2. shelter selection and bat movement activity
3. emergence and return flight activity at roost entrance
4. anti-predation behavior during emergence and return flight activity
5. heavy metals and white-nose syndrome – potential threats for bat populations

Obsah

1. Struktura a zaměření habilitační práce

Habilitační práce je předkládána jako soubor originálních vědeckých prací, který je doplněn obecným komentářem k jednotlivým studovaným problematikám. Celkem se jedná o 28 publikovaných prací, z toho je 20 publikací v ISI časopisech tzn. indexovaných impakt faktorem včetně jednoho rukopisu v recenzním řízení, a jedna kapitola v knize. Nejedná se přitom o všechny vědecké publikace autora, ale pouze o výběr prací, jejichž jednotícím tématem je výzkum ekologie netopýrů převážně v oblasti Moravského krasu a jeho okolí.

Výsledky těchto studií jsou ve shrnujícím komentáři zasazeny do obecného kontextu dané problematiky, přičemž většina studií významně doplňuje aktuální znalosti ekologických aspektů života netopýrů, které je možné studovat v jejich přirozených úkrytech nebo v jejich blízkém okolí. Rozdělení prací do pěti studovaných problematik (viz níže) zároveň odráží obrovskou variabilitu života netopýrů (hibernace vs. období aktivity, odpočinek v úkrytu vs. energeticky náročný lov apod.). Důležitým faktorem přitom u všech výzkumů byla snaha o minimalizaci rušení studovaných zvířat, jelikož všechny druhy letounů (*Chiroptera*) jsou v Evropě zařazeny mezi chráněné druhy živočichů.

Problematiky studované u netopýrů* v jejich úkrytech v Moravském krasu a okolí:

1. změny početnosti a struktura společenstva netopýrů
2. výběr úkrytů a letová aktivita netopýrů
3. výletová a návratová aktivita u vchodu do úkrytu
4. antipredační chování během výletové a návratové aktivity
5. těžké kovy a syndrom bílého nosu - potenciální hrozby pro populace netopýrů

**Autor v dalším textu používá pojem „netopýr" jako ekvivalent pro celou skupinu letounů zahrnujíce jak druhy z podřádu Vespertilioniformes, tak i Pteropodiformes.*

2. Úvod

Netopýři jsou nejen pro laickou veřejnost, ale i pro vědce velice zajímavou a tajuplnou skupinou živočichů. Už pouhé vyslovení slova „netopýr" vzbuzuje u mnoha lidí představu něčeho odpudivého, přitom se s ním přímo setkal jen málokdo. Způsobuje to zejména noční aktivita netopýrů a jejich schopnost aktivního letu, které významně omezují i možnosti jejich studia (Kunz & Parsons 2009). Proto je vazba terénního výzkumu netopýrů na úkryty nebo jejich vchodové části jedním z jeho specifických metodických rysů. Netopýři zde mohou být chytáni (a díky tomu měřeni a značkováni), pozorováni nebo nahráváni, a to i opakovaně, jelikož vykazují velmi vysokou a dlouhodobou věrnost využívaným úkrytům (Findley 1993). U řady druhů netopýrů dokonce pochází naše znalosti o jejich ekologii a chování téměř výhradně z výzkumů jedinců v úkrytech (Kunz 1982). Teprve technický rozvoj ultrazvukových detektorů a telemetrických souprav v posledních 10–15 letech významně rozšířil možnosti výzkumu i do období lovecké aktivity netopýrů (Kunz & Parsons 2009).

Dostupnost úkrytů je přitom spolu s dostatkem potravy také jedním z významných limitujících zdrojů pro netopýry, zejména v oblastech mírného pásma. Ovlivňuje nejen jejich rozšíření, ale i lovecké, sociální a rozmnožovací chování, velikost populací, diverzitu společenstev a dokonce také morfologii nebo fyziologii netopýrů (Altringham 1996). Netopýři stráví v úkrytech velkou část svého života a v různých částech roku, ale i v různých částech dne, jsou jejich nároky kladené na úkryty zcela rozdílné. Proto je diverzita úkrytů využívaných netopýry obrovská. Přibližně polovina z více než 1200 druhů netopýrů využívá tzv. stabilní typy úkrytů, jako jsou lidské stavby, jeskyně, štoly, dutiny stromů nebo štěrbiny ve skalách, přičemž takovéto typy úkrytů jsou typické také pro všechny evropské druhy netopýrů (Wilson & Reeder 1993). Úkryty přitom poskytují netopýrům řadu výhod, např. ochranu před špatným počasím, ochranu před predátory, efektivnější termoregulaci, vyšší pravděpodobnost páření, menší náklady na lov, vyšší pravděpodobnost odchovu mláďat nebo transfer informací, a proto je výběr správného úkrytu zásadním selekčním tlakem, který rozhoduje o přežití a reprodukčním úspěchu každého jedince.

Území Moravského krasu je nejrozsáhlejším zkrasovělým územím České republiky. Zdejší krajina je značně diverzifikovaná a poskytuje tak netopýrům velké množství úkrytových možností. Nejedná se přitom pouze o cca 1200 jeskyní, ale i o rozsáhlé porosty listnatých lesů nebo o lidské stavby ve vesnicích a městech. Proto patří Moravský kras a jeho okolí k chiropterologicky nejlépe prozkoumaným oblastem (Gaisler, Řehák & Zukal 2006). Počátky výzkumu netopýrů v Moravském krasu jsou pochopitelně úzce svázané se speleologickými průzkumy podzemí, přičemž první zprávy o netopýrech se objevují již ve 2. polovině 19. století. V té době zde působili tři významní vědci, a to prof. Dr. Friedrich Anton Kolenati, Dr. Jindřich Wankel a o něco později i jeho vnuk prof. Dr. Karel Absolon. Zejména příchod prvního z nich do Brna je významným časovým mezníkem pro vývoj české, ale i evropské chiropterologie. Kolenati i díky výzkumu netopýrů v Moravském krasu publikoval první monografii o evropských netopýrech, v níž se mu podařilo shrnout dostupné poznatky o netopýrech včetně rozšíření jednotlivých druhů (Kolenati 1860).

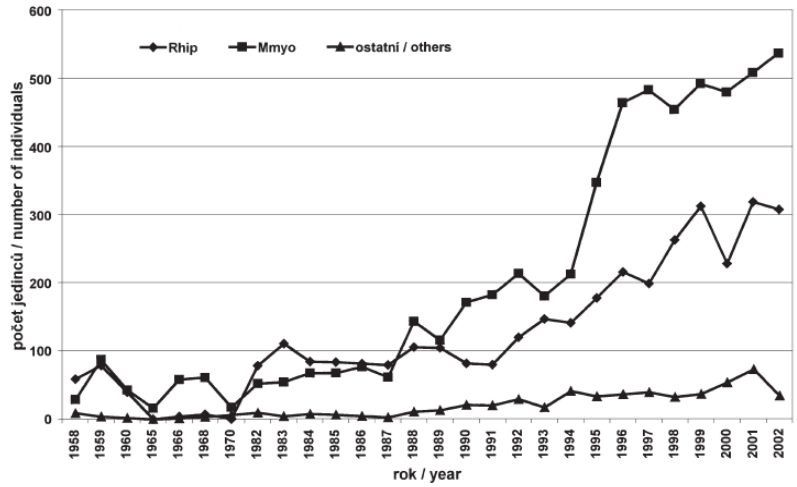
Moderní ekologický výzkum netopýrů v Moravském krasu je spojen s příchodem dalšího českého rodáka, a to prof. RNDr. Jiřího Gaislera, DrSc., v roce 1957. Ke svému výzkumu ekologie, rozmnožování a postnatálního vývoje vrápence malého (*Rhinolophus hipposideros*), narůstání srsti netopýrů nebo poměru pohlaví u fetů a mláďat získával materiál opět v úkrytech netopýrů, a to jak v zimních (jeskyně, štoly, kasematy, sklepy) tak i letních (půdy, sklepy apod.). V roce 1969 se podílel na zahájení soustavného monitoringu hibernujících netopýrů, do něhož bylo zařazeno i několik jeskyní z Moravského krasu (Erichova jeskyně, Kateřinská jeskyně a sedm jeskyní v údolí Říčky u Ochozu) (Bárta et al. 1981). Na tyto ekologické výzkumy navazuje i práce autora, která je rozšiřuje o řadu dalších aspektů života netopýrů studovaných v jejich úkrytech a blízkém okolí.

3. Shrnující komentář

3.1. Změny početnosti a struktura společenstva netopýrů

Dlouhodobě jsme svědky významných negativních změn početnosti u řady druhů živočichů (Primack 2010). Proto se nejprve v Evropě (Battersby 2010) a později i v Severní Americe (O'Shea & Bogan 2003) rozjely rozsáhlé monitorovací projekty, které hodnotí změny početnosti netopýrů v jejich úkrytech zejména na zimovištích. Výsledky těchto výzkumů přitom potvrdily významné změny v početnosti různých druhů evropských netopýrů. Rychlý pokles početnosti byl pozorován během 60. a 70. let zejména u *Rhinolophus ferrumequinum*, *R. hipposideros, Plecotus austriacus* a *Myotis myotis* (Řehák 1997). Během posledních tří desetiletí však byl zejména u termofilních druhů netopýrů zaznamenán výrazný nárůst početnosti a stabilizace jejich populací (např. Horáček, Hanák & Gaisler 2005, Uhrin et al. 2010; Piksa & Nowak 2013; Presetnik, Podgorelec & Šalamun 2013).

Výsledky z námi sledovaných lokalit v Moravském krasu, jež patří mezi celoevropsky významná zimoviště netopýrů, potvrzují tento obecný trend u obou dominantních druhů, tedy *R. hipposideros* a *M. myotis* (Řehák, Zukal & Kovařík 1994; Zima et al. 1994; Zukal, Řehák & Kovařík 2003). Růst početnosti byl také potvrzen u *Myotis emarginatus*, což je také teplomilný druh, který dosahuje na území České republiky severní hranice svého rozšíření. Tento druh byl v ještě 90. letech dvacátého století zaznamenáván v jeskyních Moravského krasu zcela výjimečně, ale v posledních letech je zde pravidelným hibernantem (Zukal et al. 2001). U dalších druhů



Obr. 1 Dlouhodobý vývoj početnosti netopýrů zimujících na lokalitě Sloupsko-šošůvské jeskyně. Zkratky: Rhip – *R. hipposideros*, Mmyo – *M. myotis* a ostatní – všechny zbývající zastižené druhy netopýrů. U dvou nejpočetnějších druhů je zřetelný nárůst početnosti od počátku 90. let, který je v obou případech statisticky významný (Pearsonův korelační koeficient, $p < 0{,}001$). Převzato z publikace Zukal, Řehák & Kovařík 2003.

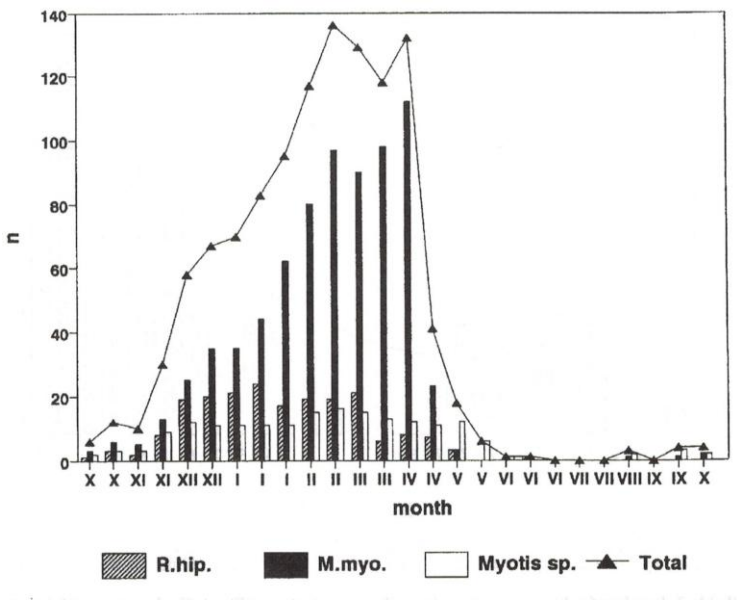
zimujících netopýrů jako jsou *Barbastella barbastellus*, druhy rodu *Plecotus* nebo malé druhy rodu *Myotis* (zejména *Myotis daubentonii*, *Myotis nattereri* a *Myotis bechsteinii*) již trendy ve změnách početnosti nejsou jednoznačné (Zima et al. 1994). Ačkoliv je složité určit konkrétní faktory, které způsobují takový nárůst početnosti zimujících netopýrů, jeví se omezení rušení netopýrů na zimovištích, globální oteplování a změny ve způsobu managementu krajiny po roce 1989 (včetně omezení používání pesticidů) jako jedny z hlavních (Horáček, Hanák & Gaisler 2005).

Typickými druhy společenstva netopýrů hibernujících v jeskyních Moravského krasu jsou *M. myotis* a *R. hipposideros* (Řehák, Zukal & Kovařík 1994; Zima et al. 1994; Zukal et al. 2001). Oba druhy tvoří více než 80 % všech zimních nálezů ve většině kontrolovaných jeskyní. Celkově však zde bylo během zimování nalezeno 17 druhů netopýrů včetně vzácných hibernantů jako *Rhinolophus ferrumequinum*, *Eptesicus nilssonii*, *Myotis dasycneme* nebo *Pipistrellus pipistrellus*. Území Moravského krasu se tím řadí v celoevropském měřítku mezi oblasti velmi významné pro zimování netopýrů. Fylogeografické studie ukazují, že se zde mohli střetnout dvě možné trasy postglaciální kolonizace střední Evropy netopýry z jižních refugií (Bogdanowicz et al. 2009; Bryja et al. 2010). Druhově stejně bohaté společenstvo má jen chiropterofauna zimující v jeskyních Slovenského krasu a Muránské Planiny (Uhrin et al. 2010), které podobně jako Moravský kras leží na severní hranici rozšíření některých druhů netopýrů.

Obr. 2 Zimující vápenec malý (*Rhinolophus hipposideros*).

Odchyty netopýrů ve vchodech jeskyní během jarních a podzimních přeletů ovšem potvrzují mnohem vyšší diverzitu společenstva zimujících netopýrů a vyšší relativní zastoupení dalších druhů netopýrů než výsledky sčítání. Jedná se zejména o jedince malých druhů rodu *Myotis*, jejichž počty jsou při zimních kontrolách rozsáhlých podzemních prostor pravděpodobně podhodnoceny. Často totiž využívají nepřístupné typy úkrytů (hluboké štěrbiny a pukliny apod.) (Bauerová & Zima 1988; Řehák, Zukal & Kovařík 1994), a to v závislosti na teplotě a intenzitě

proudění vzduchu na dané lokalitě, na konkrétním druhu netopýra, ročním období a počasí. Naše výsledky sledování krátkodobých změn početnosti netopýrů v jeskyních Moravského krasu ovšem potvrdily, že podíl „viditelných" jedinců je ovlivněn i u dobře sledovatelných druhů a nelze jej považovat za konstantní (Řehák, Zukal & Kovařík 1994; Zukal, Řehák & Kovařík 2003). Celkový počet zimujících netopýrů roste kontinuálně od října a nejvyšších hodnot dosahuje během února nebo března v závislosti na struktuře společenstva zimujících netopýrů. Nicméně tento růst početnosti je ovlivněn příletem nových jedinců z úkrytů mimo sledovanou lokalitu pouze v tzv. pre-hibernačním období. V období hluboké hibernace již na zimoviště noví jedinci nepřilétají a dochází pouze k přesunům netopýrů v rámci lokality, to znamená k přesunům z míst nedostupných pro člověka do míst, kde je jejich sledování možné (Berková & Zukal 2006). V dubnu dochází k postupnému, ale relativně rychlému opouštění jeskyní a početnost netopýrů klesá k minimu. Výzkum v jeskyních Moravského krasu navíc ukázal, že přesuny netopýrů uvnitř jeskynního systému a tím i zaznamenané změny početnosti jsou u jednotlivých druhů rozdílně časovány. Početnost *R. hipposideros* se pozvolna (6 – 8 týdnů) zvyšuje do poloviny prosince a stejně pozvolna klesá od poloviny března. Maximální početnost se však během hluboké hibernace mění jen minimálně, v závislosti na změnách počasí (viz kap. 3.3). U *M. myotis* je nárůst početnosti během celé zimy plynulý a na konci zimování tento druh již ve společenstvu dominuje. Následně dochází k velmi rychlému opouštění zimoviště, kdy během cca 2 týdnů většina jedinců odletí (Nagel & Nagel 1989; Zukal, Řehák & Kovařík 2003).



Obr. 3 Krátkodobý vývoj početnosti netopýrů na lokalitě Kateřinská jeskyně během zimy 1992/93. Zkratky: R.hip. – *R. hipposideros*, M.myo. – *M. myotis*, Myotis sp. – druhy rodu *Myotis* mimo *M. myotis* a Total – všechny druhy zimujících netopýrů. Převzato z publikace Řehák, Zukal & Kovařík 1994.

3.2. Výběr úkrytů a letová aktivita netopýrů

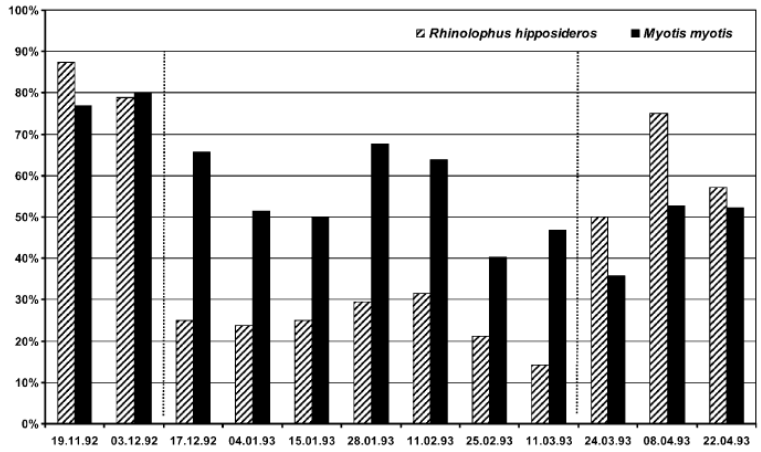
Již v úvodní kapitole bylo konstatováno, že diverzita úkrytů využívaných netopýry mírného pásma je vysoká. Jejich potřeby se totiž významně mění dle specifických nároků v jednotlivých fázích ročního cyklu života netopýra: 1. hibernace, 2. jarní přelety ze zimovišť na letní úkryty, 3. porody a výchova mláďat na letních koloniích (samci vytvářejí bakalářské kolonie nebo žijí soliterně) a 4. podzimní migrace na místa rozmnožování (někdy označovaná jako „swarming sites“) a na zimoviště (Kovařík, Zukal & Řehák 2000; Berková & Zukal 2006). V našem výzkumu jsme se zaměřili na výběr úkrytů, resp. jejich specifických částí během hibernace netopýrů a výběr úkrytů letními koloniemi samic, včetně hodnocení letové aktivity netopýrů v okolních biotopech. Jelikož výběr správného typu úkrytu se správnými parametry je zásadní, jak pro úsporné využití energetických zásob a úspěšné přežití nepříznivého období, tak i pro správné načasování a efektivitu lovecké aktivity netopýrů.

Pro netopýry mírného pásma je charakteristickou součástí ročního cyklu aktivity hibernace, jako optimální adaptace na období s extrémním nedostatkem potravy a nepříznivými klimatickými podmínkami (Ransome 1990). Podzemní prostory jako jsou jeskyně v Moravském krasu, opuštěná důlní díla, pevnosti a tunely jsou významnými zimovišti netopýrů a nejvýznamnější lokality mohou být využívány tisícovkami netopýrů. Nicméně i lokality využívané jen malým počtem netopýrů, mohou být regionálně významné. Výběr úkrytu se v průběhu hibernačního období mění se změnami mikroklimatických podmínek na těchto zimovištích a různé druhy přitom mají různé požadavky (Altringham 1996).

Náš výzkum ukázal, že teplomilný *R. hipposideros* byl během zimování zaznamenán prakticky ve všech definovaných částech sledovaných jeskyní Moravského krasu (Kateřinská jeskyně a Sloupsko-šošůvské jeskyně) s jedinou výjimkou, kterou jsou vchodové části s dynamickým mikroklimatem (Zukal, Řehák & Kovařík 2003; Zukal, Berková & Řehák 2005). Tento druh přitom zimuje téměř výhradně volně visící na exponovaných místech, a to bez ohledu na část sezóny nebo část zimoviště. Přeletová aktivita *R. hipposideros* je v průběhu hluboké hibernace

minimální a toto období je jasně odděleno od pre- resp. post-hibernačního období s vysokou úrovní aktivity (Obr. 4). Dá se tedy předpokládat, že na změny úkrytů v průběhu zimy má u vrápenců vliv spíše fáze ročního cyklu (pre-, hluboká a post-hibernace), jejich fyziologický stav nebo chování (Saint Girons, Brosset & Saint Girons 1969; Brosset & Poillet 1985; Ransome 1990).

Také euryvalentní *M. myotis* byl nalézán během hibernace prakticky v celém jeskynním systému, přičemž využívá náhodně všechny typy úkrytů (exponované vs. chráněné, umístěné na stropě vs. na stěně). Nicméně, na rozdíl od *R. hipposideros*, byla potvrzena výrazná sezónní dynamika ve využívání jednotlivých částí jeskyní Moravského krasu (cf. Hanzal & Průcha 1988). V období od poloviny prosince do začátku dubna opouští *M. myotis* zadní klimaticky stabilní části jeskyní a jeho početnost se naopak zvyšuje ve vchodových částech. Například ve Sloupsko-šošůvské jeskyni je nalezeno v jarních měsících přes 80 % všech jedinců *M. myotis* v oblasti tzv. Eliščiny jeskyně a jejího blízkého okolí (Zukal, Řehák & Kovařík 2003). Přeletová aktivita *M. myotis* uvnitř zimoviště je přitom relativně vysoká během celého období hibernace. Výběr částí zimoviště s konstantní teplotou uprostřed hibernačního období pravděpodobně zajišťuje největší úsporu energie v období, kdy je největší nedostatek potravy. Naopak obsazení dynamických částí jeskyně, jejichž teplota odráží teplotní změny vně zimoviště, pomáhá netopýrům na konci zimního období synchronizovat probouzení z hibernace s aktuálním stavem počasí a zahájit loveckou aktivitu za příznivých klimatických podmínek (Ransome 1990; Zukal, Berková & Řehák 2005). Srovnání hibernačního chování *M. myotis* ve dvou přirozených jeskyních Moravského krasu navíc ukázalo, že netopýři využívají rozdílné strategie na lokalitách s rozdílným mikroklimatickým



Obr. 4 Přeletová aktivita netopýrů na lokalitě Kateřinská jeskyně během zimy 1992/93. Vysvětlivky: svislé tečkované čáry – oddělení tři částí hibernačního období tj. pre-, hluboké a post-hibernace. Převzato z publikace Zukal, Berková & Řehák 2005.

profilem (dynamický vs. stabilní). Sledované parametry těchto strategií (úroveň aktivity, preference různých typů úkrytů, shlukovací chování) jsou specifické pro danou lokalitu, aby zajistily zimujícím netopýrům optimální spotřebu energetických zásob během hibernace a úspěšné přezimování (Zukal et al. 2010).

Řada druhů netopýrů se adaptovala a v letním období využívá jako úkryty různé lidmi vybudované stavby (půdy, sklepy, doly, tunely, mosty, budky), které jsou preferovány před jinými typy úkrytů zejména při tvorbě mateřských kolonií. Poskytují většinou teplejší prostředí než přirozené úkryty a díky tomu mohou samice v těchto úkrytech vytvářet větší seskupení, více šetří energii a mláďata rychleji rostou (Rodriguez et al. 2003; Lausen & Barclay 2006).

V Moravském krasu a blízkém okolí (oblasti o celkové rozloze 2826 km^2) jsme zjistili, že lidské stavby využívá devět druhů netopýrů, přičemž nejčastěji byly nalézány druhy, které vykazující silnou vazbu na lidské stavby v celé střední Evropě, tzn. *M. myotis*, *R. hipposideros*, *Eptesicus serotinus* a *P. austriacus*. U nejpočetnějšího druhu (*M. myotis*) bylo při kontrolách 187 různých objektů nalezeno celkem 17 mateřských kolonií. Celková početnost byla odhadnuta na 3700 dospělých samic (květen 2002), což je více než třikrát tolik jedinců, kolik bylo v daném roce napočítáno na zimovištích celého Moravského krasu (Pokorný et al. 2003). Podobná struktura společenstva je společná pro všechna stejně intenzivně zkoumaná území České (podhůří Šumavy, Podyjí) i Slovenské republiky (Západní a Východní Slovensko) a představuje tak všeobecný stav (Krátká & Krátký 1985; Lehotská & Lehotský 1998; Danko et al. 2000; Reiter et al. 2003).

Výše zmíněné výsledky se staly základem pro analýzu vybraných charakteristických parametrů úkrytů mateřských kolonií *M. myotis*, které mohou být určující pro jejich výběr. Srovnáním využívaných úkrytů mateřských kolonií a náhodně vybraných, potenciálně vhodných lokalit neosídlených netopýry jsme potvrdili, že samičky jsou vybíravé při obsazování úkrytů (Berková, Pokorný & Zukal 2014). Tato selekce se však projevuje spíše na výběru celkového charakteru lokality včetně okolní krajiny než na úrovni jednotlivých parametrů. Žádný ze strukturálních parametrů úkrytů totiž není významně odlišný od kontrolních lokalit,

převážná většina úkrytů se nacházela v neobydlených budovách (např. kostely, zámky) pravděpodobně díky absenci rušení (Rudolph & Liegl 1990) a budovy obývané koloniemi netopýrů byly spíše samostatně stojící a vyvýšené nad okolní domy. Úkryty se však lišily od kontrolních lokalit spíše ve struktuře a propojenosti okolních biotopů. Větší plocha urbánních biotopů a listnatých resp. smíšených lesů v blízkosti úkrytů a jejich propojení liniovými elementy, ovlivňují významně loveckou aktivitu netopýrů (Bartonička & Zukal 2003) a pravděpodobně i její časování (Jenkins et al. 1998).

Obr. 5 Kostel Jména Panny Marie ve Křtinách – úkryt letní kolonie *M. myotis Zdroj: Wikipedie*

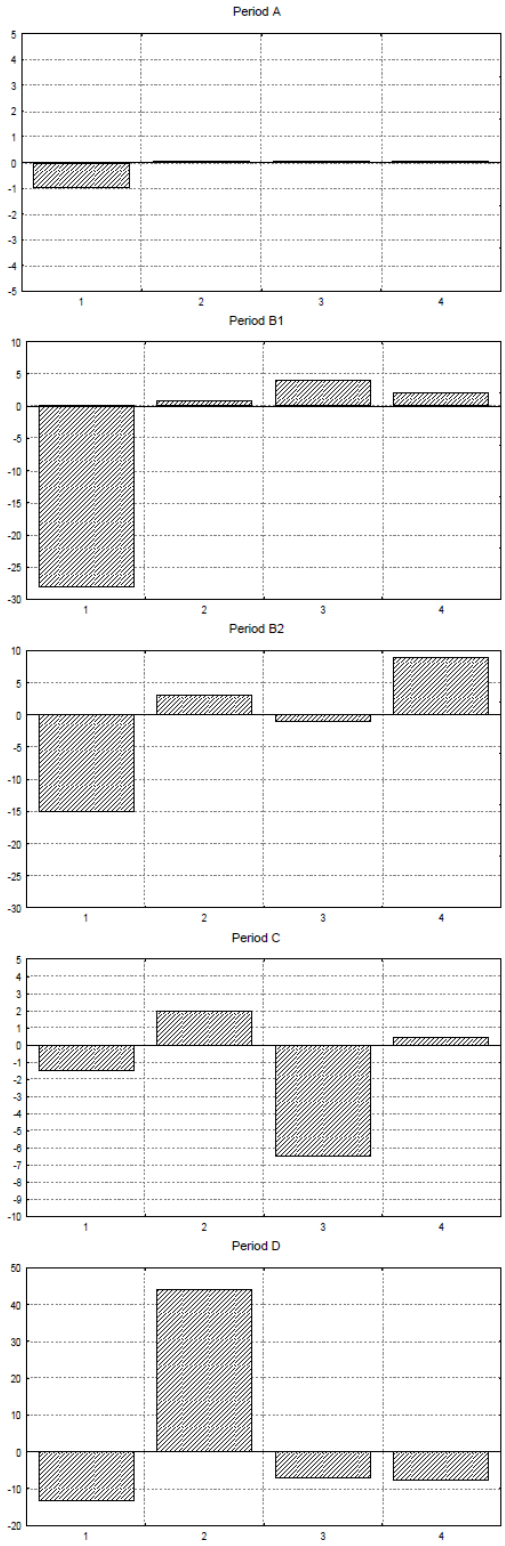
Letová aktivita netopýrů v biotopech Moravského krasu je, i při srovnání s jejich aktivitou v urbánním prostředí města Brna, velmi vysoká. Například aktivita typicky synantropního druhu jako je *E. serotinus*, který je téměř výhradně vázán na úkryty v lidských stavbách (Pokorný et al. 2003) a svou loveckou strategií se významně liší od ostatních druhů netopýrů (Catto et al. 1996), je až trojnásobně vyšší v Moravském krasu než v Brně (Gaisler et al. 1998; Zukal & Řehák 2006). Podobně při srovnání aktivity na nejvyužívanějším typu biotopu, tj. nad vodními plochami, je letová aktivita netopýrů v Moravském krasu asi čtyřikrát vyšší. Je tedy zřejmé, že kombinace dostupnosti vhodných úkrytů a dostatečné potravní nabídky zvyšuje významně loveckou aktivitu netopýrů v dané oblasti (Walsh & Harris 1996).

3.3. Výletová a návratová aktivita u vchodu do úkrytu

Aktivita a lovecké chování netopýrů jsou v oblastech mírného pásma významně ovlivněny sezónními změnami v délce noční části dne a také klimatickými faktory (Erkert 1982). Jejich denní i roční změny proto vyžadují úpravu časování letové aktivity netopýrů tak, aby byla správně synchronizována s časovači těchto rytmů a zůstala téměř výhradně noční (nokturnální). Dlouhodobě jsme proto studovali pomocí infračervené průletové brány výletovou a návratovou aktivitu netopýrů u vchodu do Kateřinské jeskyně, která patří mezi dlouhodobě monitorovaná významná zimoviště (Řehák, Zukal & Kovařík 1994; Zima et al. 1994).

Úroveň letové aktivity netopýrů se mění v závislosti na období roku a na jejím základě jsme definovali pět období ročního cyklu aktivity. Všechna tato období vykazují nenáhodné rozložení letové aktivity netopýrů a její koncentraci v určitém specifickém čase (Berková & Zukal 2006). Navíc její úroveň je také významně ovlivňována klimatickými faktory, a to jak v průběhu sezóny, tak i v průběhu dne. Nicméně, efekt jednotlivých faktorů (roční cyklus života netopýrů vs. klimatické faktory) a úroveň jejich vlivu na variabilitu letové aktivity se během roku mění (Berková & Zukal 2010).

Prvním obdobím je **hibernace** (listopad – začátek března) s velmi nízkou až nulovou aktivitou. Jen zřídka dochází k výletům z jeskyně, přičemž se jedná o netopýry, u nichž došlo k přerušení letargie nejčastěji díky změně podmínek vnějšího prostředí, fyziologickým stavem hibernujícího netopýra (např. dehydratace), případně přímým vyrušením (Speakman & Racey 1989, Thomas 1995). Během hibernace jsou klíčovými exogenními faktory průměrná teplota a rozpětí denních teplot (rozdíl mezi denní maximální a minimální teplotou), které nejlépe predikují celkovou úroveň letové aktivity netopýrů (Ransome 1990, Park et al. 1999). Procento nocí, ve kterých jsme zaznamenali aktivitu, se zvyšuje s rostoucí teplotou a zároveň větší kolísání teploty během dne vyvolává probouzení netopýrů a jejich zvýšenou letovou aktivitu. Nicméně, aktivita netopýrů u vchodu Kateřinské jeskyně byla výjimečně zaznamenána i při teplotách nižších než 0°C (Tmin = -13,2°C) (cf. Boyles, Dunbar & Whitaker 2006). Všechny záznamy byly pozitivní při maximální denní teplotě nad 6.2°C, kdy mohou jedinci některých druhů již i aktivně lovit (Ransome 1990). Rozporuplné jsou názory na úroveň desynchronizace aktivity se západem Slunce a tedy ztráta nokturnality během hibernace. Výsledky výzkumů jsou totiž nekonzistentní, některé podporují desynchronizaci, jiné ji naopak popírají (např. Thomas 1993, 1995; Nagel & Nagel 1997; Park et al. 1999). Naše výsledky však ukazují, že výlety z jeskyně jsou i v zimním období synchronizované se Západem Slunce a aktivita byla soustředěna do doby asi 3 až 3,5 hod po Západu Slunce. Aktivita netopýrů se také nezměnila ani po objevení WNS v Evropě, což naznačuje, že popsaný model hibernačního chování netopýrů včetně změn jejich aktivity, může



Obr. 6 Rozdíl mezi příletovou a výletovou aktivitou ve čtyřech částech noci a definovaných obdobích. Vysvětlivky: pozitivní hodnoty – převaha příletů, negativní hodnoty – převaha výletů, A – hibernace, B1 – pozdní hibernace, B2 – jarní přelety, C – léto a D – podzimní swarming. Převzato z publikace Berková & Zukal 2006.

představovat behaviorální adaptaci, která zabraňuje fatálním dopadům onemocnění, jak je můžeme pozorovat v Severní Americe (Zukal, Berková & Madaraszová submitted).

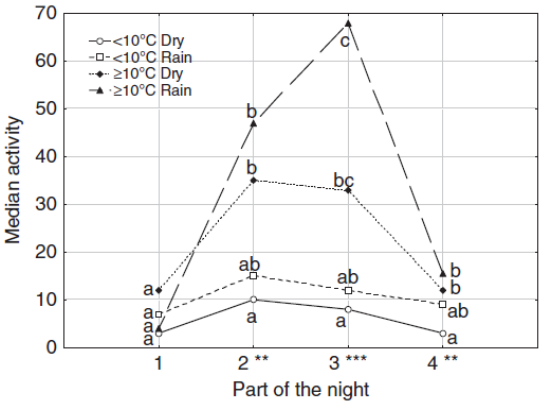
Od začátku března do poloviny dubna trvá druhé období tzv. **pozdní hibernace** s intenzivní výletovou aktivitou na začátku noci. Zároveň je v tomto období poměrně vysoká přeletová aktivita i uvnitř úkrytu a netopýři se pravděpodobně již připravují na opuštění zimoviště (Zukal, Berková & Řehák 2005). Celkově byla letová aktivita netopýrů opět silně pozitivně závislá na průměrné denní teplotě. Netopýři velmi rychle reagovali na změny teploty ze dne na den snížením aktivity při poklesu teploty o více než 2°C nebo zvýšením letové aktivity s teplotou rostoucí o více než 2°C. Tyto rychlé změny v úrovni aktivity jsou možné díky přesunu netopýrů do vchodových částí zimoviště, kde mohou velmi dobře registrovat kolísání venkovní teploty (Ransome 1990; Zukal, Berková & Řehák 2005) a tím i potenciální změny v abundanci hmyzu. Netopýři jsou ovšem schopni lovit i při velmi nízkých teplotách například *Myotis daubentonii* při teplotách až -3,3°C (Ciechanowski et al. 2007), přičemž v období pozdní hibernace byla zjištěna vyšší aktivita při stejných teplotách než v období

hibernace. Dá se tedy předpokládat, že v pozdní hibernaci je již dostupné větší množství potravy, jejíž lov dostatečně kompenzuje ztrátu energie (Andreas 2002), a proto mohou být netopýři nebo alespoň některé druhy netopýrů aktivní.

Relativně vysoká je letová aktivita ještě i v období od poloviny dubna až do začátku června, které je možno označit za **jarní přelety**. Jeskyně je v tomto období využívána jako přechodný úkryt při jarních přeletech a od května pravděpodobně zejména samci, jelikož samice jsou již na letních koloniích. Vysoká výletová aktivita probíhá v 1. čtvrtině noci, ale v poslední části noci se netopýři vrací do jeskyně. I v období jarních přeletů byl zjištěn významný pozitivní vliv průměrné denní teploty a také průměrného denního barometrického tlaku na celkovou letovou aktivitu. Nicméně, množství variability v aktivitě, která může být vysvětlena pomocí klimatických faktorů, je v tomto období nejnižší. To naznačuje, že teplota je již v tomto období dostatečně vysoká a není tedy limitujícím faktorem, nebo že na odlet ze zimoviště mají silný vliv endogenní rytmy (Degn, Andersen & Baagoe 1995; Berková & Zukal 2010). Využití podzemních prostor v jarním období je ovšem odlišné druhově, regionálně, a pravděpodobně závisí i na struktuře úkrytu (Skiba 1987; Park, Jones & Ransome 1999; Perry 2013).

Během **letního období** (polovina června – konec července) je jeskyně využívána velmi málo, přičemž netopýři úkryt navštěvují v průběhu celé noci, tj. před půlnocí vletují do jeskyně a po půlnoci ji zase opouští. Tento typ aktivity nasvědčuje tomu, že malý počet jedinců využívá pravděpodobně jeskyni jako úkryt mezi vrcholy lovecké aktivity nebo jako přechodný denní úkryt (Degn, Andersen & Baagoe 1995; Park, Jones & Ransome 1999). Pro vchody jeskyní je přitom v letním období charakteristické téměř výhradní zastoupení samců (Bauerová & Zima 1988; Whitaker & Rissler 1992), jelikož dospělé samice se v průběhu laktace vracejí mezi vrcholy lovecké aktivity do mateřské kolonie, kde kojí mláďata a noční úkryty využívají jen sporadicky a krátce (např. Anthony, Stack & Kunz 1981; Lučan & Hanák 2011). Na jejich letovou aktivitu ve vchodu do nočního úkrytu neměla vliv absolutní úroveň okolní teploty, ale její kolísání. Čím větší je rozdíl mezi maximální a

minimální denní teplotou, tím vyšší je aktivita. To odpovídá celkovému modelu změn aktivity insektivorních netopýrů v mírném pásmu, který má odrážet aktivitu hmyzu (Erkert 1982). Pokud je abundance denního hmyzu vyšší díky teplejším nocím, může lovecká aktivita netopýrů probíhat celou noc a u vchodu do úkrytu se neobjeví (nízká aktivita), ale během chladnějších nocí (kdy se zvyšuje rozpětí denních teplot) netopýři tráví více času v nočním úkrytu. Lovecká aktivita mimo úkryt je tedy nejvyšší na začátku noci, resp. před Východem Slunce s následným návratem do denního úkrytu (Anthony, Stack & Kunz 1981). Tento model je ještě navíc podpořen v kombinaci s dešťovými srážkami, při kterých je letová aktivita vyšší, a to jak při teplých, tak i chladných nocích (Obr. 7).



Obr. 7 Kombinovaný vliv rozpětí teplot a přítomnosti dešťových srážek na průběh aktivity během noci v letním období. Významné rozdíly mezi částmi noci jsou označeny hvězdičkami a písmena (a, b, c) indikují významné rozdíly mezi skupinami. Vysvětlivky: číslovky označují čtvrtiny noci. Převzato z publikace Berková & Zukal 2010.

Poslední období (konec července – konec října) je charakterizované velmi vysokou letovou aktivitou a typicky se označuje jako **podzimní migrace** případně **„swarming"**. Aktivita netopýrů u vchodů jeskyní se začíná postupně zvyšovat v souvislosti s rozpadem letních kolonií, a proto se zde také začínají objevovat i adultní samice a juvenilní jedinci (např. Horáček & Zima 1978; Řehák, Zukal & Kovařík 1994). Vrchol letové aktivity je posunut až do doby kolem půlnoci, kdy netopýři vlétávají do jeskyně často v malých skupinkách (2 až 12 jedinců). Podzimní aktivita u vchodů jeskyní pravděpodobně umožňuje seznámení juvenilních jedinců s potenciálními zimovišti a setkání jedinců opačného pohlaví, kteří v letním období žijí odděleně (např. Rivers, Butlin & Altringham 2006). Úroveň aktivity je přitom pozitivně ovlivněna maximální teplotou, atmosférickým tlakem i srážkami. To znamená, pokud jsou noci teplé s velkou aktivitou hmyzu (vysoký atmosférický tlak) a netopýři uloví dostatek kořisti, vyhledávají vchody do jeskyní (tzv. swarming site) pro páření s potenciálními partnery a v případě, že začne pršet, netopýři využívají potenciální zimoviště k ukrytí před nepříznivým počasím (Parsons, Jones &

Greenaway 2003; Berková & Zukal 2010).

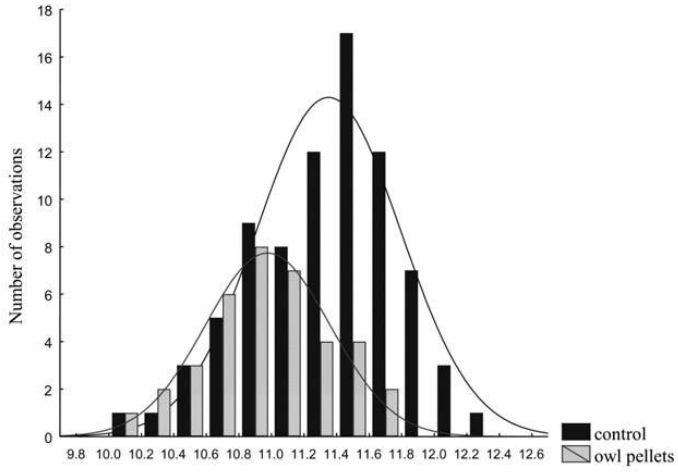
3.4. Antipredační chování během výletové a návratové aktivity

V letním období vytváří samičky všech druhů netopýrů žijících v mírném pásmu tzv. letní kolonie, ve kterých rodí a vychovávají svá mláďata. Velikost kolonií dosahuje od několika jedinců až po několik tisíc podle druhu netopýrů, a kolonie využívají jako úkryty různé tmavé prostory jako jsou jeskyně, půdy budov, hluboké štěrbiny ve skalách i na budovách, dutiny stromů apod. (Kunz 1982). Život v kolonii má řadu výhod (např. účinnější termoregulaci, transfer informací), ale i nevýhod, z nichž tou nejdůležitější je zvýšení rizika predace. Větší seskupení netopýrů jsou „viditelnější" pro predátory (Speakman 1991; Fenton et al. 1994), a proto je predace netopýrů dravci považována za významný evolučně selektivní faktor. Ovlivnila pravděpodobně vznik nokturnality u netopýrů a ovlivňuje také časování a model výletové aktivity netopýrů (Speakman, Stone & Kerslake 1995; Speakman 2001). Správné načasování večerního výletu totiž může významně ovlivnit fitness netopýrů. Pokud vyletí příliš pozdě, mohou netopýři minout vrchol aktivity a abundance létajícího hmyzu, ale příliš brzký výlet zvyšuje významně riziko predace (Rydell, Entwistle & Racey 1996).

Náš výzkum se zaměřil právě na toto kritické období, tedy na opouštění úkrytu (výlet) resp. na návrat do úkrytu (přílet), kdy jsou netopýři ohroženi zejména dravci a částečně i sovami. K výletu netopýrů z úkrytu kolonie dochází po relativně dlouhém období takzvané předletové aktivity (až 3 hodiny), která je spojena s výraznými hlasovými projevy (McAney & Fairley 1988) a zahrnuje zejména intenzivní čištění a zvýšenou pohybovou aktivitou (Burnett & August 1981, Zukal 1994). Před zahájením výletu bylo často pozorováno takzvané průzkumové chování (light sampling), kdy se netopýři přesunují z tmavých míst úkrytu do oblasti východu (u druhů štěrbinových) nebo konají krátké výlety z úkrytu s okamžitým návratem (Gaisler 1963). Tímto způsobem zjišťují netopýři aktuální intenzitu světla, přičemž existují druhově specifické rozdíly v množství světla nutného k odstartování výletu. Doposud nebylo potvrzeno, zda případně tento typ chování souvisí i se

zjišťováním přítomnosti predátora v okolí úkrytu a tedy s hodnocením míry rizika predace (Jones & Rydell 1994).

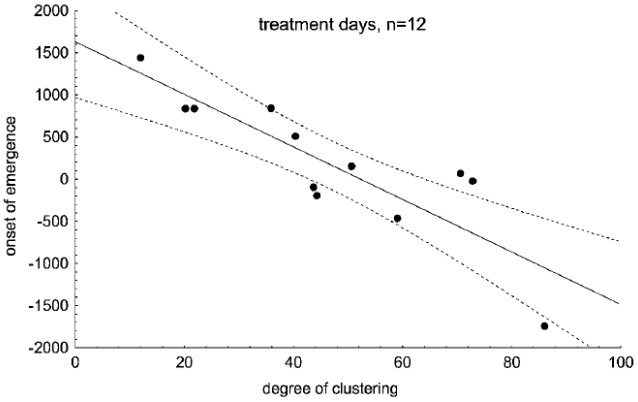
Začátek, medián i konec výletu jsou velmi silně korelovány se Západem Slunce a tyto parametry se posunují paralelně se změnou doby Západu Slunce během roku (Petrželková & Zukal 2001). Obecně je však model výletové aktivity v jednotlivých letech velmi stabilní a také je druhově specifický, například studovaný modelový druh *E. serotinus* začíná vylétat na lov velmi krátce po Západu Slunce (5 až 20 minut po Západu Slunce) na rozdíl od *P. pipistrellus* a *M. daubentonii*, jež opouštějí úkryty později (15 až 30 minut po Západu Slulnce) (Rydell, Entwistle & Racey 1996). Výletovou aktivitu samic netopýrů z jejich letních úkrytů ovšem ovlivňuje reprodukční období, během nějž se mění celková délka výletu. V laktačním a postlaktačním období, kdy jsou již vzletná tohoroční mláďata, je výlet netopýrů z úkrytu kolonie delší, a to výhradně posunem konce výletu. Vzletná mláďata, která jsou v této době již téměř stejně velká jako dospělci, jsou přitom preferována lovícími sovami (Petrželková, Obuch & Zukal 2004), jelikož nemají dostatek zkušeností. Jsou méně obratná v letu, a proto jsou pro predátory snadnější kořistí zejména během výletu z úkrytu kolonie nebo krátce po něm, kdy se jejich letová aktivita soustřeďuje do blízkosti úkrytu.



Obr. 8 Histogram hodnot rozměrů IM_3 u kontrolní skupiny lebek netopýra velkého *M. myotis* a u skupiny z vývržků. Sovy preferovali menší jedince pravděpodobně vzletná mláďata. Vysvětlivky: number of observations – počet pozorování, control – kontrolní soubor, owl pellets – soví vývržky. Převzato z publikace Petrželková, Obuch & Zukal 2004.

Časování výletového chování je ovlivňováno také takzvanou nárazovou aktivitou (outbrust activity) (Speakman et al. 1992), kdy netopýři vylétají z kolonie rychle za sebou v určitých skupinách oddělených časovou mezerou, během které vylétá pouze malý počet jedinců. Výsledky výzkumu kolonie *E. serotinus* potvrdily, že nárazová aktivita se vyskytuje téměř ve všech výletech sledované kolonie.

Přítomnost makety dvou predátorů (*Tyto alba* nebo *Falco tinnunculus*) spolu s playbackem jejich hlasů či živého ptačího predátora (*T. alba*) během výletu mateřské kolonie netopýrů vyvolávala změny ve vztazích mezi jednotlivými výletovými parametry. V přítomnosti potenciálního predátora úroveň nárazové aktivity silně korelovala s většinou sledovaných parametrů výletu (začátek, konec, medián či délka výletu, intenzita světla apod.) na rozdíl od výletů během kontrolních nocí bez přítomnosti predátora, kdy žádná korelace zaznamenána nebyla. Zásadním zjištěním však je, že dřívější výlet kolonie způsobil i zvýšení nárazové aktivity, čímž se snižuje riziko jedince být napaden predátorem za relativně vysoké intenzity světla. Nárazová aktivita může být tedy považována za důležitou anti-predační strategii během výletu netopýrů z úkrytu (Speakman, Stone & Kerslake 1995; Petrželková & Zukal 2001 a 2003).



Obr. 9 Korelace mezi začátkem výletu netopýrů z úkrytu (onset of emergence) a úrovní jejich shlukování během výletu (degrese of clustering) ve dnech, kdy byla použita maketa predátora. Netopýři zvyšovali úroveň shlukování v případě, že vylétávali dříve a tedy za větší intenzity světla, kdy jsou ohroženější predátory. Převzato z publikace Petrželková & Zukal 2001.

Využití nárazové aktivity netopýry při výletu také umožňuje výlet z úkrytu za vyšších světelných intenzit, než při kterých probíhá návrat do úkrytu. Časové parametry výletu jsou tedy posunuty blíže k Západu Slunce než parametry návratu vzhledem k Východu Slunce. Návratová aktivita netopýrů je totiž mnohem zřetelnější pro predátory, jelikož netopýři se postupně shlukují před vchodem do úkrytu a opakovaně se snaží dostat do jeho vchodu (tzv. swarming). Brzký přílet netopýrů do úkrytu za nižších světelných intenzit tedy snižuje riziko jejich napadení ze strany denních dravců a představuje pravděpodobně další anti-predační adaptaci netopýrů během jejich letové aktivity (Petrželková et al. 2006).

3.5. Těžké kovy a syndrom bílého nosu – potenciální hrozby pro populace netopýrů

Netopýři jsou v současnosti ohroženi řadou negativních vlivů, od těch tradičních jako je ničení přirozených biotopů a úkrytů (zejména díky odlesňování a rekonstrukcím budov), až po chemické kontaminanty, světelné znečištění, syndrom bílého nosu nebo kolize s větrnými elektrárnami. Většina těchto hrozeb pro netopýry je přímo spojena s celosvětově se zvyšující početností lidské populace a jejím vlivem na životní prostředí. Jejich dopady na populace netopýrů ovšem nejsou vždy zcela zřejmé (např. postupné snížení porodnosti) a okamžité (např. dlouhodobé působení subletálních dávek). Teprve dlouhodobá expozice a působení určitého faktoru, nebo spolupůsobení různých negativních vlivů mohou nakonec vést k významnému úbytku početnosti netopýrů, jelikož dojde k překročení kritické hranice daného vlivu. Podobnými riziky jsou i námi studované účinky toxických polutantů (např. těžké kovy) a specifických patogenů (např. syndrom bílého nosu).

Netopýři jsou považováni s ohledem na řadu svých ekologických charakteristik (rychlý metabolismus, hibernace, synantropizace, dlouhý věk, rozmanitost využívaných úkrytů a potravních zdrojů atd.) za vhodné bioindikátory změn životního prostředí (Jones et al. 2009), včetně jeho zatížení emisemi těžkých kovů. Navíc úkryty letních kolonií netopýrů představují ideální místa pro analýzu přítomnosti kontaminantů v potravním řetězci, jelikož netopýři loví v širokém okolí úkrytů, na kterých se následně shromažďují (Clark 1988). Na druhou stranu je zde celosvětová přísná ochrana všech druhů netopýrů (Mickleburgh, Hutson & Racey 2002), která, s ohledem na velikost vzorků nutných k chemické analýze, ekotoxikologický výzkum limituje.

V poslední době se však díky využití přesných moderních přístrojů a neletálních metod odběru vzorků, včetně analýz guána nebo nalezených mrtvých jedinců, zvýšil zájem o výzkum zátěže netopýřích populací těžkými kovy, a to zejména v Severní Americe a Evropě (Zukal, Banďouchová & Pikula 2015). Přesto naše meta-analýza doposud publikovaných dat ukázala, že výsledky výzkumů jsou stále velmi omezené. Těžké kovy sice byly studovány u 65 druhů netopýrů, ale

pouze čtyři druhy netopýrů (*Eptesicus fuscus*, *Myotis grisescens*, *M. myotis* a *P. pipistrellus* sensu lato) byly analyzovány více než pětkrát. Obsah těžkých kovů přitom je velmi variabilní a liší se mezi druhy, pohlavími, věkovými skupinami, roky odběru nebo lokalitami, a to bez zjevného vztahu k jednotlivým faktorům. Obecně však platí, že insektivorní druhy mají celkově nižší hodnoty zátěže než fruktivorní a nektarivorní druhy netopýry, ale také než vzorky guána. Podobně velká variabilita vlivem různých faktorů byla zjištěna i u zátěže organickými polutanty (Bayat et al. 2013).

V České republice je situace velmi podobná jako v celé Evropě, přičemž náš výzkum potvrdil, že jedinci většiny z jedenácti druhů netopýrů nalezených na lokalitách v Moravském krasu a okolí (tj. *M. myotis*, *M. daubentonii*, *M. brandtii*, *M. nattereri*, *M. emarginatus*, *M. mystacinus*, *P. pipistrellus, Pipistrellus nathusii*, *Pipistrellus pygmaeus*, *N. noctulla* a *E. serotinus*) byli vystaveni expozici těžkými kovy (Pikula at el. 2010). Analýza obsahu metallothioneinu a tří těžkých kovů (olovo, kadmium a zinek), z nichž první dva patří mezi jedenáct těžkých kovů s nejvyšším rizikem pro životní prostředí, ukázala vysokou variabilitu mezi pohlavími i věkovými skupinami. Navíc poprvé výzkum prokázal rozdíly také mezi skupinami netopýrů s rozdílnou loveckou strategií. U druhů, které jsou při lovu vázány na vodní biotopy např. *P. pipistrellus* (Zukal & Řehák 2006), byly zjištěny nejvyšší hodnoty metallothioneinu a zároveň prakticky nulové hodnoty kadmia na rozdíl od druhů lovících pouze v terestrických biotopech nebo druhů, které loví v obou typech biotopů. Zátěž těžkými kovy tedy představuje pro některé skupiny netopýrů vysoké riziko, jehož negativní důsledky se mohou objevit teprve v okamžiku, kdy se u dané skupiny střetne několik stresových faktorů, které způsobí jejich úhyn.

Syndrom bílého nosu (white-nose syndrome) je plísňové onemocnění, které již 8 let decimuje populace některých druhů netopýrů na východě USA a Kanady (Cryan et al. 2013). V únoru 2006 vyfotografoval americký speleolog ve veřejně přístupné jeskyni (Howe Caverns) asi 50 km od města Albany ve státě New York přezimující jedince *Myotis lucifugus* s nápadně bílými povlaky na uších, křídlech a zadních končetinách (Blehert et al. 2009). Mnozí z nich jevili atypické chování, zmateně

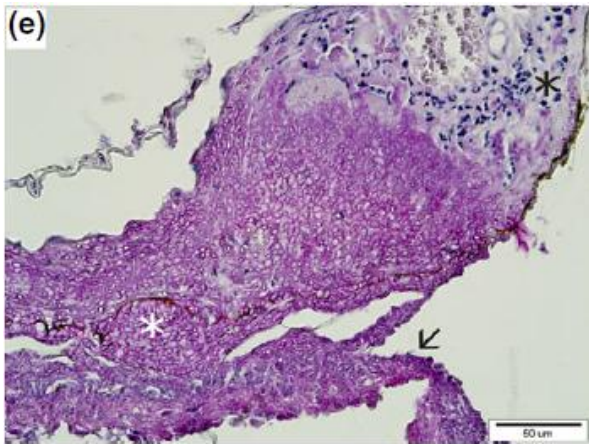
poletovali i mimo úkryt a někteří leželi mrtví na zemi, hlavně poblíž jeskynních vchodů. Onemocnění se následně začalo rychle epizooticky šířit po hlavních zimovištích na severovýchodě USA a v Kanadě (Turner, Reeder & Coleman 2011), přičemž matematické modely predikovaly, že k vyhynutí některých lokálních populací *Myotis lucifugus*, jednoho z nejpočetnějších druhů netopýrů severní Ameriky, může dojít za méně než 20 let (Frick et al. 2010). Již v roce 2009 byl popsán a potvrzen jediný patogen, který onemocnění způsobuje, nový druh plísně *Geomyces destructans* (Gargas et al. 2009), která byla později překlasifikována na *Pseudogymnoascus destructans* (Minnis & Lindner 2013). Předpokládá se, že tato plíseň byla introdukována do severní Ameriky z Evropy (Turner et al. 2011). Plíseň poškozuje severoamerickým netopýrům zejména létací blánu, což vede k dehydrataci netopýrů a také ke ztrátě elektrolytů nutných pro funkční fyziologické pochody během hibernace. Ztráta homeostatické rovnováhy způsobuje častější probouzení netopýrů ze zimního spánku a předčasné spotřebování tukových zásob nutných k úspěšnému přežití celého období hibernace. Netopýři umírají na celkové vyčerpání organizmu (Reeder et al. 2012).

Obr. 10 Jedinec *Myotis lucifugus* napadený plísní *Pseudogymnoascus destructans*. Převzato z publikace Blehert et al. 2009.

V Evropě je situace podobná jako v severní Americe, s jediným rozdílem, nedochází zde prozatím k masovému úhynu (Puechmaille et al. 2011a). První nález netopýra s nárůstem plísně *P. destructans* byl zaznamenán 12. března 2009 na zimovišti v jeskyni u města Périgueux ve Francii, kde byl nalezen jedinec *M. myotis* s plísní na stejných obličejových partiích jako u postižených amerických netopýrů. Vykultivovaná plíseň odpovídala morfologicky nálezům z USA (Puechmaille et al. 2010). Naše výzkumy realizované na zimovištích v Moravském krasu potvrdily v Evropě jak přítomnost patogenu tj. plísně *Pseudogymnoascus destructans* (Martínková et al. 2010), která onemocnění způsobuje, tak histopatologicky i přítomnost vlastního syndromu bílého nosu (Pikula et al. 2012). Využití nové metody UV detekce lézí, tj. poškozených částí létacích blan (Turner et al. 2014), nám umožnilo nalezení

pozitivních jedinců u 11 druhů evropských netopýrů. U řady z nich přitom léze prorůstaly celou tloušťkou létací blány a způsobovaly tedy stejně závažné onemocnění jako je tomu u netopýrů v severní Americe (Banďouchová et al 2014).

Jedním z možných vysvětlení proč evropští netopýři masově nehynou na syndrom bílého nosu, je jejich dřívější historická expozice tomuto onemocnění, která vedla k vytvoření ochranné adaptace. Výzkum hibernačního chování netopýrů z Moravského krasu naznačuje, že evropští netopýři využívají specifické hibernační chování, které představuje výhodnou evoluční adaptaci, jež jim umožňuje přežívat napadení plísní *Pseudogymnoascus destructans* (Zukal, Berková & Madaraszová submitted). Zároveň však naše dosavadní výzkumy také dokazují, že plíseň může napadnout jakýkoliv druh netopýra, který využívá pro hibernaci podzemní prostory s mikroklimatickými podmínkami, které jsou vhodné pro růst plísně (Zukal et al. 2014). Syndrom bílého nosu je proto rozšířen prakticky po celé Evropě (Puechmaille et al. 2011b), a přestože nebyla doposud v Evropě zaznamenána masivní mortalita zimujících netopýrů, potvrzuje se, že většina evropských druhů netopýrů je stále potenciálně v ohrožení.



Obr. 11 Léze létací blány u netopýra druhu Plecotus auritus s pozitivním nálezem onemocnění syndrom bílého nosu. Extenzivní plísňová infekce, která prorůstá celou tloušťkou létací blány. Vysvětlivky: černá hvězdička – příznaky zánětu, bílá hvězdička – cupping-like eroze kůže a šipka – zbytky buněk na povrchu kůže. Převzato z publikace Banďouchová et al. 2014.

4. Závěr

Ve shrnujícím komentáři přiložených publikací jsem se snažil ukázat, že i přes intenzivní rozvoj moderních technologií a metod výzkumu, zůstávají úkryty netopýrů stále prostorem, kde je možné studovat tak pohyblivá zvířata jako jsou právě netopýři. Přímo v jejich úkrytech nebo ve vchodech do těchto úkrytů získáváme s vysokou pravděpodobností dostatečný materiál pro testování obecných hypotéz jako je například vztah predátor – kořist nebo specifických parametrů života netopýrů, například hibernace, ale dají se zde řešit i problémy, které odrážejí životní strategie jednotlivých druhů. Oblast Moravského krasu je přitom ideálním prostředím pro netopýry s dostatkem přirozených úkrytů a tedy i s velkým potenciálem pro výzkum jejich ekologie a chování.

5. Literatura

Altringham J.D., 1996: Bats. Biology and Behaviour. Oxford University Press, Oxford. 262 pp.

Andreas M., 2002: Potravní ekologie společenstva netopýrů. Dizertační práce. Česká zemědělská univerzita, Praha. 163 pp.

Anthony E.L.P., Stack M.H. & Kunz T.H., 1981: Night roosting and the nocturnal time budget of the little brown bat, *Myotis lucifugus*: effects of reproductive status, prey density, and environmental conditions. Oecologia 51: 151-156.

Banďouchová H., Bartonička T., Berková H., Brichta J., Černý J., Kováčová V., Kolařík M., Köllner B., Kulich P., Martínková N., Řehák Z., Turner G.G., Zukal J. & Pikula J., 2015: *Pseudogymnoascus destructans*: Evidence of Virulent Skin Invasion for Bats Under Natural Conditions, Europe. Transboundary and Emerging Diseases, 62: 1-5.

Bárta Z., Červený J., Gaisler J., Hanák P., Hanák V., Horáček I., Hůrka L., Miles P., Nevrlý M., Rumler Z., Sklenář J. & Žalman J., 1981: Výsledky zimního sčítání netopýrů v Československu: 1969-1979. Sborník Okresního Muzea v Mostě, řada přírodovědná 3: 71-116.

Battersby J. (comp.), 2010: Guidelines for Surveillance and Monitoring of European Bats. EUROBATS Publication Series No. 5. UNEP/EUROBATS Secretariat, Bonn. 95 pp.

Bayat S., Geiser F., Kristiansen P. & Wilson S.C., 2014: Organic contaminants in bats: Trends and new issues. Environment International 63: 40–52.

Bartonička T. & Zukal J., 2003: Flight activity and habitat use of four bat species in a small town revealed by bat detectors. Folia Zoologica 52: 155-166.

Bauerová Z. & Zima J., 1988: Seasonal changes in visits to a cave by bats. Folia Zoologica 37: 97-111.

Berková H., Pokorný M. & Zukal J., 2014: Selection of Buildings as Maternity Roosts by Greater Mouse-eared Bats (*Myotis myotis*). Journal of Mammalogy 95, in print.

Berková H. & Zukal J., 2006: Flight activity of bats at the entrance of a natural cave. Acta Chiropterologica 8: 187-195.

Berková H. & Zukal J., 2010: Cave visitation by temperate zone bats: effects of climatic factors. Journal of Zoology, London 280: 387-395.

Blehert D.S., Hicks A.C., Behr M., Meteyer C.U., Berlowski-Zier B.M., Buckles E.L., Coleman J.T.H., Darling S.R., Gargas A., Niver R., Okoniewski J.C., Rudd R.J. & Stone W.B., 2009: Bat white-nose syndrome: an emerging fungal pathogen? Science 323: 227.

Bogdanowicz W., Van Den Bussche R.A., Gajewska M., Postawa T. & Harutyunyan M., 2009: Ancient and contemporary DNA sheds light on the history of mouse-eared bats in Europe and the Caucasus. Acta Chiropterologica 11: 289–305.

Boyles J.G., Dunbar M.B. & Whitaker Jr. J.O., 2006: A review of activity following

arousal in winter in North American vespertilionid bats. Mammal Review 36: 267–280.

Brosset A. & Poillet A., 1985: Structure d´une population hibernante de grands rhinolophes *Rhinolophus ferrumequinum* dans l´est de la France. Mammalia 49: 221-233.

Bryja J., Uhrin M., Kaňuch P., Bémová P., Martínková N. & Zukal J., 2010: Mitochondrial DNA confirms low genetic variation of the greater mouse-eared bats, *Myotis myotis*, in Central Europe. Acta Chiropterologica 12: 73-81.

Burnett C.D. & August P.V., 1981: Time and energy budgets for day roosting in a maternity colony of *Myotis lucifugus*. Journal of Mammalogy 62: 758-766.

Catto C.M.C., Hutson A.M., Racey P.A. & Stephenson P.J., 1996: Foraging behaviour and habitat use of the serotine bat (*Eptesicus serotinus*) in southern England. Journal of Zoology, London 238: 623–633.

Ciechanowski M., Zajac T., Bilas A. & Dunajski R., 2007: Spatiotemporal variation in activity of bat species differing in hunting tactics: effects of weather, moonlight, food abundance, and structural clutter. Canadian Journal of Zoology 85: 1249-1263.

Clark Jr. D.R., 1988: Environmental contaminants and management of bat populations in the United States. 409-413. In R.C. Czaro, Tech. Coord. Management of amphibians, reptiles and small mammals in North America. Proc. of Symposium, U.S. Forest Service, Ft. Collins, CO. Gen. Tech. Rept. RM-166.

Cryan P.M., Meteyer C.U., Boyles J.G & Blehert D.S., 2013: White-nose syndrome in bats: illuminating the darkness. BMC Biology 11: 47.

Danko Š., Uhrin M., Pjenčák P. & Matis Š., 2000: Netopiere Východoslovenskej roviny, Východoslovenskej pahorkatiny a Zemplínských vrchov. Vespertilio 4: 37-58.

Degn H.J., Andersen B.B. & Baagoe H., 1995: Automatic registration of bat activity through the year at Monsted Limestone Mine, Denmark. Zeitschrift für Säugetierkunde 60: 129-135.

Erkert H.G., 1982: Ecological aspects of bat activity rhythms. 201-242. In: Kunz T.H., ed., Ecology of bats. Plenum Press, New York.

Fenton M.B., Rautenbach I.L., Smith S.E., Swanepoel C.M., Grosell J. & van Jaarsveld J., 1994: Raptors and bats: threats and opportunities. Animal Behaviour 48: 9-18.

Findley J.S., 1993: Bats: A Community perspective. Cambridge University Press, Cambridge. 167 pp.

Frick W.F., Pollock J.F., Hicks A.C., Langwig K.E., Reynolds D.S., Turner G.G., Butchkoski C.M. & Kunz T.H., 2010: An emerging disease causes regional population collapse of a North American bat species. Science 329: 679–682.

Gaisler J., 1963: Nocturnal activity in the lesser horseshoe bat, *Rhinolophus hipposideros* (Bechstein, 1800). Zoologické listy 12: 223-230.

Gaisler J., Řehák Z. & Zukal J., 2006: Výzkum netopýrů v Moravském krasu: historie a současnost. Vespertilio 9-10: 75-85.

Gaisler J., Zukal J., Řehák Z. & Homolka M., 1998: Habitat preference and flight activity of bats in a city. Journal of Zoology, London 244: 439-445.

Gargas A., Trest M.T., Christensen M., Volk T.J. & Blehert D.S., 2009: *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. Mycotaxon 108: 147–154.

Hanzal V. & Průcha M., 1988: Sezónní dynamika netopýřích společenstev na zimovištích Českého krasu v letech 1984-1986. Lynx (Praha), n.s. 24: 15-35.

Horáček I., Hanák V. & Gaisler J., 2005: Dlouhodobé změny biodiversity netopýrů: zpráva o nejrozsáhlejším monitorovacím programu v ČR 1969–2004. 105–115. In: Vačkář D. (ed.), Ukazatele změn biodiverzity, Academia, Praha.

Horáček I. & Zima J., 1978: Net-revealed cave visitation and cave-dwelling on European bats. Folia Zoologica 27: 135 - 148.

Jenkins E.V., Laine T., Morgan S.E., Cole K.R. & Speakman J.R., 1998: Roost selection in the pipistrelle bat, *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae), in northeast Scotland. Animal Behaviour 56: 909-917

Jones G., Jacobs D.S., Kunz T.H., Willig M.R. & Racey P.A., 2009: Carpe noctem: the importance of bats as bioindicators. Endangered Species Research 8: 93–115.

Jones G. & Rydell J., 1994: Foraging strategy and predation risk as factors influences emergence time in echolocating bats. Philosophical Transactions of the Royal Society of London Series B 346 : 445-455.

Kolenati F.A., 1860: Monographie der europäischen Chiropteren. Jarhesh. natw. Sect. k.k. Mähr.-Schl. Gesellsch. Förd. Ackerbaues, Brünn. 156 pp.

Kovařík M., Zukal J. & Řehák Z., 2000: Aktivita netopýrů v Moravském krasu. Estavela 2: 5-6.

Krátká D. & Krátký J., 1985: Letní výskyt netopýrů (Chiroptera) vázaných na lidské stavby na Šumavě III. Prachaticko, Strakonicko, Českokrumlovsko. Zpravodaj Muzeí Západočeského Kraje – Příroda 30-31: 69-78.

Kunz T.H. (ed.), 1982: Ecology of bats. Plenum Press, New York. 425 pp.

Kunz T.H. & Parsons S. (eds.), 2009: Ecological and Behavioral Methods for the Study of Bats. The Jonhs Hopkins University Press, Baltimore. 901 pp.

Lausen C.L. & Barclay R.M.R., 2006: Benefits of living in a building: big brown bats (Eptesicus fuscus) in rocks versus buildings. Journal of Mammalogy 87: 362-370.

Lehotská B. & Lehotský R., 1998: Výskyt letných kolónií netopierov na území západného Slovenska. Vespertilio 3: 57-64.

Lučan R. & Hanák V., 2011: Population structure of Daubenton's bats is responding to microclimate of anthropogenic roosts. Biologia 66: 690-695.

Martínková N., Bačkor P., Bartonička T., Blažková P., Červený J., Falteisek L., Gaisler J., Hanzal V., Horáček D., Hubálek Z., Jahelková H., Kolařík M., Korytár L.,

Kubátová A., Lehotská B., Lehotský R., Lučan R., Májek O., Matějů J., Řehák Z., Šafář J., Tájek P., Tkadlec E., Uhrin M., Wagner J., Weinfurtová D., Zima J., Zukal J. & Horáček I., 2010: Increasing Incidence of *Geomyces destructans* Fungus in Bats from the Czech Republic and Slovakia. PLoS ONE 5: e13853

McAney C.M. & Fairley J.S., 1988: Activity patterns of the lesser horseshoe bat *Rhinolophus hipposideros* at summer roosts. Journal of Zoology, London 216: 325-338.

Mickleburgh S.P., Hutson A.M. & Racey P.A., 2002: A review of the global conservation status of bats. Oryx 36: 18–34.

Minnis A.M. & Lindner D.L. 2013: Phylogenetic evaluation of *Geomyces* and allit reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. Fungal Biology 117: 638–649.

Nagel A. & Nagel R., 1989: Bestandsentwicklung winterschlafender Fledermäuse auf der Schwäbischen Alb bis zum Winter 1987/88 und ihr Schutz. Mitt. Verb. dt. Höhlen- und Karstforsch. 35: 17-23.

Nagel A. & Nagel R., 1997: Nutzung eines Untertagequartieres durch die Kleine Hufeisennase (*Rhinolophus hipposideros*). Tagungsband: „Zur Situation der Hufeisennasen in Europa" Nebra, den 26. - 28. Mai 1995, Arbeitskreis Fledermäuse Sachsen-Anhalt e.V. 1997: 97-108.

O'Shea T.J. & Bogan M.A. (eds.), 2003: Monitoring trends in bat populations of the United States and territories: problems and prospects. U.S. Geological Survey, Biological Resources Discipline, Information and Technology Report, USGS/BRD/ITR--2003–0003. 274 pp.

Park J.K., Jones G. & Ransome R.D., 1999: Winter activity of a population of greater horseshoe bats (*Rhinolophus ferrumequinum*). Journal of Zoology, London 248: 419-427.

Parsons K.N., Jones G. & Greenaway F., 2003: Swarming activity of temperate zone microchiropteran bats: effects of season, time of night and weather conditions. Journal of Zoology, London 261: 257-264.

Perry R.W., 2013: A review of factors affecting cave climates for hibernating bats in temperate North America. Environmental Research 21: 28-39.

Petrželková K., Downs N.C., Zukal J. & Racey P.A., 2006: A comparison between emergence and return activity in pipistrelle bats Pipistrellus pipistrellus and P. pygmaeus. Acta Chiropterologica 8: 381-390.

Petrželková K., Obuch J. & Zukal J., 2004: Does the barn owl (*Tyto alba*) selectively predate individual great mouse-eared bats (*Myotis myotis*)? Lynx (Praha) n.s. 35: 123-132.

Petrželková K. & Zukal J., 2001: Emergence behaviour of the serotine bat (Eptesicus serotinus) under predation risk. Netherlands Journal of Zoology 51: 395-414.

Petrželková K. & Zukal J., 2003: Does a live barn owl (*Tyto alba*) affect emergence

behavior of serotine bats (*Eptesicus serotinus*)? Acta Chiropterologica 5: 177-184.
Piksa K. & Nowak J., 2013: The bat fauna hibernating in the caves of the Polish Tatra Mountains, and its long-term changes. Central European Journal of Biology 8: 448-460.
Pikula J., Banďouchová H., Novotný L., Meteyer C.U., Zukal J., Irwin N.R., Zima J. & Martínková N., 2012: Histopathology Confirms White-Nose Syndrome in Bats in Europe. Journal of Wildlife Diseases 48: 207-211.
Pikula J., Zukal J., Adam V., Banďouchová H., Beklová M., Hájková P., Horáková J., Kížek R. & Valentíková L., 2010: Heavy metals and metallothionein in vespertilionid bats foraging over aquatic habitats in the Czech Republic. Environmental Toxicology and Chemistry 29: 501-506.
Pokorný M., Berková H., Gaisler J., Řehák Z. & Zukal J., 2003: Letní výskyt netopýrů v lidských stavbách v Moravském krasu a širším okolí. Vespertilio 7: 161-168.
Presetnik P., Podgorelec M. & Šalamun A., 2013: Odkup in obdelava podatkov monitoringa populacij izbranih ciljnih vrst netopirjev za leto 2013 (Končno poročilo). Center za kartografijo favne in flore, Miklavž na Dravskem polju. 110 pp.
Primack R.B., 2010: Essentials of Conservation Biology, Sinauer Associates, Inc. Publishers, Sunderland. 601 pp.
Puechmaille S.J., Frick W.F., Kunz T.H., Racey P.A., Voigt C.C., Wibbelt G. & Teeling E.C., 2011a: White-nose syndrome: is this emerging disease a threat to European bats? Trends in Ecology and Evolution 26: 570–576.
Puechmaille S.J., Verdeyroux P., Fuller H., Ar Gouilh M., Bekaert M. & Teeling E.C., 2010: White-Nose Syndrome Fungus (*Geomyces destructans*) in Bat, France. Emerging Infectious Diseases 16: 290-293.
Puechmaille S.J., Wibbelt G., Korn V., Fuller H., Forget F., Mühldorfer K., Kurth A., Bogdanowicz W., Borel C., Bosch T., Cherezy T., Drebet M., Görföl T., Haarsma A.J., Herhaus F., Hallart G., Hammer M., Jungmann C., Le Bris Y., Lutsar L., Masing M., Mulkens B., Passior K., Starrach M., Wojtaszewski A., Zöphel U. & Teeling E.C., 2011b: Pan-European Distribution of White-Nose Syndrome Fungus (*Geomyces destructans*) Not Associated with Mass Mortality. PLoS ONE 6: e19167.
Ransome R.D., 1990: The natural history of hibernating bats. Christopher Helm, London. 256 pp.
Reeder D.M., Frank C.L., Turner G.G., Meteyer C.U., Kurta A., Britzke E.R., Vodzak M.E., Darling S.R., Stihler C.W., Hicks A.C., Jacob R., Grieneisen L.E., Brownlee S.A., Muller L.K. & Blehert D.S., 2012: Frequent Arousal from Hibernation Linked to Severity of Infection and Mortality in Bats with White-Nose Syndrome. PLoS ONE 7: e38920.
Reiter A., Hanák V., Benda P. & Barčiová L., 2003: Netopýři (Chiroptera) jihozápadní Moravy. Lynx (Praha), n.s. 34: 79-180.

Rivers N.M., Butlin R.K. & Altringham J.D., 2006: Autumn swarming behaviour of Natterer´s bats in the UK: Population size, catchment area and dispersal. Biol. Conserv. 27: 215-226.

Rodrigues L., Zahn A., Rainho A.& Palmeirim J.M., 2003: Contrasting the roosting behaviour and phenology of an insectivorous bat (*Myotis myotis*) in its southern and northern distribution ranges. Mammalia 67: 321-335.

Rudolph B.U. & Liegl A., 1990: Sommerverbreitung und Siedlungsdichte des Mausohrs (*Myotis myotis*) in Nordbayern. Myotis 28: 19-38.

Rydell J., Entwistle A. & Racey P.A., 1996: Timing of foraging flights of three species of bats in relation to insect activity and predation risk. Oikos 76: 243–252.

Řehák Z., 1997: Trendy ve vývoji početnosti netopýrů ve střední Evropě. Vespertilio 2: 81–96.

Řehák Z., Zukal J. & Kovařík M., 1994: Long- and short-term changes in the bat community of the Kateřinská cave (Moravian Karst) - a fundamental assessment. Folia Zoologica 43: 425-436.

Saint Girons H., Brosset A. & Saint Girons M. C., 1969: Contribution a la connaissance du cycle annuel de la chauve-souris, *Rhinolophus ferrumequinum* (Schreber, 1774). Mammalia 33: 357-470.

Skiba R., 1987: Bestandsentwicklung und Verhalten von Fledermäusen in einem Stollen des Westharzes. Myotis 25: 95-103.

Speakman J.R., 1991: The impact of predation by birds on bat population in the British Isles. Mammal Review 21: 123-142.

Speakman J.R., 2001: The evolution of flight and echolocation in bats: another leap in the dark. Mammal Review 31: 111–130.

Speakman J.R., Bullock D.J., Eales L.A. & Racey P.A., 1992: A problem defining temporal pattern in animal behaviour: clustering in the emergence behaviour of bats from maternity roosts. Animal Behaviour 43: 491-500.

Speakman J.R., Stone R.E. & Kerslake J.E., 1995: Temporal patterns in the emergence behavior of pipistrelle bats, *Pipistrellus pipistrellus*, from maternity colonies are consistent with an antipredator response. Animal Behaviour 50: 1147–1156.

Speakman J.R. & Racey P.A., 1989: Hibernal ecology of the pipistrelle bat: energy expenditure, water requirements and mass loss, implications for survival and the function of emergence flights. Journal of Animal Ecology 58: 797-813.

Thomas D.W., 1993: Lack of evidence for a biological alarm clock in bats (*Myotis* spp.) hibernating under natural conditions. Canadian Journal of Zoology 71: 1-3.

Thomas D.W., 1995: Hibernating bats are sensitive to nontactile human disturbance. Journal of Mammalogy 76: 940-946.

Turner G.G., Meteyer C.U., Barton H., Gumbs J.F., Reeder D.A., Overton B., Banďouchova H., Bartonička T., Martínková N., Pikula J., Zukal J. & Blehert D.S., 2014: Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence

to detect lesions indicative of white-nose syndrome. Journal of Wildlife Diseases 50: 566-573.

Turner G.G., Reeder D.M. & Coleman J.T.H., 2011: A five-year assessment of mortality and geographic spread of white-nose syndrome in North American bats and a look to the future. Bat Research News 52: 13–27.

Uhrin M., Benda P., Obuch J. & Urban P., 2010: Changes in abundance of hibernating bats in central Slovakia (1992-2009). Biologia 65: 349-361.

Walsh A. L. & Harris S., 1996: Factors determining the abundance of vespertilionid bats in Britain: geographical, land class and local habitat relationships. Journal of Applied Ecology 33: 519-529.

Whitaker J.O. Jr. & Rissler L.J., 1992: Seasonal activity of bats at Copperhead cave. Proceedings of the Indiana Academy of Science 101: 127-134.

Wilson D.E. & Reeder D.A.M. (eds.), 1993: Mammal species of the world. A taxonomic and geographic reference. Smithsonian Inst. Press, Washington. 1206 pp.

Zima J., Kovařík M., Gaisler J., Řehák Z. & Zukal J., 1994: Dynamics of the number of bats hibernating in the Moravian Karst in 1983 to 1992. Folia Zoologica 43: 109-119.

Zukal J., 1994: Aktivita, echolokace a lovecké chování *Myotis emarginatus*. Dizertační práce. Masarykova univerzita, Brno. 103 pp.

Zukal J., Banďouchová H., Bartonička T., Berková H., Brack V., Brichta J., Dolinay M., Jaroň K.S., Kováčová V., Kovařík M., Martínková N., Ondráček K., Řehák Z., Turner G.G. & Pikula J., 2014: White-Nose Syndrome Fungus: A Generalist Pathogen of Hibernating Bats. PLoS ONE 9: e97224.

Zukal J., Banďouchová H. & Pikula J. (submitted): Bats as bioindicators of heavy metal pollution: history and prospect. Mammalian Biology.

Zukal J., Berková H. & Madaraszová J. (submitted): Flying or sleeping: Influence of WNS on flight activity of bats during deep hibernation. Folia Zoologica.

Zukal J., Berková H. & Řehák Z., 2005: Activity and shelter selection by *Myotis myotis* and *Rhinolophus hipposideros* hibernating in Kateřinská cave (Czech Republic). Mammalian Biology 70: 271-281.

Zukal J., Berková H., Řehák Z. & Kovařík M., 2010: Activity and ecological parameters of bat hibernation in caves of the Moravian karst. 313-314. In: Anděra M. et al. (eds.), A tribute to bats. Lesnická práce, Kostelec nad Černými lesy.

Zukal J., Kovařík M., Řehák Z. & Berková H., 2001: Početnost netopýrů zimujících ve dvou jeskyních v severní části Moravského krasu a její dlouhodobé změny. Vespertilio 5: 321-328.

Zukal J. & Řehák Z., 2006: Flight activity and habitat preference of bats in a karstic area, as revealed by bat detectors. Folia Zoologica 55: 273-281.

Zukal J., Řehák Z. & Kovařík M., 2003: Netopýři Sloupsko - šošůvských jeskyní (Moravský kras). Lynx (Praha) n.s. 34: 205-220.

6. Publikace autora předkládané jako součást habilitační práce

(v případě publikací v časopisech s IF je uvedena jeho poslední zveřejněná hodnota, tj. za rok 2014)

Změny početnosti a struktura společenstva netopýrů

1. Řehák, Z., Zukal, J. & Kovařík, M. (1994) Long- and short-term changes in the bat community of the Kateřinská cave (Moravian Karst) - a fundamental assessment. Folia Zoologica 43, 425-436. IF = 0,724
2. Zima, J., Kovařík, M., Gaisler, J., Řehák, Z. & Zukal, J. (1994) Dynamics of the number of bats hibernating in the Moravian Karst in 1983 to 1992. Folia Zoologica 43, 109-119. IF = 0,724
3. Zukal, J., Kovařík, M., Řehák, Z. & Berková, H. (2001) Početnost netopýrů zimujících ve dvou jeskyních v severní části Moravského krasu a její dlouhodobé změny. Vespertilio 5, 321-328.
4. Zukal, J., Řehák, Z. & Kovařík, M. (2003) Netopýři Sloupsko - šošůvských jeskyní (Moravský kras). Lynx (Praha) n.s. 34, 205-220.
5. Bryja, J., Uhrin, M., Kaňuch, P., Bémová, P., Martínková, N. & Zukal, J. (2010) Mitochondrial DNA confirms low genetic variation of the greater mouse-eared bats, *Myotis myotis*, in Central Europe. Acta Chiropterologica 12, 73-81. IF = 1,133

Výběr úkrytů a letová aktivita netopýrů

6. Gaisler, J., Zukal, J., Řehák, Z. & Homolka, M. (1998). Habitat preference and flight activity of bats in a city. Journal of Zoology (London) 244, 439-445. IF = 1,883
7. Kovařík, M., Zukal, J. & Řehák, Z. (2000) Aktivita netopýrů v Moravském krasu. Estavela 2, 5-6.
8. Pokorný, M., Berková, H., Gaisler, J., Řehák, Z. & Zukal, J. (2003) Letní výskyt netopýrů v lidských stavbách v Moravském krasu a širším okolí. Vespertilio 7, 161-168.
9. Zukal, J., Berková, H. & Řehák, Z. (2005) Activity and shelter selection by *Myotis myotis* and *Rhinolophus hipposideros* hibernating in Kateřinská cave (Czech

Republic). Mammalian Biology 70, 271-281. IF = 1,478

10. Zukal, J. & Řehák, Z. (2006) Flight activity and habitat preference of bats in a karstic area, as revealed by bat detectors. Folia Zoologica 55, 273-281. IF = 0, 724
11. Zukal, J., Berková, H., Řehák, Z. & Kovařík, M. (2010) Activity and ecological parameters of bat hibernation in caves of the Moravian karst. Pp. 313-314 In: Horáček et al. A Tribute to Bats. Lesnické práce s.r.o., Praha.
12. Berková H., Pokorný, M. & Zukal, J. (2014) Selection of Buildings as Maternity Roosts by Greater Mouse-eared Bats (*Myotis myotis*). Journal of Mammalogy 95, 1011-1017. IF = 1,840

Výletová a návratová aktivita u vchodu do úkrytu

13. Berková, H. & Zukal, J. (2004) Sezónní změny letové aktivity netopýrů u vchodu do jeskyně zjištěné pomocí automatického monitorovacího systému. Vespertilio 8, 45-54.
14. Berková, H. & Zukal, J. (2006) Flight activity of bats at the entrance of a natural cave. Acta Chiropterologica 8, 187-195. IF = 1,133
15. Berková, H. & Zukal, J. (2010) Cave visitation by temperate zone bats: effects of climatic factors. Journal of Zoology (London) 280, 387-395. IF = 1,883
16. Zukal, J., Berková, H. & Madaraszová, J. (submitted) Flying or sleeping: Flight activity of bats in natural cave with confirmed WNS. Folia Zoologica. IF = 0,724

Antipredační chování během výletové a návratové aktivity

17. Petrželková, K. & Zukal, J. (2001) Emergence behaviour of the serotine bat (*Eptesicus serotinus*) under predation risk. Netherlands Journal of Zoology 51, 395-414. IF = 0,614
18. Petrželková, K. & Zukal, J. (2003) Does a live barn owl (Tyto alba) affect emergence behavior of serotine bats (*Eptesicus serotinus*)? Acta Chiropterologica 5, 177-184. IF = 1,133
19. Petrželková, K., Obuch, J. & Zukal, J. (2004) Does the barn owl (*Tyto alba*) selectively predate individual great mouse-eared bats (*Myotis myotis*)? Lynx (Praha) n.s., 35: 123-132.

20. Petrželková, K., Downs, N.C., Zukal, J. & Racey, P.A. (2006) A comparison between emergence and return activity in pipistrelle bats *Pipistrellus pipistrellus* and *P. pygmaeus*. Acta Chiropterologica 8, 381-390. IF = 1,133

Těžké kovy a syndrom bílého nosu - potenciální hrozby pro populace letounů

21. Martínková, N., Bačkor, P., Bartonička, T., Blažková, P., Červený, J., Falteisek, L., Gaisler, J., Hanzal, V., Horáček, D., Hubálek, Z., Jahelková, H., Kolařík, M., Korytár, L., Kubátová, A., Lehotská, B., Lehotský, R., Lučan, R., Májek, O., Matějů, J., Řehák, Z., Šafář, J., Tájek, P., Tkadlec, E., Uhrin, M., Wagner, J., Weinfurtová, D., Zima, J., Zukal, J. & Horáček, I. (2010) Increasing Incidence of *Geomyces destructans* Fungus in Bats from the Czech Republic and Slovakia. PLoS One 5, e13853. IF = 3,234
22. Pikula, J., Zukal, J., Adam, V., Banďouchová, H., Beklová, M., Hájková, P., Horáková, J., Kížek, R. & Valentíková, L. (2010) Heavy metals and metallothionein in vespertilionid bats foraging over aquatic habitats in the Czech Republic. Environmental Toxicology and Chemistry 29, 501-506. IF = 3,225
23. Pikula, J., Banďouchová, H., Novotný, L., Meteyer, C.U., Zukal, J., Irwin, N.R., Zima, J. & Martínková, N. (2012) Histopathology Confirms White-Nose Syndrome in Bats in Europe. Journal of Wildlife Diseases 48, 207-211. IF = 1,355
24. Zukal, J., Pikula, J. & H. Banďouchova (2012) Bats and toxic pollutants. Pp. 75-79 in: Flaquer, C. & Puig-Montserrat, X. eds. Proceedings of the International Symposium on the Importance of Bats as Bioindicators. Museum of Natural Sciences Edicions, Granollers.
25. Banďouchová, H., Bartonička, T., Berková, H., Brichta, J., Černý, J., Kováčová, V., Kolařík, M., Köllner, B., Kulich, P., Martínková, N., Řehák, Z., Turner, G. G., Zukal, J., Pikula, J. (2014) *Pseudogymnoascus destructans*: Evidence of Virulent Skin Invasion for Bats Under Natural Conditions, Europe. Transboundary and Emerging Diseases 62, 1-5. IF = 2,944
26. Turner, G. G., Meteyer, C. U., Barton, H., Gumbs, J. F., Reeder, D. A., Overton, B., Banďouchova, H., Bartonička, T., Martínková, N., Pikula, J., Zukal, J., & Blehert, D. S. (2014). Nonlethal screening of bat-wing skin with the use of ultraviolet

fluorescence to detect lesions indicative of white-nose syndrome. Journal of Wildlife Diseases 50, 566-573. IF = 1,355

27. Zukal, J., Banďouchová, H., Bartonička, T., Berková, H., Brack, V., Brichta, J., Dolinay, M., Jaroň, K. S., Kováčová, V., Kovařík, M., Martínková, N., Ondráček, K., Řehák, Z., Turner, G. G. & Pikula, J. (2014) White-Nose Syndrome Fungus: A Generalist Pathogen of Hibernating Bats. PLoS One 9, e97224. IF = 3,234
28. Zukal, J., Banďouchová, H. & Pikula, J. (2015) Bats as bioindicators of heavy metal pollution: history and prospect. Mammalian Biology 80, 220-227. IF = 1,478

Změny početnosti a struktura společenstva netopýrů

Řehák, Z., Zukal, J. & Kovařík, M. (1994) Long- and short-term changes in the bat community of the Kateřinská cave (Moravian Karst) - a fundamental assessment. Folia Zoologica 43, 425-436.

FOLIA ZOOLOGICA – 43 (4) : 425–436 (1994)

LONG- AND SHORT-TERM CHANGES IN THE BAT COMMUNITY OF THE KATEŘINSKÁ CAVE (MORAVIAN KARST) – A FUNDAMENTAL ASSESSMENT

Zdeněk ŘEHÁK[1], Jan ZUKAL[2],
Miroslav KOVAŘÍK[3]

Received March 18, 1994
Accepted August 18, 1994

[1] Faculty of Sciences, Masaryk University, Brno
[2] Institute of Landscape Ecology, AS CR, Brno
[3] Nature Conservancy Moravský kras, Blansko

Abstract

In 1992–1993, the bat community of a natural karst cave (Kateřinská cave, Moravian Karst) was investigated by means of a regular winter census of hibernating bats and of nettings in the cave entrance. In total, 14 species were ascertained. The maximum abundance of the bat community in the cave during hibernation was in late February (25. 2. 93 – 136 ind.). The number of bats was lowest in late June and July. The highest intensity of cave visitation occurred in late August and the first half of September, with a minor peak recorded in April. The species composition changed markedly during the whole year. The hibernating bat community was characterised by significant dominance of *Myotis myotis* whereas small *Myotis* species (especially *Myotis daubentoni, M. nattereri, M. emarginatus* and *M. bechsteini*) dominated the spring and autumn catching samples. The Kateřinská cave has also been included in long-term monitoring of bat communities in Moravian Karst caves. The changes in numbers of hibernating bats has shown a similar trend to that found in other caves, viz. a strong increase of *M. myotis* and a weak increase of *Rhinolophus hipposideros.*

Introduction

Underground sites (caves, mines, tunnels etc.) are the most important roost sites for most species of bats. They present a significant factor influencing the abundance and structure of bat communities in a region. Many authors have been interested in the study of long-term and short-term changes in bat communities (Daan et al. 1980; Nagel & Nagel 1987, 1989; Gaisler 1991; Anděra et al. 1992; Hanzal & Průcha 1992; Urbanczyk 1992 and others), especially in winter. The long-term monitoring of hibernating bats has been carried out in different caves of the Moravian Karst since 1973 (Bárta et al. 1981; Bauerová et al. 1989; Zima et al. 1994). The results of these studies have revealed a general increase in the numbers of *Myotis myotis*. A stabilisation or slight upward trend, was also recorded in *Rhinolophus hipposideros.* However, little attention has been given to the study of short-term changes in bat communities within the framework of a particular locality. The only exception is the research of Gaisler (1973 and 1975), who studied population changes throughout the year by the regural netting of bats on the southern edge of the Moravian Karst, an area rich in caves. Work has also been produced by Bauerová & Zima (1988a,b), who netted bats at the

entrances of the Hladomorna and Býčí skála caves in the northern and central part of the Moravian Karst. However, their results represent a summary of studies from several growing seasons.

The aim of the present paper is to evaluate the fundamental synecological data obtained both by the twenty five year winter census of bats in the Kateřinská cave and also by the monitoring of bat community in underground spaces and the netting of bats at cave entrances during the season of 1992–1993. The results of the long-term monitoring studies obtained throughout 1973–1979 and 1983–1987, have already been reported by B á r t a et al. (1981) and B a u e r o v á et al. (1989).

Study Area

The Kateřinská cave is situated in the northern part of the „Moravian Karst" Protected Landscape Area (Czech Republic) (49°21' N, 16°48' E). The cave represents an abandoned effluence of the underground Punkva River, which flowed from the Macocha Abyss via an unknown routs southwards and through the Kateřinská cave, appearing at the surface in the Suchý žleb valley. The main part of the cave consists of three large domes, one of which, the „Hlavní dome", is the biggest underground space in the Moravian Karst (length 96 m, width 44 m and height 20 m). The total length of the cave is about 500 m and is moderately dynamic with regard to climate.

The only entrance to the cave is oriented to the south-west and lies at an elevation of 345 m a.s.l., formed by a high „gothic" portal with a rock wall above it. The entrance corridor is closed by an iron gate and there is a hole (30 × 15 cm) in its upper part. Throughout the whole year the cave is visited by tourists and the sightseeing route is about 300 m long.

Material and Methods

The long-term changes in numbers of hibernating bats have been evaluated on the basis of one control shelter per year (in the second half of January or February, B á r t a et al. 1981). During 1970–1979 the bats under study were taken down and banded. Since 1983 the bats have been checked by a simple visual census without any disturbing influence (B a u e r o v á et al. 1989). The bats were only exceptionally taken down for the purpose of species identification, even so very few of the individuals remained unidentified.

From 24th September 1992 to 7th October 1993, both the dynamic of the bat community inside the cave and the activity of bats flying at the cave entrance were studied. The census of bats visible on the walls and in fissures in the accessible spaces of the caves (cca 400 m of route) was undertaken in the same manner as that for long-term monitoring (see above). The activity of bats at the cave entrance was assessed by means of netting and trapping (G a i s l e r 1973; H o r á č e k & Z i m a 1978). A mist-net 2.2 × 3 m, fastened directly across the gates to the cave was used, cca 50 cm near by the wall. The bats leaving the cave were captured in special harp-trap, placed in front of the exit hole from the cave (cf. Č e r v e n ý & B ü r g e r 1989). Catching was usually started 30–45 minutes prior to civil twilight and ended about midnight. The trapping period

progressed over midnight on exceptions (spring, autumn) and in this case, individuals caught up to midnight only (Central European Time) are included in the results. On occasion (winter, summer) catching was finished earlier if nothing was caught during the last three hours or if activity at the entrance was minimal. The bats were sexed, weighed, measured, banded, and then immediatelly released. Both the controls and trappings were carried out approximately once every two weeks over one or two days (first day netting and second day census). In three winter terms (3. 12. 1992, 4. 1. 1993 and 28. 1. 1993) bats were only evaluated in the cave. In total, 14 species of bats were ascertained (*Rhinolophus hipposideros, Myotis myotis, M. blythi, M. dasycneme, M. mystacinus, M. brandti, M. emarginatus, M. nattereri, M. bechsteini, M. daubentoni, Eptesicus serotinus, Plecotus auritus, P. austriacus, Barbastella barbastellus*); recaptured bats were also included in the results (Table 1).

Statistical analyses were performed using the SYSTAT program (W i l k i n s o n 1990). Third order regression analysis ($y = a + bx + cx^2 + dx^3$) was used to describe the effects of year on the abundance, both of the whole bat populations and of two dominant species (*Rhinolophus hipposideros* and *Myotis myotis*), this giving the best expression of long-term changes and their possible development. Relationships between the years and the abundance were tested by both Pearson's and Spearman's correlation coefficient. The critical values of Spearman's rank coefficient for $n = 20$ are $r_s^{0.01} = 0.534$ and $r_s^{0.05} = 0.377$. Pearson's chi-square test was used to test for differences, both between the numbers of bats leaving the cave and flying in the cave entrance during a season, and between flying activity of each species. Significance in the differences between the dominance of bat species in netting and census samples was evaluated by means of contingency tables, again using Pearson's chi-square statistics. Cluster analysis was used to assess differences in samples with $n > 15$. Complete linkage clustering was applied to the matrix of Renkonen's index of dominance similarity (L o s o s et al. 1984). Undetermined bats were excluded from the statistical analysis.

Results

L o n g - t e r m C h a n g e s

The hibernating bat community was characterised by a significant dominance of *Myotis myotis* (Fig. 1). This species formed almost three quarters (71.2 %) of all 1,125 individuals checked. The abundance of *Myotis myotis* had a statistically significant upward trend ($r = 0.75$, $p < 0.001$; $r_s = 0.77$, $p < 0.01$) even after considerable decrease during 1970–1977 (Fig. 1). Another situation was found with *Rhinolophus hipposideros*. Since 1984 *R. hipposideros* exhibited a stabilized or slightly upward trend, nevertheless, up until 1994, this species has not matched the values of 1970 (30 ind.). The changes in numbers of this species are significantly correlated by Spearman's rank coefficient ($r = 0.25$, $p = 0.29$; $r_s = 0.46$, $p < 0.05$). The total abundance of bats has increased significantly ($r = 0.68$, $p = 0.001$; $r_s = 0.72$, $p < 0.01$), in line with the increasing trend of both dominant species (Fig. 1). The correlation between the total number of hibernating bats and the abundance of *R. hipposideros* and *M. myotis* is highly significant (Table 2).

Table 1. Survey of total samples obtained by winter census (C) and nettings (N). Explanations: [1] extra dates for netting (see Material and Methods), [2] undetermined individuals of small *Myotis* species

Date	*R. hip*		*M. myo*		*M. nat*		*M. ema*		*M. dau*		*M. mys*		*M. das*		*M.* sp.[2]		*M. bra*	*M. bech*	*P. aur*		*B. bar*	*E. ser*	Total	
	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N	N	N	C	N	C	N	C	N
10. 10. 92[1]	1	0	3	21	0	34	0	10	0	5	0	0	0	0	2	0	0	16	0	5	0	0	6	91
22. 10. 92	3	0	6	1	0	20	1	1	0	2	0	0	0	0	2	0	0	2	0	0	0	0	12	26
05. 11. 92	2	2	5	4	0	11	1	0	0	1	0	0	0	0	2	3	0	0	0	0	0	0	10	21
19. 11. 92	8	0	13	0	0	0	3	0	0	0	0	0	0	0	6	0	0	0	0	1	0	0	30	1
03. 12. 92	19	–	25	–	2	–	3	–	1	–	0	–	0	–	6	–	–	–	1	–	1	–	58	–
17. 12. 92	20	0	35	1	1	0	3	0	0	0	0	0	1	0	6	0	0	0	0	0	1	0	67	1
04. 01. 93	21	–	35	–	2	–	3	–	0	–	0	–	2	–	4	–	–	–	2	–	1	–	70	–
15. 01. 93	24	0	44	0	1	0	3	0	0	0	0	0	1	0	6	0	0	0	3	0	1	0	83	0
28. 01. 93	17	–	62	–	1	–	4	–	0	–	0	–	2	–	4	–	–	–	4	–	1	–	95	–
11. 02. 93	19	0	80	0	1	0	5	0	0	1	0	0	2	0	7	0	0	0	3	0	0	0	117	1
25. 02. 93	19	0	97	0	2	0	5	0	1	0	0	0	3	0	5	0	0	0	3	0	1	0	136	0
11. 03. 93	21	0	90	0	2	0	5	0	1	0	1	0	1	0	5	0	0	0	2	3	1	0	129	3
24. 03. 93	6	2	98	0	2	5	5	0	1	4	1	0	1	0	3	0	0	0	0	1	1	0	118	12
08. 04. 93	8	0	112	4	2	15	4	0	2	10	0	0	0	0	4	3	0	1	0	1	0	0	132	34
22. 04. 93	7	5	23	4	0	0	5	0	1	4	0	3	0	0	5	1	0	8	0	0	0	0	41	25
06. 05. 93	3	6	3	1	0	0	6	1	1	0	0	3	0	0	5	0	0	0	0	0	0	0	18	11
22. 05. 93	0	0	0	0	0	8	3	7	0	12	0	0	0	0	3	8	0	17	0	0	0	0	6	52
04. 06. 93	0	0	0	0	1	1	0	3	0	2	0	0	0	0	0	0	0	11	0	0	0	0	1	17
16. 06. 93	1	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	1	7
30. 06. 93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15. 07. 93	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
29. 07. 93	0	0	0	0	0	0	0	2	0	3	0	1	0	0	0	0	1	0	0	0	0	0	0	7
12. 08. 93	0	0	1	5	0	0	0	7	0	21	0	0	0	0	2	1	0	1	0	1	0	0	3	36
02. 09. 93[1]	0	2	0	6	0	17	0	14	0	9	0	1	0	0	0	0	1	4	0	1	0	0	0	55
25. 09. 93	0	1	1	44	0	29	0	4	0	9	0	1	0	1	3	1	0	10	0	2	0	0	4	102
08. 10. 93	0	1	2	6	0	17	0	3	0	2	0	0	0	0	2	0	0	5	0	3	0	1	4	38
TOTAL	199	23	735	97	17	157	59	52	8	85	2	9	13	1	82	17	2	78	18	18	8	1	1141	540

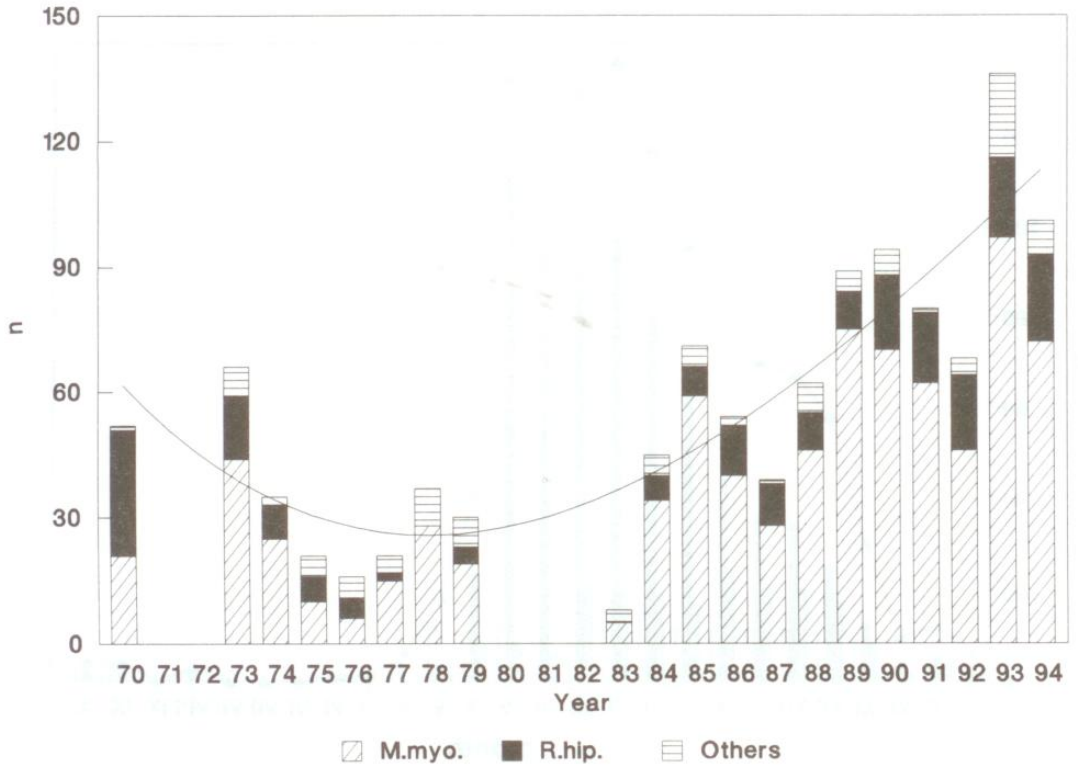


Fig. 1. Trends in numbers of bats hibernating in the Kateřinská cave from 1970 to 1994. Explanations: *M. myo. – Myotis myotis, R. hip. – Rhinolophus hipposideros,* solid line – cubic regression.

Table 2. Results of cubic regression and correlation analysis, see Fig. 1. Explanations: a, b, c, d – regression coefficients, r_p – Pearson's correlation coefficient, r_s – Spearman's correlation coefficient, p – probability

	a	b	c	d	r_p	p	r_s	p
Myotis myotis	4140.7	–139.2	1.5	–0.01	0.75	< 0.001	0.77	< 0.01
R. hipposideros	6089.7	–211.5	2.4	–0.01	0.25	0.290	0.46	< 0.05
Total	7166.9	–236.8	2.6	–0.01	0.68	0.001	0.72	< 0.01

Short-term Changes

The dominance of *Myotis myotis* in the winter community of bats in the Kateřinská cave has also been confirmed by numerous censuses undertaken in 1992/93. The number of all bats hibernating in the cave increased gradually from October, the peak was recorded at the end of February and March. From the middle of April to the end of May the abundance dropped and, during the early summer (June, July), bats did not occur in the cave (Fig. 2). Analysis by means of contingency tables showed significant differences between samples ($\chi^2 = 128.65$, $p = 0.002$, D.F. = 85).

Flying activity at the cave portal and the species structure of the netting samples also show significant differences ($\chi^2 = 374.92$, $p < 0.001$, D.F. = 99) (Fig. 3). Bat visitations to the entrance of the cave were highest (59.6 % of total netted bats) during autumn movements (from August to the first half of Octo-

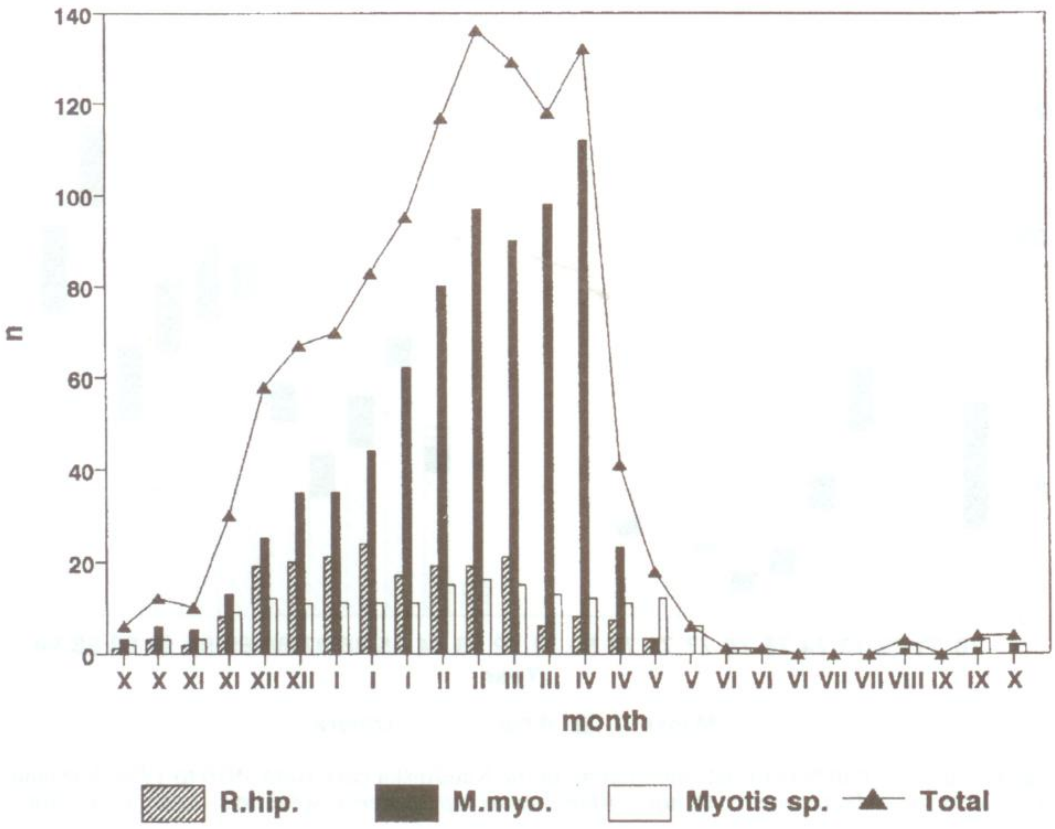


Fig. 2. Changes in numbers of hibernating bats throughout the season of 1992/1993 (results of visual census). Explanations: *Myotis* sp. – individuals of small *Myotis* species. For other see Fig. 1.

ber). In the winter season (from November to the first half of March) the activity of bats was minimal. Activity increased again in spring (from April to May) but its values were lower than those of autumn.

Cluster analysis was used to classify differences amongst netting and census samples ($n > 15$) according to dominance of bat species. Four distinct clusters were formed: 1. the winter censuses, characterized by a remarkable predominance of *M. myotis*, 2. the winter censuses with equal dominance of *R. hipposideros* and *M. myotis*, 3. netting samples with a high dominance (more than 25 % of all) of *Myotis nattereri* from the first half of April, September, October and November, and 4. netting samples, dominated by *Myotis bechsteini* and *Myotis daubentoni,* from the second half of April, May and June (possibly July) (Fig. 4). As opposed to the results of visual monitoring, small *Myotis* species (*Myotis emarginatus, M. bechsteini, M. daubentoni* and *M. nattereri* especially), with one exception, dominated in the samples of trapped bats (Table 1). This fact also influenced the values of species diversity for both samples (censuses $H' = 1.45$, catches $H' = 2.67$).

The ratio of the number of bats leaving and entering the cave changed in connection with the cycle of activity during the whole year. The number of individuals flying out of the cave was lowest (up to 50 %) during the autumn migrations (Table 3, Fig. 3) but very high in spring (nearly 100 %) and summer. The

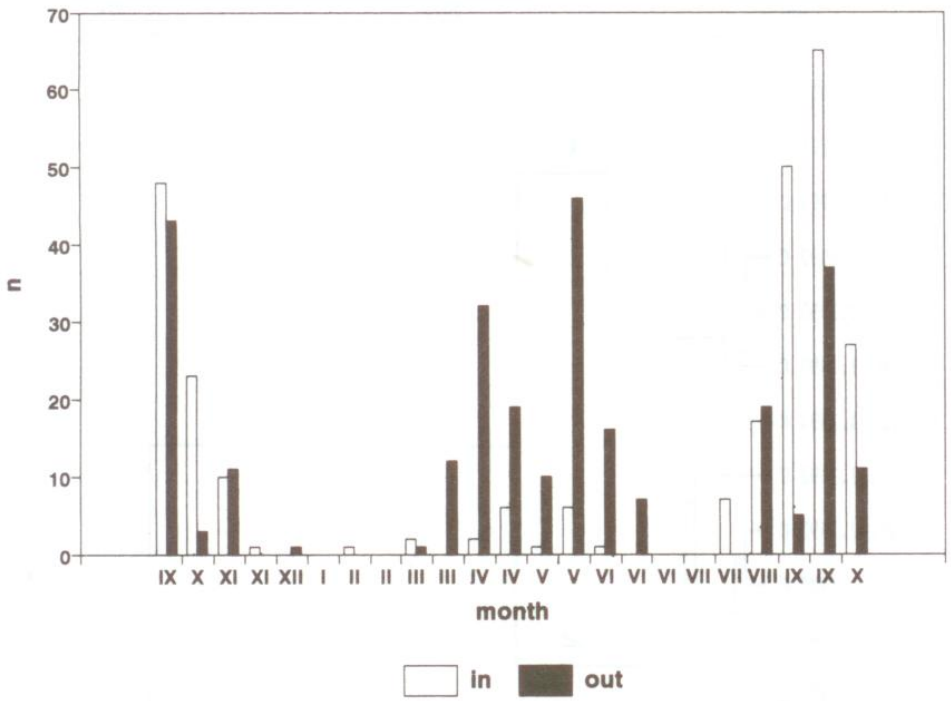


Fig. 3. Changes in numbers of bats visiting (in) or leaving (out) the cave throughout the season of 1992/1993 (results of trapping and netting).

difference was significantly higher for bats entering the cave in three autumn samples (22. 10. 92, 6. 9. 93 and 24. 9. 93). However, for the total sample, no significant differences were found ($\chi^2 = 0.015$, $p = 0.903$, D.F. = 1). Differences were observed in the flying activity of particular bat species, however, only three species (*R. hipposideros, M. myotis* and *M. emarginatus*) proved significant (Table 3). *M. myotis* and *M. emarginatus* were the dominant species entering the cave except for two samples from September (24. 9. 92 and 24. 9. 93).

Discussion

Long-term Changes

Census data of hibernating bats from various sites around Europe has demonstrated considerable changes in numbers of various bat species during the last three decades. A rapid decline was observed particularly of *Rhinolophus ferrumequinum, R. hipposideros* and *Myotis myotis,* during the 1960's and 70's (Gaisler & Bauerová 1977; Roer 1981; Kokurewicz 1990; Rudolph 1990). However a conspicuous increase in the abundance of some bat species has been recorded during the last decade (Nagel & Nagel 1989; Bauerová et al. 1989; Červený & Bürger 1990; Gaisler 1991; Hanzal & Průcha 1992; Urbanczyk 1992). Our results, from the Kateřinská cave, support the observed trends for two dominant species i.e. *R. hipposideros* and *M. myotis*. The hibernating population of *M. myotis* has

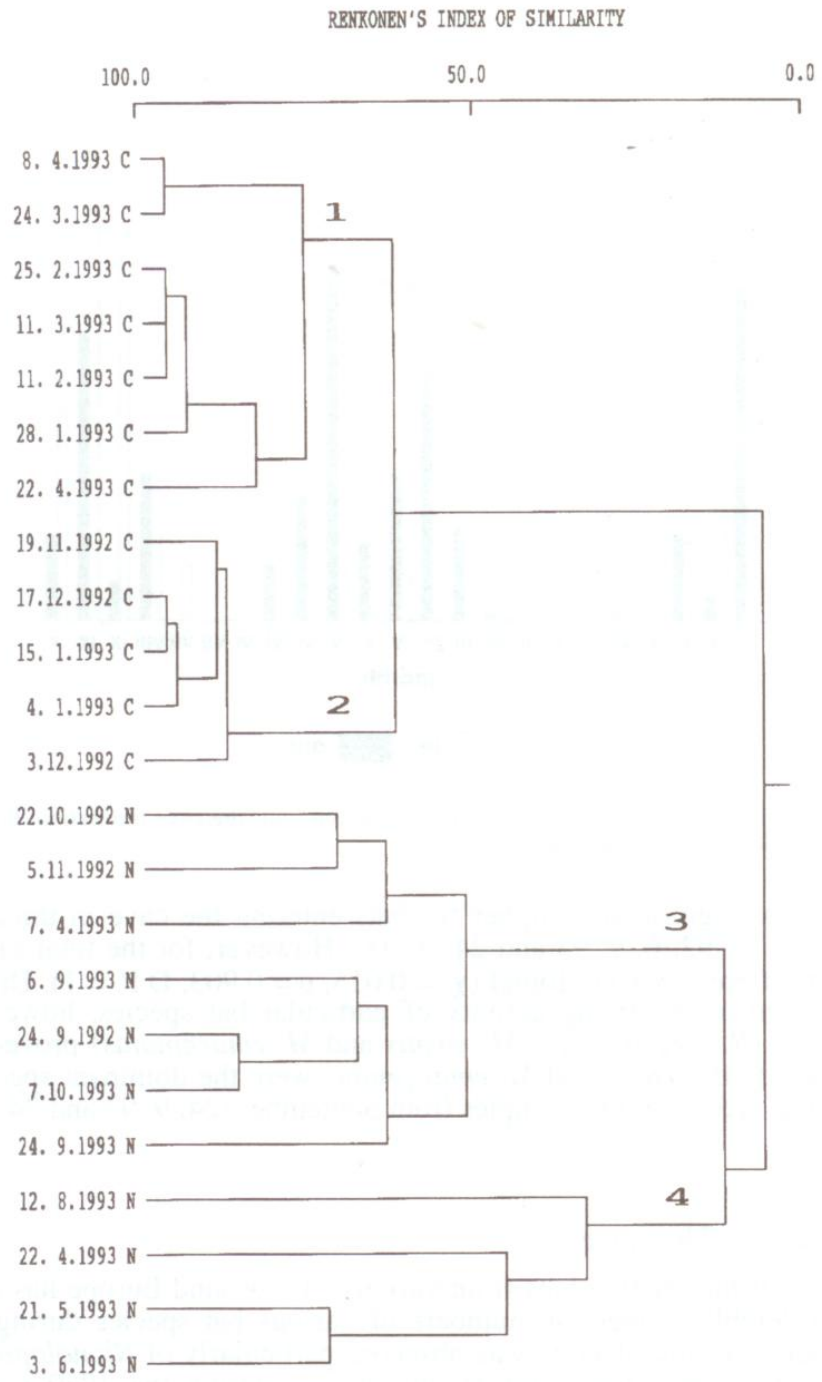


Fig. 4. Phenogram based on complete linkage cluster analysis of Renkonen's similarity index estimated among samples with n > 15. Explanations: C – winter cenus, N – netting, 1, 2, 3, 4 – number of particular clusters, see „Results".

demonstrated an upward trend in abundance and similar trends have recently been recorded from the surrounding regions – the Bohemian Karst (H a n z a l & P r ů c h a 1992) and Northern Moravia (G a i s l e r et al. 1993). *R. hipposideros* is a relatively common species in Southern Moravia and especially in the

Moravian Karst (G a i s l e r 1991). The population of this species in the Kateřinská cave has stabilized and is even showing a slight upward trend. A similar trend generally holds for populations of *R. hipposideros* over the whole Moravian Karst (Z i m a et al. 1994). Although it is difficult to explain the influences causing the increase in the numbers of bats hibernating in the Kateřinská cave, we presume that the changes have been caused mainly by minimal disturbance. This possibility was also discussed by N a g e l & N a g e l (1989) for the caves in south Germany. They found an increase in numbers of bats hibernating in newly closed caves, even if they excluded the increasing general trend in numbers of hibernating bats.

Table 3. Pearson's chi-square statistics examining the changes in numbers of bats visiting (in) or leaving (out) the cave throughout the season of 1992–93. Explanations: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, N. S. not significant

Date	out	in	χ^2	p
24. 09. 1992	43	48	0.14	N.S.
22. 10. 1992	3	23	9.03	**
05. 11. 1992	11	10	0.02	N.S.
19. 11. 1992	0	1	–	–
17. 12. 1992	1	0	–	–
15. 01. 1993	0	0	–	–
11. 02. 1993	0	1	–	–
25. 02. 1993	0	0	–	–
10. 03. 1993	1	2	0.14	N.S.
24. 03. 1993	12	0	8.00	**
07. 04. 1993	32	2	16.43	***
22. 04. 1993	19	6	3.56	N.S.
05. 05. 1993	10	1	4.30	*
21. 05. 1993	46	6	18.06	***
03. 06. 1993	16	1	8.09	**
16. 06. 1993	7	0	4.55	*
30. 06. 1993	0	0	–	–
15. 07. 1993	0	0	–	–
29. 07. 1993	0	7	4.55	*
12. 08. 1993	19	17	0.06	N.S.
06. 09. 1993	5	50	21.99	***
24. 09. 1993	37	65	3.92	*
07. 10. 1993	11	27	3.52	N.S.
Species	out	in	χ^2	p
R. hipposideros	20	3	7.17	**
M. myotis	31	66	6.50	**
M. bechsteini	46	32	1.27	N.S.
M. nattereri	94	63	3.08	N.S.
M. daubentoni	44	41	0.05	N.S.
M. emarginatus	12	40	8.13	**
M. mystacinus	5	4	0.05	N.S.
M. brandti	0	2	1.33	N.S.
M. dasycneme	0	1	–	–
E. serotinus	0	1	–	–
P. auritus	4	14	3.01	N.S.
Total	273	267	0.02	N.S.

S h o r t - t e r m C h a n g e s

The majority of studies about short-term changes in communities of bats are confined to the period of hibernation (B a g r o w s k a – U r b a n c z y k & U r b a n c z y k 1983; P r ů c h a & H a n z a l 1989; and others). Where

authors have concerned themselves with seasonal changes, their results were a summary of a number of seasons (Červený 1982; Bauerová & Zima 1988 a,b; Anděra et al. 1992).

Our study, in the Kateřinská cave, gave regular cover to the whole season, from October 1992 to October 1993. There were considerable differences between the results obtained by means of netting and trapping and the results of the visual controls (Table 1). A similar result was noted by Gaisler (1973) and Bauerová & Zima (1988a), when they used the same methods of research. The small *Myotis* species (*M. emarginatus, M. daubentoni, M. nattereri* and *M. bechsteini*) dominate in the trapping samples. It is also possible that they belonged to the hibernating community but their numbers were underestimated during the visual census. This underestimating could presumably be brought about by the choice of shelter type by various bat species in natural underground spaces. *M. daubentoni* and *M. nattereri* are species, which use highly protected shelters in hibernaculum (Bogdanowicz & Urbanczyk 1983; Lesinski 1986; Hanzal & Průcha 1988), *M. bechsteini* probably employ similar spaces because individuals of this species caught leaving the cave (May and June) were muddy on the forearms and wings.

Cluster and chi-square analysis verify the differences in the motivation of cave visitation by bats of particular species. The predominance of *M. nattereri* in late autumn (from late September to early November) and early spring (March and early April) netted samples may be connected with both searching for suitable hibernation shelters and departure from hibernaculum. These periods correspond to the third phase of the "autumn roaming movements" and early "spring roaming movements" described by Horáček & Zima (1978) for bats of the Northern Temperate Zone.

The summer samples were poor from the point of view of species diversity and abundance and they could not be included in statistic analysis (Fig. 4), with the exception of some samples from „border" months (late April, May and August). The relatively rare *M. bechsteini* dominated in samples from late April to May, as this species' departure from the hibernaculum is retarded in comparison with the more numerous *M. nattereri* (Table 1). On the other hand, the high dominance of both *M. bechsteini* and *M. emarginatus* in samples from September is presumably influenced by mating activity and the seeking of inter-individual contacts within a given population.

R. hipposideros and *M. myotis* are typical of the community of bats hibernating in the Kateřinská cave system. Both species dominate in the majority of Moravian Karst caves during the winter season (Bauerová et al. 1989; Gaisler et al. 1990; Zima et al. 1994). The changes in the ratio of these two species in community were found to affect the differences between census samples (Fig. 4). The ratios changed partly due to differences in the time of arrival in, and departure from, the hibernaculum and partly due to the dynamics of flying activity inside the cave. *M. myotis* was one of the dominant species in nettings from September (24. 9. 92 – 23.1 % and 24. 9. 93 – 43.6 %) (cf. Bauerová & Zima 1988b), and it had equal dominance in the census with *R. hipposideros* in the first half of winter (from mid-November to mid-January) (Table 1). From mid-January, the abundance of *M. myotis* increase considerably until early April (cf. Hanzal & Průcha 1988). However, the increase was not influenced by immigration of bats from neighbouring shelters as the nettings were practically zero during the winter (Fig. 3). This is more likely to be due to movements within the cave, on more exposed sites, where we are more able to

control the bats exactly. This conclusion was also reached by Nagel & Nagel (1987), who found a maximum abundance of *M. myotis* at the beginning of March, but only secondary abundance during the first half of April. Similar results to ours have been reported by Červený (1982) and Valenciuc (1989). Nagel & Nagel (1993) showed that, from the middle of February, flight activity of *Myotis myotis* inside of a cave at Swabian Alb (Germany) increased till the end of April.

R. hipposideros exhibited a stable hibernating community (cca 20 ind.) from the beginning of December to mid-March (cf. Gaisler 1963), whereupon a rapid disintegration took place. The main departure from the hibernaculum was ascertained as the period from late April to early May, however, the sample caught of *R. hipposideros* was, as a whole, very low. There was also a significant difference between the entering and leaving of the cave by this species, believed to be influenced by the method of catching. *R. hipposideros* is a species with high manoeuvrability (Norberg & Rayner 1987), therefore it is difficult to catch it by mist net, though it has difficulty escaping from trap.

Acknowledgement

We are obliged to Prof. RNDr. Jiří Gaisler, DrSc. for valuable comments on the manuscript. We also thank all collaborators and friends from the Management of the Protected Landscape Area „Moravský Kras" and the Czech Bat Conservation Trust who participated in the field work. This work was supported by CBCT Project and by Grant GA ČR 204/93/2241.

LITERATURE

ANDĚRA, M., ZBYTOVSKÝ, P., BÜRGER, P., 1992: Bat community of the Chýnovská jeskyně cave (southern Bohemia, Czechoslovakia) in 1981–1986. In: Horáček, I. and Vohralík, V. (eds.): Prague studies in mammalogy. *Charles University Press, Praha: 1–11.*

BAGROWSKA-URBANCZYK, E. & URBANCZYK, Z., 1983: Structure and dynamics of a winter colony of bats. *Acta theriol., 28: 183–196.*

BÁRTA, Z., ČERVENÝ, J., GAISLER, J. (ed.), HANÁK, P., HANÁK, V. (ed.), HORÁČEK, I. (ed.), HŮRKA, L., MILES, P., NEVRLÝ, M., RUMLER, Z., SKLENÁŘ, J., ŽALMAN, J., 1981: Results of the winter census of bats in Czechoslovakia; 1969–1979. *Sborn. Okr. Muz. v Mostě, ř. Přír., 3/1981: 71–116 (in Czech, with a summary in English).*

BAUEROVÁ, Z., GAISLER, J., KOVAŘÍK, M., ZIMA, J., 1989: Variation in numbers of hibernating bats in the Moravian karst: results of visual censuses in 1983–1987. In: Horáček, I. and Vohralík, V. (eds.): European bat research 1987. *Charles Univ. Press, Praha: 499–505.*

BAUEROVÁ, Z. & ZIMA, J., 1988a: Bat research and census in the Býčí skála cave in 1977–1986. *Čs. kras, 39: 51–59 (in Czech, with a summary in English).*

BAUEROVÁ, Z. & ZIMA, J., 1988b: Seasonal changes in visits to a cave by bats. *Folia Zool., 37: 97–111.*

BOGDANOWICZ, W. & URBANCZYK, Z., 1983: Some ecological aspects of bats hibernating in city of Poznan. *Acta theriol., 28: 371–385.*

ČERVENÝ, J., 1982: Results of investigation of bats (Chiroptera) at Loreta near Klatovy. *Lynx, n.s., 21: 41–65.*

ČERVENÝ, J. & BÜRGER, P., 1989: Density and structure of the bat community occupying an old park at Žihobce (Czechoslovakia). In: Horáček, I. and Vohralík, V. (eds.): European bat research 1987. *Charles Univ. Press, Praha: 475–488.*

ČERVENÝ, J. & BÜRGER, P., 1990: Changes in bat population sizes in the Šumava Mts. (south-west Bohemia). *Folia Zool., 39: 213–226.*

DAAN, S., GLAS, G. H., VOUTE, A. M. (eds.), 1980: Long term changes in bat populations in the Netherlands. *Lutra, 22: 1–118.*

GAISLER, J., 1963: The ecology of lesser horseshoe bat (Rhinolophus hipposideros Bechstein, 1800) in Czechoslovakia, Part I. *Věst. Čs. spol. Zool., 27: 211–233.*

GAISLER, J., 1973: Netting as a possible approach to study bat activity. *Period. biol. Zagreb, 75: 129–134.*

GAISLER, J., 1975: A quantitative study of some populations of bats in Czechoslova-

kia (Mammalia: Chiroptera). *Acta Sc. Nat. Brno, 9 (5): 1–44.*

GAISLER, J., 1991: The status of Rhinolophus hipposideros in S-Moravia (CS). *Myotis, 29: 105–108.*

GAISLER, J. & BAUEROVÁ, Z., 1977: The bat community at Květnice in the course of thirty years. *Lynx, n.s., 19: 17–28 (in Czech, with a summary in English).*

GAISLER, J., CHYTIL, J., VLAŠÍN, M., 1990: The bats of S-Moravian lowlands (Czechoslovakia) over thirty years. *Acta Sc. Nat. Brno, 24 (9): 1–50.*

GAISLER, J., ŠEBELA, M., DUNGEL, J., 1993: Variation in numbers of bats hibernating in abandoned mine galleries near Malá Morávka (Czechoslovakia). *Acta Mus. Moraviae, Sci. nat., 77: 255–264 (in Czech, with a summary in English).*

HANZAL, V. & PRŮCHA, M., 1988: Seasonal dynamics of bat communities at winter shelters of Český kras (central Bohemia) during 1984–1986. *Lynx (Praha), n.s., 24: 15–35 (in Czech, with a summary in English).*

HANZAL, V. & PRŮCHA, M., 1992: Changes in the numbers of bats hibernating in the Bohemian karst during 1969–1987. In: Horáček, I. and Vohralík, V. (eds.): Prague studies in mammalogy. *Charles University Press, Praha: 71–74.*

HORÁČEK, I. & ZIMA, J., 1978: Net-revealed cave visitation and cave-dwelling in European bats. *Folia Zool., 27: 135–148.*

KOKUREWICZ, T., 1990: The decrease in abundance of the lesser horseshoe bat Rhinolophus hipposideros Bechstein, 1800 (Chiroptera, Rhinolophidae) in winter quarters in Poland. *Myotis, 28: 109–118.*

LESIŃSKI, G., 1986: Ecology of bats hibernating underground in central Poland. *Acta theriol., 31: 507–521.*

LOSOS, B. et al., 1984: Animal ecology. *SPN, Praha: 316 pp. (in Czech).*

NAGEL, A. & NAGEL, R., 1987: Veränderungen des Bestandes winterschlafender Fledermäuse in Winter 1982/83 in Württemberg. *Myotis, 25: 91–94.*

NAGEL, A. & NAGEL, R., 1989: Bestandsentwicklung winterschlafender Fledermäuse auf der Schwäbischen Alb bis zum Winter 1987/88 und ihr Schutz. *Mitt. Verb. dt. Höhlen- und Karstforsch., 35: 17–23.*

NAGEL, A. & NAGEL, R., 1993: Activity of hibernating bats in their natural habitat recorded by automatic data logging. *VI European Bat Research Symposium, Abstracts: 37–38.*

NORBERG, U. M. & RAYNER, M. V., 1987: Ecological morphology and flight in bats (Mammalia: Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Phil. Trans. R. Soc. Lond. B., 316: 335–427.*

PRŮCHA, M. & HANZAL, V., 1989: Some aspects of hibernation of bats wintering in the Bohemian Karst (Central Bohemia, Czechoslovakia). *Acta Univ. Carolinae, 33: 315–333.*

ROER, H., 1980/1981: Zur Bestandsentwicklung einiger Fledermäuse in Mitteleuropa. *Myotis, 18/19: 60–67.*

RUDOLPH, B.-U., 1990: Frühere Bestandsdichte und heutige Situation der Kleinen Hufeisennase Rhinolophus hipposideros in Nordbayern. *Myotis, 28: 101–108.*

URBANCZYK, Z., 1992: Significance of the Nietoperek Reserve for central European population of Myotis myotis (Mammalia: Chiroptera). In: Horáček, I. and Vohralík, V. (eds.): Prague studies in mammalogy. *Charles University Press, Praha: 213–215.*

VALENCIUC, N., 1989: Dynamics of movements of bats inside some shelters. In: Hanák, V., Horáček, I., Gaisler J. (eds.): European bat research 1987. *Charles University Press, Praha: 511–517.*

WILKINSON, L., 1990: SYSTAT: The System for Statistics. *Evanston, IL: SYSTAT, Inc.*

ZIMA, J., KOVAŘÍK, M., GAISLER, J., ŘEHÁK, Z., ZUKAL, J., 1994: Dynamics of the number of bats hibernating in the Moravian karst in 1983 to 1992. *Folia Zool., 43: 109–119.*

Authors' addresses:

Zdeněk ŘEHÁK,

Department of Zoology and Ecology, Faculty of Sciences, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Jan ZUKAL,

Institute of Landscape Ecology, Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic

Miroslav KOVAŘÍK,

Nature Conservancy Moravský kras, Sadová 2, 678 01 Blansko, Czech Republic

Změny početnosti a struktura společenstva netopýrů

Zima, J., Kovařík, M., Gaisler, J., Řehák, Z. & Zukal, J. (1994) Dynamics of the number of bats hibernating in the Moravian Karst in 1983 to 1992.
Folia Zoologica 43, 109-119.

FOLIA ZOOLOGICA – 43 (2) : 109–119 (1994)

DYNAMICS OF THE NUMBER OF BATS HIBERNATING IN THE MORAVIAN KARST IN 1983 TO 1992

Jan ZIMA[1], Miroslav KOVAŘÍK[2], Jiří GAISLER[3],
Zdeněk ŘEHÁK[3], Jan ZUKAL[1]

Received June 18, 1993
Accepted September 20, 1993

[1] Institute of Systematic and Ecological Biology, AS CR, Brno
[2] Nature Conservancy, Moravian Karst, Blansko
[3] Faculty of Sciences, Masaryk University, Brno

Abstract

Visual censuses of hibernating bats were organised annually in 37 underground spaces, largely natural caves, in the Moravian karst between 1983 to 1992. The total number of hibernating bats increased significantly during the 10 – year period. The most striking increase in numbers was observed in the greater mouse-eared bat, *Myotis myotis,* with a less pronounced increase recorded in the lesser horseshoe bat, *Rhinolophus hipposideros.* In other bat species *(Barbastella barbastellus, Plecotus* spp., small *Myotis* spp.) no apparent trends were observed. Possible explanations for the observed changes in the abundance of wintering bats are discussed.

Introduction

The bats of the temperate zone present an animal group very appropriate for monitoring the long-term changes in population densities. Such long-term monitoring can be performed easily during hibernation, which provides relatively standard conditions at a low financial cost. Censuses of wintering bats in various European regions have already demonstrated considerable changes in numbers of the various bat species (Bezem et al. 1960, Dorgelo & Punt 1969, Gaisler et al. 1980/1981, Haensel 1980/1981, Preiss 1982, Baar et al. 1986, Urbanczyk 1989, Kowalski & Lesinski 1991, Weinrich & Oude Voshaar 1992). A rapid decline in numbers and even local extinctions have been observed particularly in *Rhinolophus ferrumequinum, Rh. hipposideros* as well as *Myotis myotis* (Kraus & Gauckler 1979, Schober & Wilheim 1983/1984, Helversen et al. 1987, Kokurewicz 1990, Rudolph 1990), and various approaches to protect and manage bat hibernacula were proposed (Voute & Lina 1986). The opposite upward trend in numbers observed at hibernation sites was reported in certain other bat species during recent decades (Daan et al. 1980, Gaisler et al. 1983/1984, Tress et al. 1989, Kowalski & Lesinski 1991, Urbanczyk 1992, Weinrich & Oude Voshaar 1992). The long-term dynamics in the abundance of European bats evokes a number of interpretations and questions (Horáček 1983/1984).

In the Czech Republic, an extensive winter census of bats in selected

important hibernacula was undertaken between 1969 and 1979 (Bárta et al. 1981, Gaisler et al. 1983/1984). All the bats observed were individually banded for subsequent identification. The 1969–1979 census revealed considerable decreases in the abundance of species evaluated as mesophilous seasonal cave-dwellers of South-European origin, i.e. *Rhinolophus hipposideros*, *Myotis myotis* and *Plecotus austriacus*. A stabilised or upward trend was observed in the species of supposed Central-European origin *(Myotis mystacinus, M. daubentoni, Plecotus auritus)*. These general results have been supplemented by evaluations of the bat number dynamics in individual wintering sites in Bohemia and Moravia (Gaisler & Bauerová 1977, Sklenář 1981, Červený 1982, Rumler 1985, Jóža & Kareš 1986, Eleder & Helešic 1987, Nevrlý 1987, Hanzal & Průcha 1988, 1992, Bauerová & Zima 1989, Červený & Bürger 1990, Anděra et al. 1992, Gaisler et al. in press).

The aim of the present article is to report on results obtained during winter census of bats in the Moravian karst between 1983 to 1992. The first 5 years of this census have already been reported by Bauerová et al. (1989), and report limited to one species was published by Gaisler (1991).

Study Site, Material and Methods

A total of 37 underground spaces were selected (for a detail site description see Bauerová et al. 1989). Although 40 sites were originally selected (Bauerová et al. 1989), some sites have been combined for the present study (Table 1). Only site 32 was an artificial gallery, all the others were natural karstic limestone caves. The sites are situated within a protected landscape area of Moravian karst. This region is in central Moravia, the Czech Republic, approximately 16° 40' E; 49° 20' N (quadrate No. 6665, 6666, 6667 after mapping grid used by the Czech Zoological Society). Northern (sites No. 1–25), central (sites No. 26–33) and southern (sites No. 34–37) part of the Moravian karst may be recognised, differing in their drainage basin systems.

Census method

The annual census took place regularly in January and February, and each study site was examined using a standardised route. The census was performed visually, and wintering bats were disturbed only quite exceptionally for the purpose of species identification or recording the band number. However, no new banding efforts were applied.

Results

Five groups of bats were distinguished in the visual census:

a) Lesser horseshoe bat, *Rhinolophus hipposideros*.

b) Large *Myotis* species. This group includes *Myotis myotis* and *M. blythi,* but the former species is considered largely prevailing according to previous studies (Gaisler 1977, Bauerová 1984).

Table 1. List of the wintering roosts examined by their local names with the mean number of bats found per one check (mean), the standard deviation of the mean number (SD), and the coefficient of variation (SD/mean)

	mean	SD	SD/mean
1. Sloupsko-Šošůvské jeskyně (cave)	218.5	68.8	0.315
2. jeskyně Pustožlebská 17 (cave)	60.5	16.3	0.269
3. jeskyně Řečiště (cave)	5.3	4.0	0.755
4. jeskyně Čertova kazatelna (cave)	0.7	1.2	1.700
5. Koudelkova propast I. (chasm)	46.8	14.7	0.313
6. jeskyně Bertalánka (cave)	6.3	1.9	0.302
7. Erichova jeskyně (cave)	36.2	21.6	0.597
8. Pássovského jeskyně (cave)	10.0	10.2	1.017
9. Červíkovy jeskyně (cave)	0.7	0.7	1.000
10. Punkevní jeskyně (cave)	2.6	1.4	0.523
11. Větrná jeskyně (cave)	19.0	10.0	0.526
12. jeskyně V Bučí (cave)	4.9	2.3	0.478
13. jeskyně Nová Rasovna (cave)	39.1	16.5	0.422
14. jeskyně Piková dáma (cave)	27.7	7.6	0.276
15. jeskyně Dagmar (cave)	18.0	5.1	0.281
16. Císařská jeskyně (cave)	7.5	2.3	0.305
17. jeskyně Balcarka (cave)	12.5	7.7	0.614
18. Liščí jeskyně (cave)	2.9	2.0	0.679
19. jeskyně Malý pes (cave)	21.9	6.6	0.303
20. jeskyně Suchožlebská zazděná (cave)	0.0	0.0	0.000
21. Komínová jeskyně (cave)	0.6	0.6	1.100
22. Králova jeskyně (cave)	70.8	14.9	0.210
23. jeskyně Koňská jáma (cave)	16.7	9.5	0.568
24. Kateřinská jeskyně (cave)	63.0	20.5	0.326
25. jeskyně Horní v Chobotu (cave)	6.0	1.0	0.167
26. Rudické propadání (cave)	55.6	13.9	0.251
27. jeskyně Nová Drátenická (cave)	27.0	5.4	0.199
28. jeskyně Stará Drátenická (cave)	17.4	7.1	0.410
29. Mariánská jeskyně (cave)	12.2	4.5	0.372
30. jeskyně Jestřábka (cave)	16.7	5.2	0.310
31. jeskyně Býčí skála (cave)	156.3	59.2	0.379
32. jeskyně Jáchymka (cave)	3.6	2.5	0.694
33. štola nad Švýcarnou (gallery)	7.4	2.9	0.397
34. Málčina jeskyně (cave)	10.7	3.4	0.316
35. jeskyně Netopýrka (cave)	1.6	1.3	0.800
36. Ochozská jeskyně (cave)	29.6	12.7	0.428
37. jeskyně Pekárna (cave)	0.8	0.8	0.938

c) Barbastelle, *Barbastella barbastellus.*

d) Long-eared bats of the genus *Plecotus.* The representation of both species, *Plecotus auritus* and *P. austriacus,* may be supposed approximately equal.

e) Small *Myotis* species, inclunding *Myotis mystacinus, M. brandti, M. bechsteini, M. nattereri, M. emarginatus, M. dasycneme* and *M. daubentoni.* The actual representation of individual species is not exactly known, but *M. emarginatus* and *M. daubentoni* may be considered more common than the other species.

The abundance of wintering bats varied considerably between the hibernation sites examined (Table 1). The average number of hibernating bats per one annual check exceeded 150 individuals in only two caves (sites No. 1 and 31), it was 50 to 149 individuals in four caves (sites No. 2, 22, 24, 26), and 10 to 49 individuals in 16 caves. The average number in the other sites was lower than

10 individuals, and in one locality (site No. 19) no bats were found during the whole census period. The variation coefficient of numbers found in the sites populated by more than 150 individuals on the average was 0.347, in the sites with 50–149 individuals 0.264, in the sites with 10–49 individuals 0.435, and in the sites with less than 10 individuals 0.703.

The large *Myotis* species represented the most frequently occurring group in the assemblages of wintering bats in Moravian karst. We assume that this group consisted mainly of *Myotis myotis,* which could thus be considered the most abundant bat species in this region. The highest constancy was also revealed in this group, represented in most of the wintering sites studied. Large *Myotis* species were found at 25 (67.6 %) to 31 (97.3 %) of the wintering sites in individual years of the census. In the whole census period, large *Myotis* individuals were found at 36 sites (97.3 %). The mean variation coefficient of *M. myotis* number the 10-year period was 0.423 for the sites populated by more than 10 individuals on the average. *Rhinolophus hipposideros* was the second species in term of abundance, being found at 16 to 22 sites (43.2–59.5 %) in individual years, and at 28 sites (75,7 %) during the whole census period. The mean variation coefficient of *Rh. hipposideros* numbers during the 10 years period was 0.340 for sites populated by more than 10 individuals on the average. *Barbastella barbastellus* was found at 9 to 12 sites (24.3–32.4 %) in individual years, and at 23 sites (62.2 %) during the whole census period. The mean variation coefficient of *B. barbastellus* numbers was 0.704 during the 10 years period for sites populated by more than 10 individuals on the average. The number of wintering *M. myotis* and *Rh. hipposideros* was thus generally more stable than that of *B. barbastellus. Plecotus* species were observed at 6 to 12 sites (16.2–32.4 %) in individual years, and at 28 sites (75.7 %) in total. Small *Myotis* species were recorded at 10 to 24 sites (27.0 –64.9 %) in individual years, and at 33 sites (89.2 %) in total.

Table 2. Numbers of bats found in individual regions of the Moravian Karst between 1983 and 1992

	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992
Total numbers	703	757	857	956	874	1082	1121	1415	1399	1262
Northern part	466	511	612	594	574	750	753	931	979	871
Central part	202	199	206	296	249	304	349	449	378	345
Southern part	35	47	57	66	51	28	19	35	42	46
Myotis myotis	323	408	493	540	481	636	715	918	879	764
R. hipposideros	278	233	233	269	242	295	297	299	355	373
B. barbastellus	41	42	62	70	76	62	17	64	74	42
Plecotus spp.	7	16	6	16	10	13	18	11	12	10
Myotis spp.	30	53	79	58	60	71	72	116	77	70

In all sites coupled, 703 to 1415 wintering bats were found in individual years, and a significant upward trend in numbers was observed during the whole census (Tables 2 and 3; Figs. 1 and 2). This upward trend was apparent namely in the sites situated in northern and central part of the Moravian karst, whereas the numbers of bats were rather decreasing in the southern part. The number of

sites studied and the number of bats found in the southern part of the Moravian karst was, however, rather low so that the results from this area had little influence on the overall trend.

Tab. 3. Results of linear regression and correlation analysis , see Figs. 2 and 3 (X – independent variable, Y – dependent variable, a, b – regression coefficients, r – correlation coefficient, * – statistically significant)

X	Y	a	b	r	P
Years	Total numbers	–5860.88	78.90	0.93	0.00008 *
Years	Northern part	–4158.25	55.47	0.94	0.00007 *
	Central part	–1807.07	24.05	0.86	0.001 *
	Southern part	153.96	–1.27	–0.28	0.44
Years	*Myotis myotis*	–4724.98	61.04	0.92	0.0001 *
	R. hipposideros	–769.08	12.10	0.77	0.01 *
	B. barbastellus	17.88	0.42	0.07	0.85
	Plecotus spp.	–5.60	0.20	0.15	0.67
	Myotis spp.	–284.01	4.04	0.61	0.06

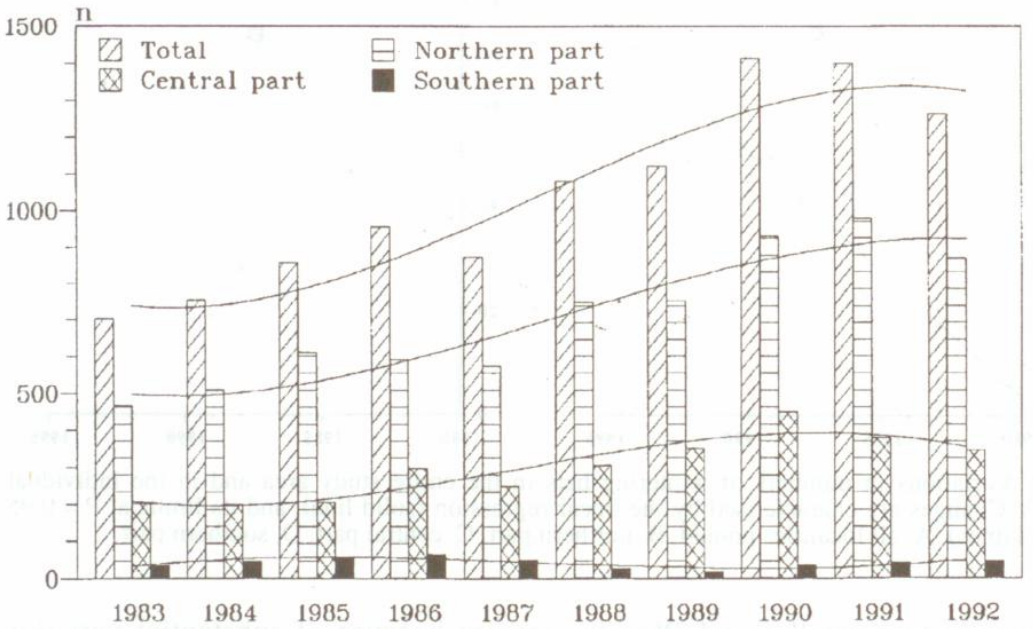


Fig. 1. Variations in the number of wintering bats in the Moravian Karst in 1983 to 1992. Changes are demonstrated by the cubic regression (solid line).

The changes in numbers of the large *Myotis* species were quite similar to those observed in the whole wintering bat assemblage. Therefore, we can conclude that the dynamics of this particular group (*M. myotis* largely prevailing) influenced substantially the overall trend. The abundance of *Rhinolophus hipposideros* was rather stable in the first half of the census period but it begun to increase afterwards, so that an upward trend in numbers of this species can be demonstrated for the whole study period. No trends in numbers were apparent in

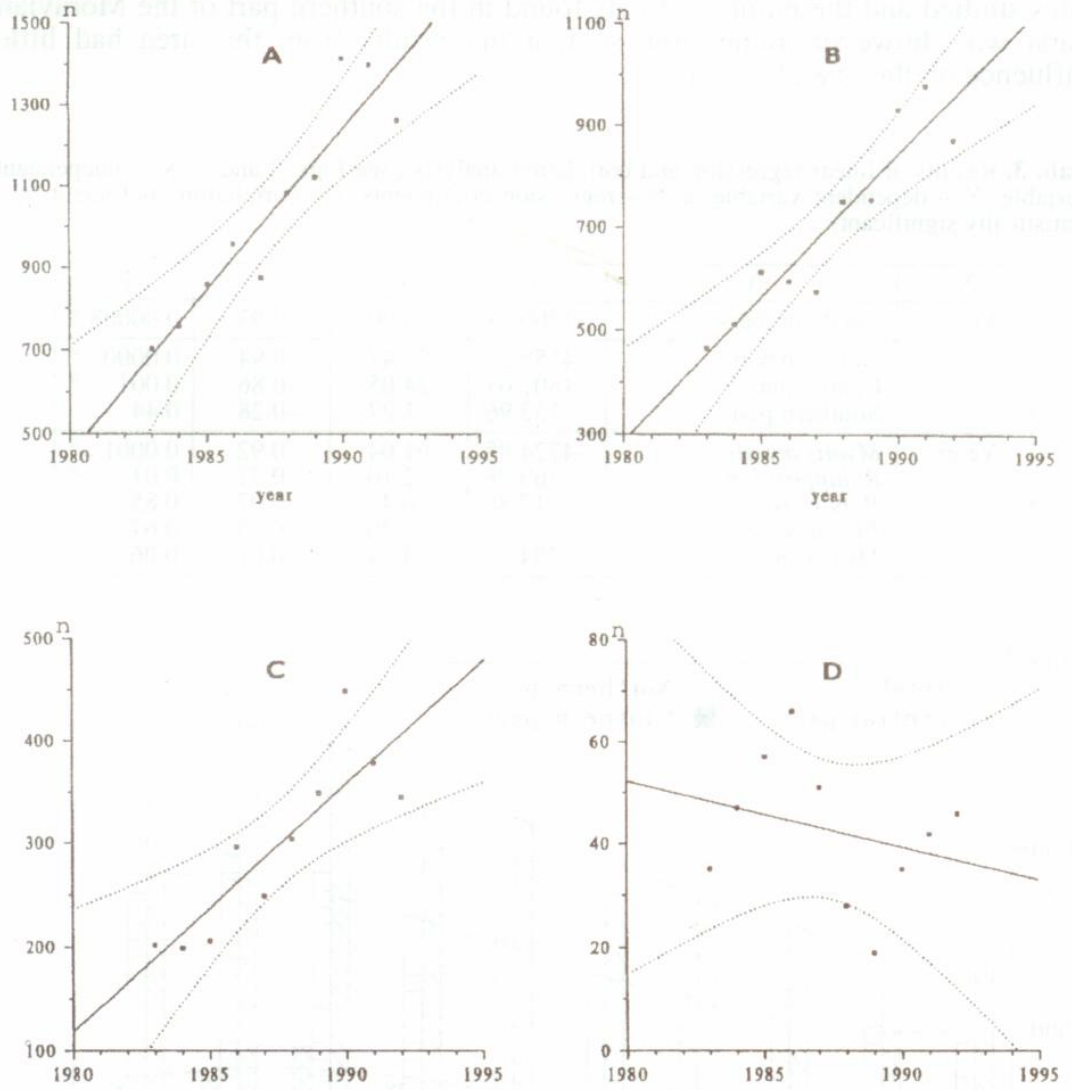


Fig. 2. Variations in numbers of wintering bats in the entire study area and in the individual regions. Changes are characterised by the linear regression (solid line), and its limits at P = 0.95 (dashed lines). A. all localities pooled, B. northern part, C. central part, D. southern part.

Barbastella barbastellus and *Plecotus* species because of substantial variation in their abundance. A moderate upward trend was apparent in numbers of wintering individuals of small *Myotis* species (Tables 2 and 3; Figs. 3 and 4).

Discussion

The population decline observed in European bats during the last decades has been usually interpreted as a general trend, which has been strikingly expressed namely in rhinolophids and *Myotis myotis* (R o e r 1980/1981, 1983/1984, H o r á č e k 1983/1984). Surprisingly, an upward trend was demonstrated either

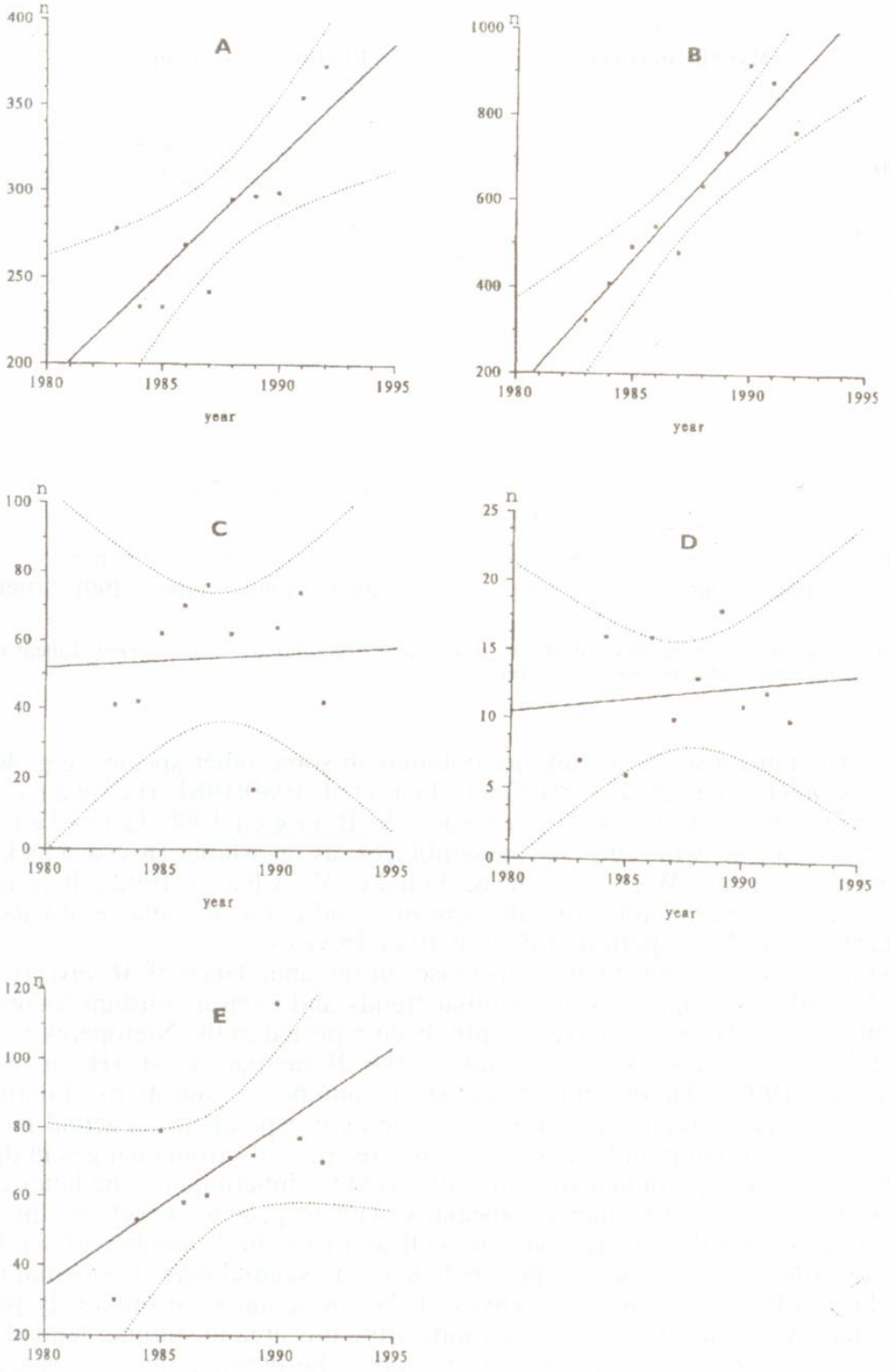


Fig. 3. Variations in numbers of individual species or species groups. For detail see Fig. 2. A. *Rhinolophus hipposideros,* B. *Myotis myotis,* C. *Barbastella barbastellus*, D. *Plecotus* spp., E. *Myotis* spp.

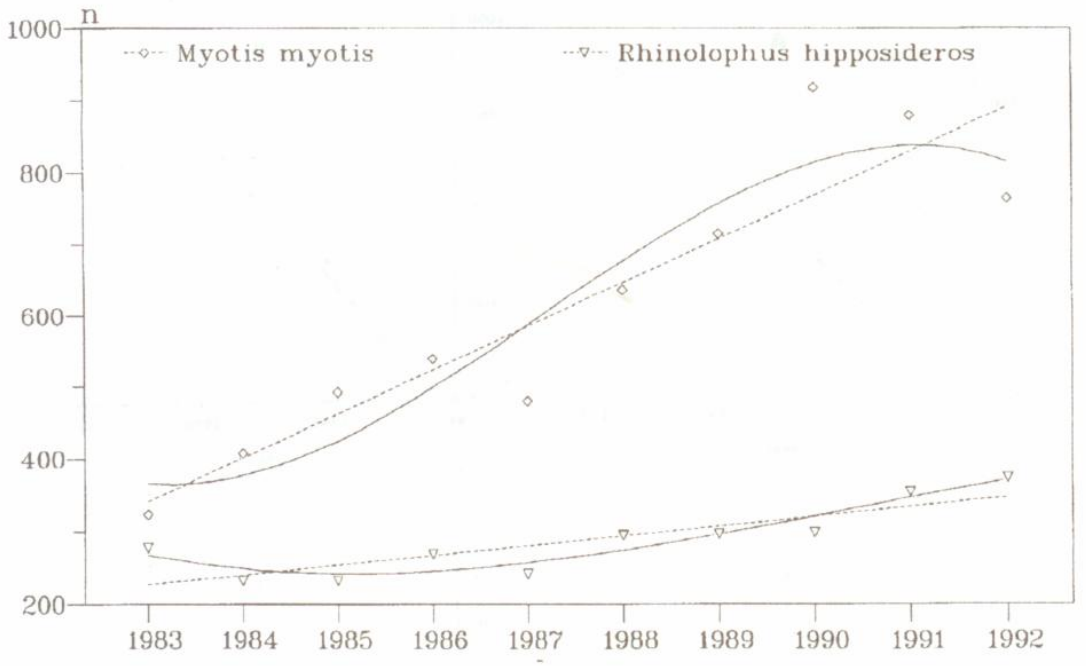


Fig. 4. Variations in the number of *Myotis myotis* and *Rhinolophus hipposideros*. Linear regression – dashed line, cubic regression – solid line.

in hibernation roosts or in summer colonies in some other species, e.g. *Myotis daubentoni* (D a a n et al. 1980, G a i s l e r et al. 1980/1981, H e l v e r s e n et al. 1987), *Eptesicus nilssoni* (Č e r v e n ý & B ü r g e r 1990, G a i s l e r et al. in press), or in wintering bat assemblages as a whole (K o w a l s k i & L e s i n s k i 1991, W e i n r i c h & O u d e V o s h a a r 1992). It is remarkable that the results indicating the upward trend in the abundance of bats have concerned mainly the period of the last 10 or 15 years.

We found a very conspicuous increase in the abundance of *M. myotis* and a slight trend in *R. hipposideros*. Similar trends and even a constant increase in the numbers of *M. myotis* have recently been reported in the Nietoperek reserve, Poland (U r b a n c z y k 1992) and in the Bohemian karst (H a n z a l & P r ů c h a 1992). These findings evoke a number of questions. Firstly, we should consider if the observed upward trend corresponds to an actual increase in the density of bat populations, and/or if it results only from changes in dispersal preferences of particular roosting sites used for hibernation. The latter explanation seems to be less likely, especially with respect to reports of the same phenomenon in different regions, as well as to the high number of wintering sites examined in this study, which included a substantial part of potential hibernacula in a broader region. The caves of the Moravian karst obviously present important wintering shelters for bats inhabiting this area in summer, and also for other populations from neighbouring regions. The premise that the increase in the numbers of hibernating bats indicates the growing population density is further supported by observations made in some nursery colonies of *M. myotis* located in the Moravian karst and adjacent regions (unpublished data).

Secondly, direct human disturbance and pursuit, damage of suitable roosting

sites, and environmental pollution affecting the food base are usually considered the common causes for a decline in European bat populations. Which of these factors could change substantially their impact in the region under study to evoke an increase in the abundance of bats? Although the area and the caves themselves have been under protection for many decades, the improvement in conservation measures could be considered a positive influence. Note that a change to the visual census method avoided any disturbance of the hibernating bats, another favourable factor. Nevertheless, it is hardly realistic to explain the increase in numbers of hibernating bat populations in the Moravian karst only by protection of the landscape and the caves and the change in census method.

It is difficult to find any other potential human factor or impact that has been essentially changed or limited in the region during the last decades. The most plausible interpretation of the reported change in numbers seems to be the natural process of the population dynamics that may be influenced by various natural factors as noticed previously by H o r á č e k (1983/1984).

A c k n o w l e d g e m e n t s

This investigation is dedicated to the memory of our friend and colleague, Dr. Zdeňka B a u e r o v á. Collaboration of our colleagues and friends who helped to collect the census data is greatly acknowledged: The late Dr. Zdeňka B a u e r o v á, I. B a l á k, J. Č e r v e n ý, J. H e l e š i c, M. H o n z a, I. H o r á č e k, J. K o k e š, M. K u b e š, R. N e p r a š, J. P e l l a n t o v á, Z. Š t r o u f o v á, M. V l a š í n, P. Z e m a n. Collaboration with the Management Office of the Protected Landscape Area of the Moravian Karst helped us much in realisation of this project. Thanks are due also to local groups of the Czech Speleological Society for their help and support in this census.

The field research was supported by the grant No. P 006 by the Ministry of Economy of the Czech Republic, and by financial support from the Czech Bat Conservation Trust.

LITERATURE

ANDĚRA, M., ZBYTKOVSKÝ, P., BÜRGER, P., 1992: Bat community of the Chýnovská jeskyně cave (southern Bohemia, Czechoslovakia) in 1981–1986. *Prague Studies in Mammalogy (I. Horáček and V. Vohralík, eds.), Charles University Press, Praha: 1–11.*

BAAR, A., MAYER, A., WIRTH, J., 1986: 150 Jahre Fledermausforschung in der Hermannshöhle. *Ann. Naturhist. Mus. Wien, 88/89, B: 223–243.*

BÁRTA, Z., ČERVENÝ, J., GAISLER, J., HANÁK, P., HANÁK, V., HORÁČEK, I., HŮRKA, L., MILES, P., NEVRLÝ, M., RUMLER, Z., SKLENÁŘ, J., ŽALMAN, J., 1981: Výsledky zimního sčítání netopýrů v Československu: 1969–1979 [Results of the winter census of bats in Czechoslovakia; 1969–1079]. *Sborn. Okr. Muz. v Mostě, ř. Přír., 3/1981: 71–116 (in Czech, with a summary in English).*

BAUEROVÁ, Z., 1984: Zur Fledermausfauna des Mährischen Karstes. *Nyctalus (N. F.), 2: 65–71.*

BAUEROVÁ, Z., GAISLER, J., KOVAŘÍK, M., ZIMA, J., 1989: Variation in numbers of hibernating bats in the Moravian karst: results of visual censuses in 1983–1987. *European Bat research 1987 (V. Hanák, I. Horáček, J. Gaisler, eds.), Charles Univ. Press, Praha: 499–505.*

BAUEROVÁ, Z., & ZIMA, J., 1988: Výzkum netopýrů v jeskyni Býčí skála v letech 1977–1986 [Bat research and census in the Býčí skála cave in 1977–1986]. *Čs. kras, 39: 51–59 (in the Czech, with a summary in English).*

BEZEM, J. J., SLUITER, J. W., van HEERDT, P. F., 1960: Population statistics of five species of the bat genus Myotis and one of the genus Rhinolophus, hibernating in the caves of S. Limburg. *Arch. Neerl. Zool., 13: 511–539.*

ČERVENÝ, J., 1982: Results of investigation

of bats (Chiroptera) at Loreta near Klatovy. *Lynx, n.s., 21: 41–65.*

ČERVENÝ, J., BÜRGER, P., 1990: Changes in bat population sizes in the Šumava Mts. (south-west Bohemia). *Folia Zool., 39: 213–226.*

DAAN, S., GLAS, G. H., VOUTE, A. M., (eds.), 1980: Long term changes in bat populations in the Netherlands. *Lutra, 22: 1–118.*

DORGELO, J. & PUNT, A., 1969: Abundance and „internal migration" of hibernating bats in an artificial limestone cave („Sibbergroeve"). *Lynx, n.s., 10: 101–125.*

ELEDER, P. & HELEŠIC, J., 1987: Výsledky šestiletého průzkumu podzemních zimovišť netopýrů (Chiroptera) v moravské části Českomoravské vrchoviny [The results of the six years research concerning the underground bat (Chiroptera) hibernation localities in the Moravian part of the Czech-Moravian Higlands]. *Acta Sci. Nat. Mus. Moraviae Occ., Třebíč, 15: 21–34 (in Czech, with a summary in English).*

GAISLER, J., 1977: Moravian caves as a natural habitat of bats (Chiroptera). *Proc. 6th Int. Congr. Speleology, Olomouc: 93–100.*

GAISLER, J., 1991: The status of Rhinolophus hipposideros in S-Moravia (CS). *Myotis, 29: 105–108.*

GAISLER, J. & BAUEROVÁ, Z., 1977: Společenstvo netopýrů (Chiroptera) na Květnici během třiceti let [The bat community at Květnice in the course of thirty years]. *Lynx, n.s., 19: 17–28 (in Czech, with a summary in English).*

GAISLER, J., HANÁK, V., HORÁČEK, I., 1980/1981: Remarks on the current status of bat populations in Czechoslovakia. *Myotis, 18/19: 68–75.*

GAISLER, J., ŠEBELA, M., DUNGEL, J., in press: Kolísání počtu netopýrů přezimujících v opuštěných štolách u Malé Morávky [Variation in numbers of bats hibernating in abandoned mine galleries near Malá Morávka (Czech Republic)]. *Acta Mus. Moravie (in Czech, with a summary in English).*

HAENSEL, J., 1980/1981: Zur Bestandsentwicklung der Fledermäuse in einigen langjährig unter Kontrolle gehaltenen Winterquartieren der DDR. *Myotis, 18/19: 45–47.*

HANZAL, V. & PRŮCHA, M., 1988: Sezónní dynamika netopýřích společenstev na zimovištích Českého krasu v letech 1984–1986 [Seasonal dynamics of bat communities at winter shelters of Český kras (central Bohemia) during 1984–1986]. *Lynx (Praha) n.s., 24/1988: 15–35 (in Czech, with a summary* in English).

HANZAL, V. & PRŮCHA, M., 1992: Changes in the numbers of bats hibernating in the Bohemian karst during 1969–1987. *Prague Studies in Mammalogy (I. Horáček and V. Vohralík, eds.), Charles University Press, Praha: 71–74.*

HELVERSEN, O., von, ESCHE, M., KRETZCHMAR, F., BOSCHERT, M., 1987: Die Fledermäuse Südbadens. *Mitt. bad. Landesver. Naturkunde u. Naturschutz, N. F., 14: 409–475.*

HORÁČEK, I., 1983/1984: Remarks on the causality of population decline in European bats. *Myotis, 21/22: 137–147.*

JÓŽA, M. & KAREŠ, M., 1986: Zimoviště netopýrů v komplexu starých štol u Nového Města pod Smrkem v Jizerských horách [Winterquartiere der Fledermäuse in alten Stollen bei Nové Město pod Smrkem im Jizerské hory (Isergebirge)]. *Sborn. Severočes. Muz. – Přír. vědy, 15: 139–163 (in Czech, with a summary in German).*

KOKUREWICZ, T., 1990: The decrease in abundance of the lesser horseshoe bat Rhinolophus hipposideros Bechstein, 1800 (Chiroptera, Rhinolophidae) in winter quarters in Poland. *Myotis, 28: 109–118.*

KOWALSKI, M. & LESINSKI, G., 1991: Changes in number of bats in Szachownica cave (central Poland) during 10 years. *Myotis, 29: 35–38.*

KRAUS, M. & GAUCKLER, A., 1979: Zur Abnahme der Kleinen Hufeisennase (Rhinolophus hipposideros) in den Winterquartieren der Frankenalb (Nordbayern) zwischen 1958–1980. *Myotis, 18: 3–12.*

NEVRLÝ, M., 1987: Dvacet pět let výzkumu netopýrů na Bílé Desné v Jizerských horách [Fünfundzwanzig Jahre der Fledermausforschung im Isergebirge]. *Sborn. Severočes. Muz. – Přír. Vědy, 16: 231–272 (in Czech, with a summary in German).*

PREISS, G., 1982: Vierjährige Bestandserhebungen überwinternder Fledermäuse in Frankischen Karstholen und Schutzmassnamen. *Myotis, 20: 11–34.*

ROER, H., 1908/1981: Zur Bestandsentwicklung einiger Fledermäuse in Mitteleuropa. *Myotis, 18/19: 60–67.*

ROER, H., 1983/1984: Zur Bestandsituation von Rhinolophus ferrumequinum (Schreber, 1774) und Rhinolophus hipposideros (Bechstein, 1800) (Chiroptera) im westlichen Mitteleuropa. *Myotis, 21/22: 122–131.*

RUDOLPH, B.-U., 1990: Frühere Bestandsdichte und heutige Situation der Kleinen Hufeisennase Rhinolophus hipposideros in Nordbayern. *Myotis, 28: 101–108.*

RUMLER, Z., 1985: Výsledky chiropterologických průzkumů některých podzemních prostorů Beskyd a Oderských vrchů v letech 1976–1982 (Mammalia, Chiroptera)

[The results of the chiropterological investigations of some underground spaces in the Beskydy and Oderské vrchy Mts. in the years 1976–1982]. *Čas. Slez. Muz. Opava (A), 34: 75–89 (in Czech, with a summary in English).*

SCHOBER, W. & WILHELM, M., 1983/1984: Zur Vebreitung und Bestandsentwicklung der Kleinen Huffeisennase (Rhinolophus hipposideros) in der DDR. *Myotis, 21/22: 132–137.*

SKLENÁŘ, J., 1981: Deset let výzkumu zimoviště netopýrů (Chiroptera) v Orlických horách [Ten years of research into the winter roosts of bats (Chiroptera) in the Orlické hory Mts.]. *Acta Mus. Reginaehrad. S. A. Sci. Nat., 16: 273–288 (in Czech).*

TRESS, C., TRESS, J., HENKEL, F.,1989: Methodik und Ergebnisse der Bestandeskontrolle von Myotis myotis. *Wiss. Beitr. Univ. Halle, 20: 139–155.*

URBANCZYK, Z., 1989: Changes in the population of bats in the „Nietopierek" Bat Reserve in 1975–1987. *European Bat Research 1987 (V. Hanák, I. Horáček, J. Gaisler, eds), Charles Univ. Press, Praha: 507–510.*

URBANCZYK, Z., 1992: Significance of the Nietoperek Reserve for central European population of Myotis myotis (Mammalia: Chiroptera). *Prague Studies in Mammalogy (I. Horáček and V. Vohralík, eds.), Charles University Press, Praha: 213–215.*

VOUTE, A. M. & LINA, P. H. C., 1986: Management effects on bat hibernacula in the Netherlands. *Biol. Conserv., 38: 163–178.*

WEINRICH, J. A. & OUDE VOSHAAR, J. H., 1992: Population trends of bats hibernating in marl caves in the Netherlands (1943–1987). *Myotis, 30: 75–84.*

Authors' addresses:

Prof. Dr. Jiří GAISLER, DrSc.,
Dr. Zdeněk ŘEHÁK,

Department of Zoology and Ecology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Dr. Miroslav KOVAŘÍK,

Nature Conservancy Moravský kras, Sadová 2, 678 01 Blansko, Czech Republic

Dr. Jan ZIMA, CSc.,

Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Veveří 97, 602 00 Brno 2, Czech Republic

Mgr. Jan ZUKAL,

Institute of Landscape Ecology, Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic

Změny početnosti a struktura společenstva netopýrů

Zukal, J., Kovařík, M., Řehák, Z. & Berková, H. (2001) Početnost netopýrů zimujících ve dvou jeskyních v severní části Moravského krasu a její dlouhodobé změny. Vespertilio 5, 321-328.

Změny početnosti a struktura společenstva netopýrů

Zukal, J., Řehák, Z. & Kovařík, M. (2003) Netopýři Sloupsko - šošůvských jeskyní (Moravský kras). Lynx (Praha) n.s. 34, 205-220.

Lynx (Praha), n. s., **34**: 205–220 (2003). ISSN 0024–7774

Netopýři Sloupsko-šošůvských jeskyní (Moravský kras)

Bats of the Sloupsko-šošůvské jeskyně cave (Moravian Karst, Central Moravia)

Jan ZUKAL[1], Zdeněk ŘEHÁK[2] & Miroslav KOVAŘÍK[3]

[1] Ústav biologie obratlovců AV ČR, Květná 8, 603 65 Brno
[2] Katedra zoologie a ekologie, PřF MU, Kotlářská 2, 611 37 Brno
[3] Správa CHKO ČR, CHKO Moravský kras, Svitavská 29, 678 01 Blansko

došlo 10. 11. 2003

Abstract. The bat community of the Sloupsko-šošůvské jeskyně cave (a natural karstic cave, Moravian Karst) was studied using two methods, a regular winter census of hibernating bats and netting at the cave entrance. In total, 15 bat species have been recorded. The bat community is characterised by a significant dominance of *Myotis myotis* in both seasons (58.5 % and 53.8 %, respectively). The changes in numbers of hibernating bats have shown a similar trend to that found in other caves or regions, viz., a strong increase of *M. myotis* and a stabilization of *Rhinolophus hipposideros* population with subsequent increase (late 1990s). The maximum abundance of bats in the cave during hibernation was recorded in early March (3 March 1993 – 482 ind.). The highest intensity of cave visitation occurred in the C period (from 15 July to 14 September). *M. myotis* individuals occupied only one specific part of the cave system during hibernation (80% of findings), while all parts of the cave were used by *R. hipposideros*.

ÚVOD

Netopýři se vyznačují unikátní kombinací ekologických adaptací, které ve svém komplexu nemají ve srovnání s jinými skupinami savců obdobu. Jsou jedinými savci schopnými aktivního letu a jejich letová aktivita spojená se získáváním potravy je posunuta do nočních hodin. Ve tmě registrují překážky a detekují kořist prostřednictvím echolokace. Morfologické a fyziologické adaptace jim přitom umožňují využívat rozmanité ekologické niky a zajišťují také jejich ekologickou rozmanitost.

Netopýři jsou významnými složkami ekosystémů, mají nezastupitelnou roli v potravních řetězcích. Jsou schopni kompenzovat vysoký energetický výdej, který aktivní let vyžaduje, obdobími s minimálními energetickými ztrátami. Pro denní odpočinek vyhledávají nejčastěji různě prostorné dutiny. Klimaticky nepříznivá období roku přečkávají v hlubokém letargickém stavu. V podmínkách mírného pásma se jedná o zimování – hibernaci, obvykle v rozsáhlých podzemních prostorech.

K územím s dostatkem podzemních prostorů patří zejména krasové oblasti. K největším z nich na území ČR náleží Moravský kras a Český kras (cf. Kolektiv 2001). Obě oblasti patří k chiropterologicky nejlépe prozkoumaným oblastem České republiky. První zprávy o netopýrech Moravského krasu se objevují v souvislosti s průzkumem krasového podzemí již ve 2. polovině 19. století. Patří k nim zejména studie Kolenatiho (1851) o netopýrech Sloupských jeskyní. Souborné informace o jeskynní netopýří fauně uvádí práce Wankela (1860), která se vedle faunistických

údajů také dotýká podmínek hibernace netopýrů v jeskyních. Na přelomu století publikuje další faunistická data Wankelův vnuk Absolon, který se však z větší části opírá právě o díla Kolenatiho a Wankela. V té době je již v Moravském krasu evidováno 12 druhů netopýrů (Absolon 1899).

Souhrn všech faunistických údajů od počátku sledování až do 50. let 20. století včetně kompletní bibliografie publikoval Gaisler (1956), který také o rok později zahájil systematický výzkum netopýrů Moravského krasu. Již od počátku měl tento výzkum faunisticko-ekologický charakter (Gaisler 1962). Sloupsko-šošůvské jeskyně byly do něj jako významná lokalita pochopitelně zahrnuty a výsledky zde získané byly publikovány v pracích o kroužkování netopýrů na území Československa (Gaisler & Hanák 1969) nebo o zimním sledování netopýrů v podzemních úkrytech (Gaisler & Hanák 1972).

Bohužel, Sloupsko-šošůvské jeskyně nebyly, pravděpodobně pro svou rozlehlost, zahrnuty do celostátního zimního sčítání netopýrů organizovaného v letech 1969–1979 (Bárta et al. 1981). Teprve v roce 1981 byly jeskyně zařazeny do navazujícího 10letého projektu, který byl prováděn jinou metodikou sledování, umožňující zapojení také amatérských chiropterologů (Zima et al. 1994). V současnosti probíhá zimní sčítání netopýrů, řízené a podporované Českou společností pro ochranu netopýrů (ČESON), na cca 18 lokalitách Moravského krasu včetně Sloupsko-šošůvských jeskyní (Kovařík 1997).

Podstatně kratší historii má výzkum netopýrů v mimohibernačním období, a to jak v celém Moravském krasu, tak i v oblasti Sloupsko-šošůvských jeskyní. První práce se zabývají téměř výhradně letními koloniemi netopýrů z území Moravského krasu (Gaisler 1962), případně přelety kroužkovaných netopýrů (Gaisler & Hanák 1969). Zájem o výzkum netopýrů v letním období ovšem výrazně vzrostl díky rozvoji metod umožňujících odchyt netopýrů v letové fázi. V roce 1971 byly poprvé v Československu použity k odchytu japonské nárazové sítě (mist nets) právě v Moravském krasu. Byly exponovány před vchody jeskyní a přes potok v jižní části krasu, v údolí Říčky (Gaisler 1973). V dalších letech byli netopýři chytáni také před vchodem do jeskyně Býčí skála (Bauerová & Zima 1988a) a Hladomorna u Holštejna (Bauerová & Zima 1988b). U Sloupsko-šošůvských jeskyní byly, i přes jejich velký význam pro společenstvo netopýrů, první výzkumy v mimohibernačním období prováděny až v letech 1991–1994 (Řehák et al. 1994, Řehák 1995). Jejich výsledky jsou prezentovány také v této práci.

Cílem naší práce je shrnout dosavadní poznatky o netopýrech využívajících Sloupsko-šošůvské jeskyně, a to jak z hlediska faunistického, tak z hlediska změn početnosti, resp. úrovně aktivity vybraných druhů.

POPIS LOKALITY

Sloupsko-šošůvské jeskyně se nacházejí na severní hranici Moravského krasu (49° 25’ s. š., 16° 45’ v. d., čtverec zoologického mapování ČR 6566). Byly vytvořeny ve vápencích svrchního devonu v ponorové oblasti Sloupského potoka. Podzemní prostory jsou rozloženy ve dvou úrovních, spojených 60–80 m hlubokými propastmi a celková zaměřená délka systému dosahuje cca 7 km. Jedná se o velice složitý jeskynní labyrint tvořený jak poměrně úzkými chodbami, tak i velkými dómy (Hlavní dóm má rozměry 70×40×10 m). Střídají se zde mikroklimaticky statické části s úseky, které se spíše projevují jako dynamické. Jeskyně mají 6 různě upravených vchodů (460,0–471,8 m n. m.), které jsou vázány na údolní nivu Sloupského potoka. Nejmohutnějším vchodem je portál, který ústí dvěma chodbami do Nicové jeskyně (23×9 m) a je upraven jako vstup pro veřejnost.

Sloupsko-šošůvské jeskyně byly v minulosti člověkem značně devastovány a často zde docházelo k rozsáhlým změnám. Jeskynní systém původně tvořily 3 samostatné celky, které byly od roku 1879

postupně spojovány. V roce 1923 byly jeskyně zpřístupněny veřejnosti jako jeden celek. Další úpravy jeskyní byly prováděny v roce 1978 a nově také v letech 1997–1999, kdy byla rekonstruována turistická trasa v jeskyních. Veřejnosti přístupná je pouze část jeskyní v oblasti horních pater (cca 3,5 km), přičemž ročně si jeskyně prohlédne kolem 70.000 návštěvníků. Od začátku listopadu do konce ledna bývají jeskyně pro veřejnost uzavřeny, celoročně jsou však využívány pro speleoterapeutickou léčbu astmatických dětí.

MATERIÁL A METODIKA

Monitoring zimujících netopýrů

K analýze dlouhodobých změn početnosti zimujících netopýrů byly použity vždy jen výsledky jedné zimní kontroly v roce, prováděné nejčastěji v druhé polovině ledna. Během sporadických kontrol v období před rokem 1981 byli netopýři odchytáváni a kroužkováni (Bárta et al. 1981). Od roku 1981 však bylo sčítání prováděno výhradně vizuálně bez rušení zimujících jedinců (Bauerová et al. 1989); pouze výjimečně byli netopýři bráni do rukou ve snaze o přesné určení druhu (zejména u vzácných hibernantů, např. *Myotis dasycneme*). Pro posouzení krátkodobých změn početnosti a významnosti různých částí jeskyní pro zimující společenstvo netopýrů byly jeskyně v zimní sezóně 1992/1993 (od 9. 10. do 12. 5.) pravidelně sledovány v intervalu 3–4 týdny. Při tomto výzkumu byly jeskyně rozděleny na 17 menších částí, které se více či méně liší svojí morfologií. Sčítání jinak probíhalo stejným způsobem jako při dlouhodobém monitoringu, tj. bez rušení zimujících netopýrů.

Protože metodou vizuálního sčítání nelze vždy provést přesnou druhovou identifikaci, byly v některých případech, vedle jednoznačně určených druhů, pouze rozlišovány tyto skupiny druhů:
(a) *Myotis mystacinus/brandtii* – neurčení jedinci druhů *Myotis mystacinus* a *M. brandtii*, tzv. "sibling species";
(b) Mspp. – sedm menších druhů rodu *Myotis* (*M. mystacinus*, *M. brandtii*, *M. bechsteinii*, *M. emarginatus*, *M. nattereri*, *M. daubentonii*, *M. dasycneme*);
(c) Pspp. – netopýři rodu *Plecotus* (*P. auritus a P. austriacus*).

V případě uváděnch počtů druhu *M. myotis* nemůžeme vyloučit, že se mezi zimujícími jedinci nevyskytl také velmi podobný a blízce příbuzný *M. blythii*, jehož odlišení na základě pouhého pozorování je velmi obtížné až nemožné.

V letech 1958 až 2002 bylo provedeno celkem 28 zimních kontrol, při nichž bylo nalezeno 9689 netopýrů (tab. 1 a 2). Během 11 kontrol v zimní sezóně 1992/1993 pak bylo zaznamenáno celkem 2676 nálezů zimujících netopýrů (tab. 3).

Sledování aktivity v mimohibernačním období

Sledování letové aktivity netopýrů probíhalo v období od srpna 1991 do října 1994 v prostoru hlavního vchodu Sloupsko-šošůvských jeskyní. K výzkumu byl použit odchyt do zdvojených japonských nárazových sítí, které byly umístěny rovnoběžně do prostoru předsíně nad schodištěm. Zatímco vnitřní síť uzavírala obě paralelní vstupní chodby, vnější síť vedla podél zábradlí nad schodištěm (Řehák 1995). V obou případech byly použity sítě o délce 9 m. Byly používány sítě se 4 horizontálními záchytnými poli o celkové výšce 1,6 m; délka a umístění sítí se v průběhu výzkumu neměnily.

Vlastní odchyt začínal před západem Slunce a končil nejčastěji ve 24 hod. SEČ. Na počátku podzimu pokračoval odchyt v některých případech až do ranních hodin. Netopýři byli zpracováváni bezprostředně po odchytu, a po vyšetření a označení hliníkovým kroužkem byli vypouštěni na místě odchytu. Vedle druhového určení bylo zjišťováno pohlaví, věk a pohlavní aktivita. Dále byla posuvným měřítkem změřena délka předloktí a pesolou stanovena hmotnost. Z dalších údajů byly zaznamenány čas odchytu a směr letu. Celkem bylo během 11 akcí (20. 8. 1991, 12. 5. 1992, 26. 8. 1992, 1. 10. 1992, 14. 4. 1993, 9. 6. 1993, 14. 9. 1993, 24. 9. 1993, 25. 9. 1993, 20. 4. 1994 a 12. 10. 1994), což představovalo celkem 68 odchytových hodin, odchyceno 437 netopýrů 14 druhů (tab. 4). Za míru aktivity byl zvolen relativní počet jedinců

vzhledem k trvání odchytu – N/t (ks/hod). Relativizace hodnot umožňuje srovnání vzorků s rozdílnou frekvencí, příp. i rozdílnou délkou trvání jednotlivých odchytů. Pro posouzení sezónních změn v aktivitě byly vymezeny v souvislosti s ročním cyklem netopýrů čtyři dvouměsíční periody, tj. období A – opouštění zimovišť a jarní přelety (od 15. 3. do 14. 5.), období B – letní kolonie (od 15. 5. do 14. 7.), období C – rozpad letních kolonií a podzimní přelety (od 15. 7. do 14. 9.) a období D – podzimní přelety a zahájení zimování (od 15. 9. do 14. 11.).

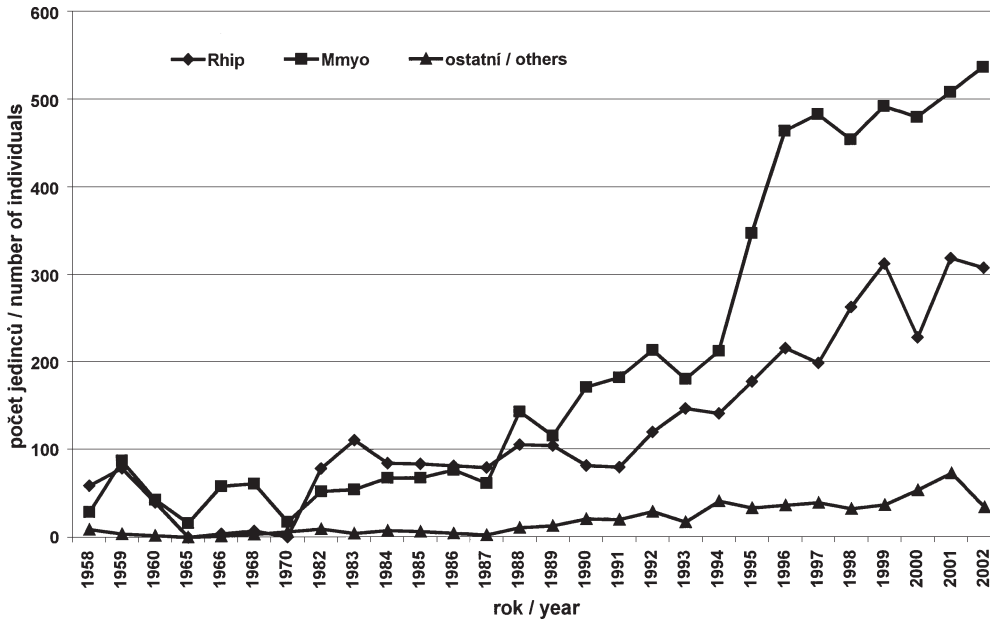
Hodnocení změn početnosti bylo provedeno pomocí statistického programu SYSTAT (Wilkinson 1990). Pro testování vztahu mezi jednotlivými roky zimního monitoringu a početností netopýrů byl použit základní Pearsonův korelační koeficient. Pro hodnocení aktivity společenstva netopýrů v jednotlivých částech mimohibernačního období byl použit medián ze všech hodnot relativní aktivity vypočtených pro jednotlivé noci. Rozdíly v aktivitě byly následně testovány Kruskal-Wallisovým testem.

V práci jsou použity jen vědecké názvy jednotlivých druhů netopýrů. V tabulkách a grafech jsou často z technických důvodů uvedeny zkratky těchto názvů. První velké písmeno je počátečním písmenem rodového názvu, následující tři malá písmena jsou počátečními písmeny názvu druhového (např. *Myotis myotis* – Mmyo, všechny zkratky viz tab. 1)

VÝSLEDKY

Struktura společenstva

Během výzkumu bylo zaznamenáno celkem 15 druhů netopýrů (tab. 1 a 4), z toho 12 během celého roku. Druhy *Myotis bechsteinii* a *Nyctalus noctula* byly zjištěny pouze v mimohibernač-



Obr. 1. Dlouhodobý vývoj početnosti netopýrů zimujících na lokalitě Sloupsko-šošůvské jeskyně.
Fig. 1. Long-term changes in numbers of bats hibernating at the Sloupsko-šošůvské cave.

Tab. 1. Celkové výsledky sčítání netopýrů zimujících ve Sloupsko-šošůvských jeskyních (27 kontrol v letech 1958–2001). Zkratky skupin druhů viz kapitola Materiál a Metodika
Tab. 1. Summary results of censuses of bats hibernating in the Sloupsko-šošůvské jeskyně caves (27 checks in 1958–2001). See chapter of Material and Methods for abbreviations of species groups

druh / species		zkratka / abbrev.	počet / no.
vrápenec malý	*Rhinolophus hipposideros*	Rhip	3494
netopýr vousatý	*Myotis mystacinus*	Mmys	9
netopýr Brandtův	*Myotis brandtii*	Mbra	5
	Myotis mystacinus / brandtii		8
netopýr brvitý	*Myotis emarginatus*	Mema	94
netopýr řasnatý	*Myotis nattereri*	Mnat	14
netopýr velký	*Myotis myotis*	Mmyo	5667
netopýr východní	*Myotis blythii*	Mbly	4
netopýr vodní	*Myotis daubentonii*	Mdau	68
netopýr pobřežní	*Myotis dasycneme*	Mdas	35
menší netopýři rodu *Myotis*		M.spp.	243
netopýr večerní	*Eptesicus serotinus*	Eser	3
netopýr černý	*Barbastella barbastellus*	Bbar	23
netopýr ušatý	*Plecotus auritus*	Paur	4
netopýr dlouhouchý	*Plecotus austriacus*	Paus	9
ušatí netopýři rodu *Plecotus*		P.spp.	9
celkem			9689

ním období, naopak během odchytových akcí nebyl zaregistrován *Myotis brandtii*. Společenstvo netopýrů je charakterizováno v obou sledovaných obdobích významnou dominancí *Myotis myotis*. Tento druh tvoří přes polovinu všech nálezů (58,5 %, resp. 53,8 %). V zimním období je druhým nejpočetnějším druhem *Rhinolophus hipposideros* (36,1 %) a zastoupení dalších druhů nepřesahuje 1 %. V mimohibernačním období je druhým nejpočetnějším druhem *Myotis daubentonii* (15,3 %). Všechny ostatní druhy s výjimkou *Myotis dasycneme* a *Myotis mystacinus* dosahují dominance mezi 1 až 5 %.

Dlouhodobé změny početnosti

Dlouhodobé změny početnosti jsou hodnoceny pouze z výsledků zimního sčítání, ze kterého jsou k dispozici kontinuální data z posledních dvaceti let (1982–2002) (tab. 2). Předchozí záznamy z let 1958 až 1970 nebyly s ohledem na velký časový odstup zahrnuty do statistické analýzy. Rostoucí trend byl zaznamenán u obou nejpočetnějších druhů, *M. myotis* ($r = 0,95$; $p < 0,001$) a *R. hipposideros* ($r = 0,90$; $p < 0,001$). Relativně stabilní počet netopýrů v 80. letech se zásadně mění počátkem let devadesátých, kdy dochází k jeho relativně rychlému nárůstu (obr. 1 a tab. 2). Tento růst je výraznější u *M. myotis*, jehož počty od té doby postupně stále zřetelněji převyšují hodnoty zaznamenané u *R. hipposideros*. Nicméně u obou druhů vedl nárůst až k rekordním počtům zjištěným během zimní sezóny 2000/2001 resp. 2001/2002 (*M. myotis* – 536 ks a *R. hipposideros* – 318 ks). Ostatní druhy netopýrů tvoří méně jak 10 % z celkového počtu nalezených zimujících netopýrů. Přesto, že jsou jejich celkové počty během zimy silně podhodnoceny (využívají převážně nedostupné typy úkrytů), vykazuje také jejich početnost statisticky významný rostoucí trend ($r = 0,87$; $p < 0,001$).

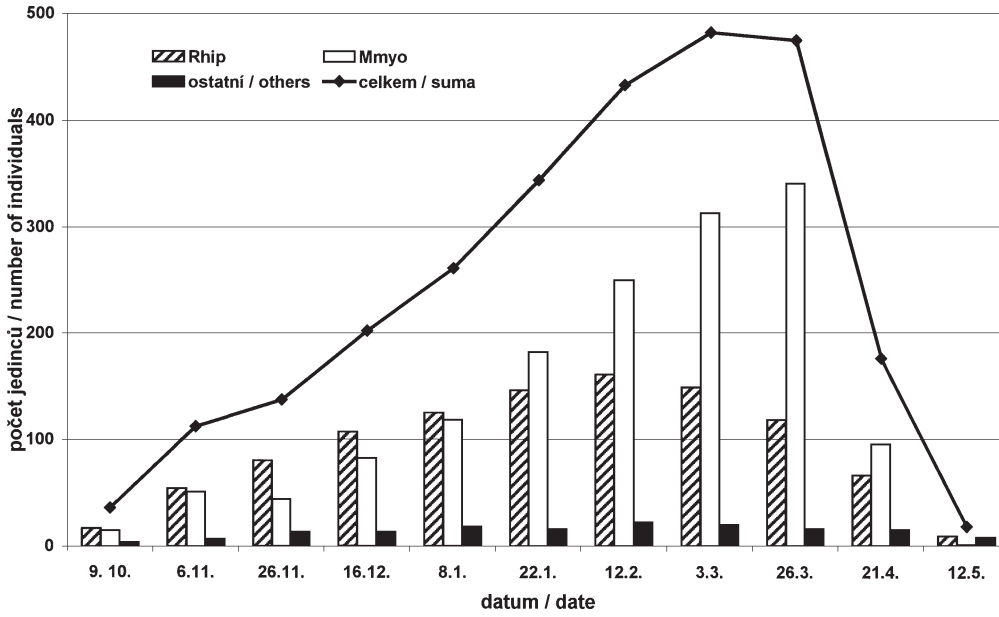
Krátkodobé změny početnosti, resp. aktivity

Hibernační období

Celkový počet zimujících netopýrů roste kontinuálně od října a nejvyšších hodnot dosahuje koncem března, netopýři přitom využívají k zimování téměř všechny části jeskyní (obr. 2 a tab. 3a, b). V dubnu dochází k postupnému, ale relativně rychlému opouštění jeskyně a početnost netopýrů klesá k minimu. Nicméně, přesněji lze průběh časových změn i přesunů uvnitř jeskynního systému hodnotit pouze u obou nejpočetnějších druhů tj. u *R. hipposideros* a *M. myotis*.

V první polovině zimního období (říjen – polovina ledna) je dominantním druhem *R. hipposideros*. Nárůst i pokles početnosti tohoto druhu je v průběhu zimy pozvolný; maxima přitom dosahuje v polovině února (12. 2. 1993 – 161 ks). Během zimování byl objeven prakticky ve všech definovaných částech jeskyní s jedinou výjimkou, kterou je krátká vchodová část s dynamickým mikroklimatem (Za mříží). Na začátku zimování využívá hlavně vstupní části jeskyní (U vchodu, Nicová, Východ v Šošůvské jeskyni), ale postupně se v průběhu zimy přesunuje do většiny jejich vnitřních úseků. 80 % nálezů přitom uskutečněno dohromady v 8 částech jeskynního systému (tab. 3a).

U *M. myotis* je nárůst početnosti během zimy plynulý a od poloviny ledna tento druh již ve společenstvu dominuje. Nárůst početnosti přitom pokračuje až do konce března (26. 3. 1993 – 341 ks). Následně dochází k velmi rychlému opouštění zimoviště, jehož průběh výrazně ovlivňuje změny celkového počtu netopýrů. Na rozdíl od *R. hipposideros* je *M. myotis* vázán téměř výhradně na oblast tzv. Eliščiny jeskyně případně jejího blízkého okolí (přes 80 % všech nálezů).



Obr. 2. Změny početnosti zimujících netopýrů během sezóny 1992/1993.
Fig. 2. Changes in the numbers of hibernating bats during the winter 1992/1993.

Tab. 2. Dlouhodobé změny početnosti dvou dominantních druhů netopýrů – *Myotis myotis* a *Rhinolophus hipposideros*
Tab. 2. Long-term changes in numbers of two dominant bat species – *Myotis myotis* and *Rhinolophus hipposideros*

rok / year	*R. hipposideros*	*M. myotis*	ostatní / others
1958	59	29	9
1959	79	87	4
1960	40	43	2
1965	0	16	0
1966	4	58	1
1968	7	61	3
1970	0	17	6
1982	78	52	9
1983	110	54	4
1984	84	67	7
1985	83	67	6
1986	81	76	4
1987	79	61	2
1988	105	143	10
1989	104	115	12
1990	81	171	20
1991	79	182	19
1992	119	213	28
1993	146	180	16
1994	140	212	40
1995	177	347	32
1996	215	464	35
1997	198	483	38
1998	262	454	31
1999	312	492	35
2000	227	479	52
2001	318	508	71
2002	307	536	32
dominance (%)	36,1	58,5	5,4

Zimující jedinci *M. myotis* v této části jeskyní tvoří velmi početná seskupení (tzv. clustery). Změny počtu v těchto shlucích potom výrazně ovlivňují i celkové změny početnosti *M. myotis* v Sloupsko-šošůvských jeskyních. V dalších částech netopýři tohoto druhu prakticky chybí (Za mříží, Gotická chodba, Východ v Šošůvské jeskyni), nebo jsou zde nalézány pouze jednotlivé kusy (tab. 3b).

Mimohibernační období
Celková intenzita letové aktivity netopýrů hodnocená ze všech odchytů v období od 15. 3. do 14. 11. dosáhla hodnoty 4,66 ks/hod. Aktivita společenstva netopýrů u vchodu Sloupsko-šošůvských jeskyní ovšem kolísá v závislosti na období roku (obr. 3). Liší se zejména období v první polovině mimohibernačního období (A, B) ve srovnání s obdobími následujícími (C, D); tyto rozdíly však nebyly statisticky významné (Kruskal-Wallisův test). Nejvyšší aktivita byla

Tab. 3a. Rozdělení nálezů *Rhinolophus hipposideros* v jednotlivých částech Sloupsko-šošůvských jeskyní během pravidelných kontrol v zimní sezóně 1992/1993
Tab. 3a. Distribution of records of *Rhinolophus hipposideros* in the particular parts of the Sloupsko-šošůvské caves during regular checks in winter 1992/1993

Rhinolophus hipposideros	9. 10.	6. 11.	26. 11.	16. 12.	8. 1.	22. 1.	12. 2.	3. 3.	26. 3.	21. 4.	12. 5.	Σ	%
U vchodu	–	13	12	7	3	3	3	2	8	2	2	55	5,3
Za mříží	–	–	–	–	–	–	–	–	–	–	–	0	0,0
Nicová	2	7	18	7	5	5	5	5	5	5	1	65	6,3
Před Eliščinou jeskyní	1	–	1	2	6	8	5	8	5	2	–	38	3,7
Eliščina jeskyně a okolí	3	5	9	34	31	38	37	40	18	10	1	226	21,9
Před chodbou U Řezaného kamene		–	3	1	2	2	1	1	1	–	–	11	1,1
U Řezaného kamene	1	1	–	5	4	10	11	9	7	2	1	51	4,9
Gotická chodba	–	1	–	–	–	–	–	–	–	3	–	4	0,4
Stupňovitá propast	1	7	7	18	23	27	26	26	15	13	–	163	15,8
U ponorů	2	3	5	3	10	2	3	5	3	4	–	40	3,9
Trámová	–	2	4	8	5	12	13	10	10	3	1	68	6,6
U Kolmé	–	2	6	9	16	17	28	22	22	7	1	130	12,6
Nagelova propast a okolí	–	2	3	5	7	5	6	5	7	4	–	44	4,3
Stříbrná	–	1	5	6	10	16	22	15	13	10	1	99	9,6
Šošůvská jeskyně	–	2	1	–	2	1	1	–	2	1	–	10	1,0
Kaple v Šošůvské jeskyni	–	1	4	2	1	–	–	–	2	–	–	10	1,0
Východ v Šošůvské jeskyni	7	7	2	–	–	–	–	1	–	–	1	18	1,7
Σ Rhip	17	54	80	107	125	146	161	149	118	66	9	1032	100

Tab. 3b. Rozdělení nálezů *Myotis myotis* v jednotlivých částech Sloupsko-šošůvských jeskyní během pravidelných kontrol v zimní sezóně 1992/1993

Tab. 3a. Distribution of records of *Myotis myotis* in the particular parts of the Sloupsko-šošůvské caves during regular checks in winter 1992/1993

Myotis myotis	9. 10.	6.11.	26.11.	16.12.	8.1.	22.1.	12.2.	3.3.	26.3.	21.4.	12.5.	Σ	%
U vchodu	–	1	2	–	–	1	1	1	4	5	–	15	1,0
Za mříží	–	–	–	–	–	–	–	–	–	–	–	0	0,0
Nicová	1	1	–	–	–	–	–	–	–	1	–	3	0,2
Před Eliščinou jeskyní	1	3	3	4	6	3	2	2	4	–	–	28	1,9
Eliščina jeskyně a okolí	10	24	24	50	81	140	208	275	304	83	1	1200	80,5
Před chodbou U Řezaného kamene	–	3	3	6	11	11	14	10	7	–	–	65	4,4
U Řezaného kamene	1	5	–	7	5	7	5	2	3	–	–	35	2,3
Gotická chodba	–	–	–	–	–	–	–	–	–	–	–	0	0,0
Stupňovitá propast	–	3	3	4	4	3	2	3	6	2	–	30	2,0
U ponorů	2	3	1	1	2	3	2	3	1	2	–	20	1,3
Trámová	–	2	2	2	3	3	5	2	3	1	–	23	1,5
U Kolmé	–	–	–	1	2	1	3	3	1	1	–	12	0,8
Nagelova propast a okolí	–	1	4	2	1	1	3	1	1	–	–	14	0,9
Stříbrná	–	4	2	1		3	4	9	7	–	–	30	2,0
Šošůvská jeskyně	–	1	–	3	1	2	1	2	–	–	–	10	0,7
Kaple v Šošůvské jeskyni	–	–	–	1	2	2	–	–	–	–	–	5	0,3
Východ v Šošůvské jeskyni	–	–	–	–	–	–	–	–	–	–	–	0	0,0
Σ Mmyo	15	51	44	82	118	180	250	313	341	95	1	1490	100

Tab. 4. Výsledky odchytů netopýrů v jednotlivých částech mimohibernačního období. Vysvětlivky: období A – od 15. 3. do 14. 5., období B – od 15. 5. do 14. 7., období C – od 15. 7. do 14. 9. a období D – od 15. 9. do 14. 11.
Tab. 4. Results of bat nettings in the particular parts of a non-hibernating period. Explanations: part A – 15. 3. – 14. 5., part B – 15. 5. – 14. 7., part C – 15. 7. – 14. 9., and part D – 15. 9. – 14. 11.

období / season	A	B	C	D	Σ	dominance (%)
Rhinolophus hipposideros	5	1	0	3	9	2,0
Myotis mystacinus	0	0	0	2	2	0,5
Myotis emarginatus	0	0	9	3	12	2,7
Myotis nattereri	0	0	7	11	18	4,1
Myotis bechsteinii	5	2	5	3	15	3,4
Myotis myotis	27	8	100	100	235	53,8
Myotis blythii	1	0	1	0	2	0,5
Myotis daubentonii	21	1	33	12	67	15,3
Myotis dasycneme	0	0	7	4	11	2,5
Eptesicus serotinus	1	1	4	0	6	1,4
Nyctalus noctula	0	1	2	3	6	1,4
Barbastellus barbastellus	0	1	9	6	16	3,7
Plecotus auritus	6	0	6	9	21	4,8
Plecotus austriacus	0	7	9	1	17	3,9
celkem	66	22	192	157	437	100,0
počet odchytů / no. of nettings	3	1	3	4	11	

zjištěna v období C. Na počátku období D je aktivita stále vysoká, postupně však klesá (tab. 4). V období A a B je letová aktivita nižší než na podzim.

DISKUSE

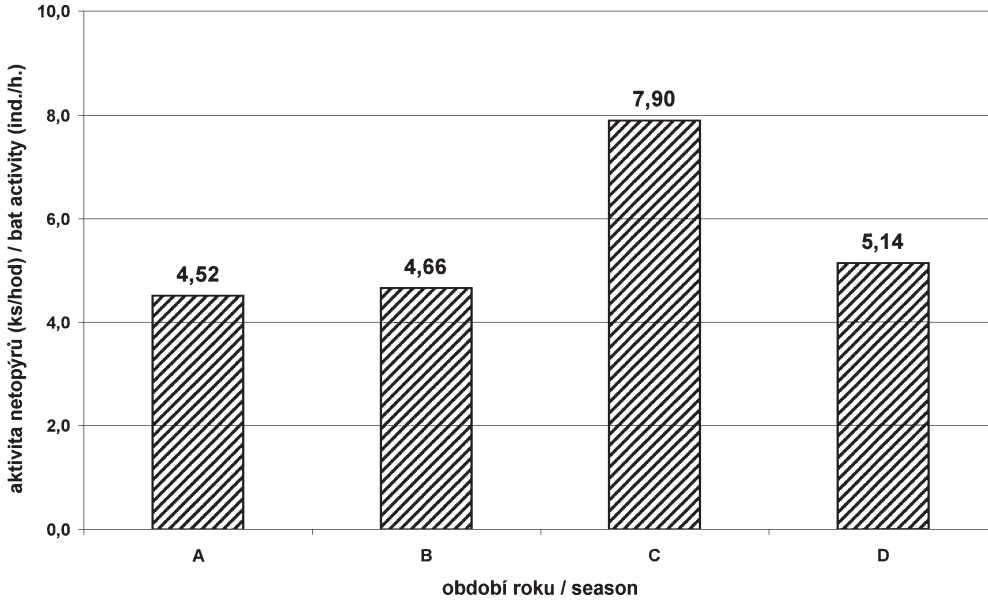
Struktura společenstva

Na území Moravského krasu bylo dosud zjištěno všech 21 z celkem 23 druhů netopýrů evidovaných na území ČR. Nebyl nalezen *Nyctalus lasiopterus*, u něhož existuje jen jediný doklad uložený ve sbírkách Jihočeského muzea v Českých Budějovicích, a je navíc sporné, zda vůbec pochází z území České republiky (Bürger & Červený 1979). V posledních letech byl opakovaně pozorován na jižní Moravě, ale doklad dosud chybí (Gaisler et al. 2002). V Moravském krasu nebyl nalezen ani *Hypsugo savii*, nedávno zjištěný na dvou lokalitách na jižní Moravě (Gaisler & Vlašín 2003). V jeskyních Moravského krasu bylo dosud v zimním období zjištěno 18 druhů netopýrů (Řehák 1995). Nálezy *Vespertilio murinus* a *Pipistrellus pipistrellus* s. l. však pocházejí z minulého století (Wankel 1860, Gaisler 1956). Druhý z nich byl po velmi dlouhé době zaznamenán při zimování v Kateřinské jeskyni (Zukal et al. 2001). Další dva druhy (*Rhinolophus ferrumequinum* a *Eptesicus nilssonii*) také nelze vzhledem k ojedinělým nálezům v tomto století považovat za stálé hibernanty (Bauerová & Zima 1988b, Zukal & Gaisler 1989). Sloupsko-šošůvské jeskyně patří počtem dosud zjištěných hibernujících druhů (15) k velmi bohatým lokalitám. Zimní sčítání v podzemních prostorech Českého krasu například prokázala přítomnost 14 druhů netopýrů (Horáček et al. 2001), v největším zimovišti netopýrů na území ČR – v Javoříčských jeskyních jen 11 druhů (Koudelka & Reiter 2001) a v pseudokrasových jeskyních

Svitavska 12 druhů (Weideinger 2001). Druhově bohatší (21 druhů) je ve srovnání s jeskyněmi Moravského krasu jen chiropterofauna zimující v jeskyních Slovenského krasu (Kolektiv 2002).

Také v mimohibernačním období je počet druhů odchycených před vchodem do jeskyní relativně vysoký. Podobné výsledky byly zjištěny také u dalších jeskyní Moravského krasu (Hladomorna 14 druhů, Býčí skála 13 druhů) (Bauerová & Zima 1988a, b, Zukal & Gaisler 1989, Řehák 1995). Území Moravského krasu se tím řadí mezi oblasti významné pro netopýry v celoevropském měřítku. Pro srovnání, Aellen (1962) zaznamenal u vchodu jeskyně ve Valisu (Švýcarsko) 12 druhů včetně migrujících jedinců, Strelkov (1971) u vchodu do Staroladožské jeskyně v Leningradské oblasti (severozápadní Rusko) dokonce jen 4 druhy. Také podobné vzorky z USA se vyznačují menšími počty druhů, např. Hall & Brenner (1968) uvádějí 5 druhů, Whitaker & Mumford (1971) 6 druhů. Podobný počet druhů jako u jeskyní Moravského krasu (16 druhů) byl zjištěn také u Ledových slují v Podyjí (Reiter et al. 1997). V ostatních oblastech České republiky je počet druhů nižší, např. Horáček (1985) a Hanzal & Průcha (1996) uvádějí z Českého krasu 9 druhů netopýrů. Řehák (1998) a Lučan (2000) uvádějí z pseudokrasových jeskyní na severní Moravě obdobný počet druhů (Beskydy – 8 až 9 druhů, Javorníky – 10 druhů, Vizovická vrchovina – 9 druhů, Hostýnské vrchy – 8 až 10 druhů).

Dominantními druhy ve společenstvu netopýrů hibernujících ve Sloupsko-šošůvských jeskyních jsou *M. myotis* a *R. hipposideros*, oba druhy tvoří téměř 95 % všech zimních nálezů.



Obr. 3. Medián aktivity společenstva netopýrů v jednotlivých částech mimohibernačního období u vchodu Sloupsko-šošůvských jeskyní.
Fig. 3. Median of activity of bat assemblages in particular parts of a non-hibernating period at the entrance of the Sloupsko-šošůvské jeskyně caves.

Podobně je tomu i ve většině jeskyní Moravského krasu (Bauerová et al. 1989, Zima et al. 1994, Kovařík 1997, Řehák et al. 1994). Poměrné zastoupení druhů netopýrů zjištěné zimním sčítáním v těchto jeskyních a letním odchytem u jejich vchodu se však výrazně liší (Bauerová & Zima 1988a,b, Řehák et al. 1994, Řehák 1995). Odchyty netopýrů ve vchodech jeskyní během mimohibernačního období potvrzují přítomnost a relativně vyšší zastoupení i dalších druhů netopýrů, zejména se jedná o jedince malých druhů rodu *Myotis* (Řehák et al. 1994). Na rozdíl od těchto výsledků, ve vzorku získaném odchytem u Sloupsko-šošůvských jeskyní dominuje *M. myotis* (53,8 %) i během mimohibernačního období. Podobná situace byla zjištěna i v Českém krasu, kde v zimě tvoří *M. myotis* 61 % hibernujícího společenstva (Hanzal & Průcha 1988) a tento druh zde má vysoké zastoupení i v letních odchytech – 41 % (Horáček 1985), resp. 63 % (Hanzal & Průcha 1996).

Rozdílné složení zimního a letního vzorku je způsobeno jednak rozdílnou efektivitou monitorovacích metod (malé druhy netopýrů využívající štěrbinové typy úkrytů unikají pozornosti při sčítání versus nízká "ulovitelnosť" některých druhů pomocí sítí), jednak skutečností, že ve vzorku získáném v období přeletů jsou navíc zastoupeny druhy netopýrů, které na daných lokalitách nemusí hibernovat (Horáček & Zima 1978, Řehák et al. 1994, Weideinger 1994).

Změny početnosti

Výsledky monitoringu zimujících netopýrů v různých částech Evropy potvrdily významné změny v početnosti různých druhů netopýrů během posledních čtyř desetiletí. Rychlý pokles početnosti byl pozorován během 60. a 70. let u *R. hipposideros* a *M. myotis* a řady dalších druhů (Rudolph 1990, Řehák 1997). Během posledních dvou desetiletí byl však u některých druhů netopýrů zaznamenán výrazný nárůst početnosti (Hanzal & Průcha 1992, Urbanczyk 1992, Řehák 1997). Výsledky ze Sloupsko-šošůvských jeskyní i z dalších lokalit Moravského krasu (Řehák et al. 1994, Zima et al. 1994) potvrzují tento obecný trend u obou nejpočetnějších druhů – *R. hipposideros* a *M. myotis*. Podobný stav je u těchto dvou druhů i v jiných regionech – Český kras (Hanzal & Průcha 1992) a Jeseníky (Řehák & Gaisler 1999).

Změny v dominanci dvou nejpočetnějších druhů (*M. myotis* a *R. hipposideros*) v průběhu sezóny jsou způsobeny jednak rozdílnou dobou příletu na zimoviště a odletu na konci hibernačního období, jednak přeletovou aktivitou netopýrů uvnitř jeskyně. Při odchytech do sítí u vchodu do Kateřinské jeskyně byl *M. myotis* jedním z dominantních druhů v září a začátkem října (Řehák et al. 1994). Také Bauerová & Zima (1988b) zachytili vrchol letové aktivity *M. myotis* u vchodu do jeskyně Hladomorna v Moravském krasu koncem září až začátkem října. Podobně jako ve Sloupsko-šošůvských jeskyních, byla i uvnitř Kateřinské jeskyně dominance tohoto druhu v první polovině zimy (od poloviny listopadu do poloviny ledna) srovnatelná s dominancí *R. hipposideros*. Nárůst početnosti *M.myotis* od poloviny ledna do začátku dubna byl na obou lokalitách vázán téměř výhradně na jedinou část jeskyně (Eliščina jeskyně resp. Chodba) (cf. Hanzal & Průcha 1988, Fuszara et al. 1996). Letová aktivita před vchodem do jeskyní však je v tomto období prakticky nulová (Řehák et al. 1994). Zvyšování početnosti *M. myotis* je tedy spíše výsledkem přeletů uvnitř jeskyně na místa blíže ke vchodu, nežli imigrací z okolních zimovišť. K podobným závěrům dospěli Nagel & Nagel (1987), kteří zaznamenali největší početnost *M. myotis* začátkem března, zatímco u ostatních druhů početnost poklesla. Toto pozdní maximum u *M. myotis* vysvětlují tím, že netopýři sice přilétají na zimoviště podstatně dříve, ale zprvu se zavěšují na místa v hlubokých štěrbinách, kde jsou při sčítání přehlédnuti. Nagel & Nagel (1993) také zjistili, že se od poloviny února až do konce dubna zvyšovala přeletová aktivita

M.myotis uvnitř zimoviště. Podobné výsledky podávají Dorgelo & Punt (1969), Červený (1982), Skiba (1987) a Valenciuc (1989).

Změny početnosti *R. hipposideros* jsou během zimního období pozvolnější (Řehák et al. 1994). Přeletová aktivita uvnitř Kateřinské jeskyně byla v období od začátku prosince do poloviny března nízká (Berková 2001). Nicméně, Baroň & Řehák (1999) sledovali letovou aktivitu *R. hipposideros* u vchodu do jeskyně Ďáblova díra (Slimrovka) na vrchu Kopce u Lidečka (Vsetínsko) a zjistili, že do poloviny prosince byla letová aktivita zvýšená a později prudce poklesla. I uprostřed zimy vyletovali někteří probuzení vrápenci ven a po krátké době se vraceli do jeskyně. Nagel & Nagel (1997) uvádějí postupný pokles početnosti *R. hipposideros* na zimovišti u Freyburgu (SRN) od konce března. K výraznějšímu úbytku zimujících jedinců však docházelo až během května. Tomu odpovídá i nízká aktivita v období listopad až duben, studovaná pomocí automatického záznamníku echolokačních signálů uvnitř zimoviště.

Sezónní dynamika ve využívání jednotlivých částí jeskyní byla u dvou sledovaných druhů rozdílná. Zatímco *R. hipposideros* využívá většinu částí jeskynního systému, *M. myotis* je po celou dobu hibernace vázán pouze na malou část jeskyní (Eliščina jeskyně a její okolí). V Kateřinské jeskyni se *M. myotis* v období od poloviny prosince do začátku dubna přesunuje ze zadních klimaticky stabilních částí jeskyně do části vchodové (Berková 2001). Podobný trend zaznamenali u *M. myotis* také Daan & Wichers (1968), Dorgelo & Punt (1969), Hanzal & Průcha (1988) a Valenciuc (1989). Naopak u *R.hipposideros* byla na řadě lokalit zjištěna preference částí zimoviště se stálým mikroklimatem (Gaisler 1963, Bezem et al. 1964, Daan & Wichers 1968). Složitý podzemní systém Sloupsko-šošůvských jeskyní poskytuje klimaticky stabilní prostředí a pravděpodobně z tohoto důvodu jsou také jedinci *R. hipposideros* schopni využívat většinu jeho částí. Baroň & Řehák (1997) zjistili, že jedinci *R. hipposideros* se zejména na podzim a na jaře zdržují v dynamických částech jeskyně, jejichž teplota je v těchto obdobích srovnatelná s teplotou ve statických částech jeskyně (11–12 °C). V zimě obsazují části jeskyně se stabilním mikroklimatem.

Hodnota celkové intenzity letové aktivity zjištěná ve vchodu Sloupsko-šošůvských jeskyní v mimohibernačním období je poměrně vysoká (4,66 ks/hod.). Ze souhrnných výsledků odchytů u vchodů jeskyní Československa a Bulharska (Horáček & Zima 1978) lze totiž dojít k daleko nižší hodnotě (1,30). Podobně nízké hodnoty uvádí také Weideinger (1994) z odchytů u pseudokrasových jeskyní ve východních Čechách (1,87) a Gaisler (1973) u 3 jeskyní v údolí Říčky (1,18) v jižní části Moravského krasu. Na druhé straně u vchodu jeskyně Great Scott Cave (Missouri, USA) dosahovala aktivita hodnot až 490 ks/hod. (LaVal & LaVal 1980). Rozdílná aktivita netopýrů u vchodů jednotlivých jeskyní je pravděpodobně zapříčiněna specifickými podmínkami lokalit, jako jsou velikost jeskyně, charakter portálu, velikost a tvar samotného vchodu do jeskyně, modulace okolního terénu, zvláště jeho vegetační pokryv, které spolu s odlišnými klimatickými podmínkami ovlivňují i odlišnou strukturu jednotlivých společenstev netopýrů.

Intenzita letové aktivity podléhá sezónním změnám v souvislosti s ročním cyklem netopýrů. Jeskynní vchody jsou využívány v období jarních a zejména podzimních přeletů (swarming). Vysoká aktivita netopýrů je v tomto období způsobena zvýšenou přeletovou aktivitou po rozpadu letních kolonií. Zaznamenané sezónní změny v aktivitě netopýrů u jeskynních vchodů jsou v plné shodě se závěry Horáčka & Zimy (1978) a Bauerové & Zimy (1988b). Anděra et al. (1992) zaznamenali v červenci u vchodu Chýnovské jeskyně (jižní Čechy) minimální aktivitu (0,23), ale o měsíc později již dosáhla letová aktivita celoročního maxima (3,13). Pozvolný pokles aktivity s postupujícím podzimem zřejmě souvisí s počátkem hibernace (Horáček & Zima 1978). Méně

výrazný jarní vrchol aktivity je ve spojitosti s opouštěním zimovišť a jarní přeletovou aktivitou. Nejnižší aktivita zaznamenaná koncem jara a počátkem léta naznačuje, že většina druhů netopýrů v tomto období jeskyně navštěvuje minimálně (Horáček & Zima 1978). Adultní samice žijí v této době mimo jeskyně v letních koloniích. Analogické změny v sezónní aktivitě byly zaznamenány i na jednotlivých lokalitách. I dřívější odchyty u vchodů jeskyní v údolí Říčky (Gaisler 1973) měly stejný roční průběh, byť s přepočtenými hodnotami letové aktivity téměř 2–3 krát nižšími (období A: 2,5–1,28, B: 1,2–0,65, C: 5,0–3,10, D: 3,0–0,75).

PODĚKOVÁNÍ

Autoři by rádi poděkovali všem kolegům a kamarádům z řad pracovníků Správy CHKO Moravský kras Blansko a Správy jeskyní Moravského krasu Blansko, pracovníků i studentů PřF MU Brno a ÚBO AV ČR Brno, členů ČESON a České speleologické společnosti, i dalším, kteří se podíleli na jednotlivých akcích. Výzkum probíhal za finanční podpory ČESON, výzkumných grantů GAČR č. 206/01/1555 a GAAV č. A6087606, a výzkumného záměru MSM 143100010.

LITERATURA

Absolon K., 1899: O zimním spánku a rozšíření netopýrů v jeskyních moravských. *Vesmír*, **28**: 219–220, 230–231.

Aellen V., 1962: Le baguement des chauves-souris an Col de Bretolet (Valais). *Arch. Sci. Phys. Natur.*, *Genève*, **14**: 365–392.

Anděra M., Zbytovský P. & Bürger P., 1992: Bat community of Chýnovská jeskyně cave (Southern Bohemia, Czechoslovakia) in 1981–1986. Pp.: 1–11. In: Horáček I. & Vohralík V. (eds.): *Prague Studies in Mammalogy*. Charles Univ. Press, Praha, 248 pp.

Baroň I. & Řehák Z., 1997: K problematice zimování vrápence malého (*Rhinolophus hipposideros*) na Vsetínsku. *Zprav. Okr. Vlastivěd. Muz. ve Vsetíně*, **1997**: 3–12.

Bárta Z., Červený J., Gaisler J. (ed.), Hanák P., Hanák V. (ed.), Horáček I. (ed.), Hůrka L., Miles P., Nevrlý M., Rumler Z., Sklenář J. & Žalman J., 1981: Výsledky zimního sčítání netopýrů v Československu: 1969–1979. *Sbor. Okr. Muz. v Mostě*, *Ř. Přírodověd.*, **3**: 71 –116.

Bauerová Z., Gaisler J., Kovařík M. & Zima J., 1989: Variation in numbers of hibernating bats in the Moravian Karst: results of visual censuses in 1983–1987. Pp.: 499–505. In: Hanák V., Horáček I. & Gaisler J. (eds.): *European Bat Research 1987*. Charles Univ. Press, Praha, 720 pp.

Bauerová Z. & Zima J., 1988a: Výzkum netopýrů v jeskyni Býčí skála v letech 1977–1986. *Čs. Kras*, **39**: 51–59.

Bauerová Z. & Zima J., 1988b: Seasonal changes in visits to a cave by bats. *Folia Zool.*, **37**: 97–111.

Berková H., 2001: *Ekologie společenstva netopýrů v Kateřinské jeskyni*. Diplomová práce. PřF MU Brno, 65 pp.

Bezem J. J., Sluiter J. W. & van Heerdt P. F., 1964: Some characteristics of the hibernating locations of various species of bats in south Limburg. *Proc. Koninkl. Nederl. Akad. Wetensch., S. C*, **67**: 325–350.

Bürger P. & Červený J., 1979: Netopýr obrovský *Nyctalus lasiopterus* (Schreber, 1780) ve sbírkách Jihočeského muzea. *Lynx*, *n. s.*, **20**: 105–106.

Červený J., 1982: Results of investigation of bats (Chiroptera) at Loreta near Klatovy. *Lynx*, *n. s.*, **21**: 41–65.

Daan S. & Wichers H. J., 1968: Habitat selection of bats hibernating in a limestone cave. *Ztschr. Säugetierk.*, **33**: 262–287.

Dorgelo J. & Punt A., 1969: Abundance and "internal migration" of hibernating bats in an artificial limestone cave ("Sibbergroeve"). *Lynx*, *n. s.*, **10**: 101–125.

Fuszara E., Kowalski M., Lesinski G. & Cygan P., 1996: Hibernation of bats in underground shelters of central and northeastern Poland. *Bonn. Zool. Beitr.*, **46**: 349–358.

Gaisler J., 1956: Faunistický přehled československých netopýrů. *Ochr. Přír.*, **11**: 161–169.

Gaisler J., 1962: Tajemní obyvatelé jeskyně Býčí skály. *Vlastivěd. Zpr. z Adamova a okolí*, **6**: 3–6.
Gaisler J., 1963: The ecology of lesser horseshoe bat (*Rhinolophus hipposideros* Bechstein, 1800) in Czechoslovakia, Part I. *Věst. Čs. Společ. Zool.*, **27**: 211–233.
Gaisler J., 1973: Netting as a possible approach to study bat activity. *Period. Biol.* (Zagreb), **75**: 129–134.
Gaisler J. & Hanák V., 1969: Summary of the results of bat-banding in Czechoslovakia, 1948–1967. *Lynx*, *n. s.*, **10**: 25–34.
Gaisler J. & Hanák V.,1972: Netopýři podzemních prostorů v Československu. *Sborn. Západočes. Muz. Plzeň, Přír.*, **7**: 1–46.
Gaisler J. & Vlašín M., 2003: Second record of the Savi's pipistrelle (*Hypsugo savii*) in the Czech Republic. *Vespertilio*, **7**: 181–182.
Gaisler J., Řehák Z. & Bartonička T., 2002: Mammalia: Chiroptera. Pp.: 139–149. In: Řehák Z., Gaisler J. & Chytil J. (eds.): *Vertebrates of the Pálava Biosphere Reserve of UNESCO. Folia Sci. Natur. Univ. Masaryk. Brunn., Biol.*, **106**: 1–162.
Hall J. S. & Brenner F. J., 1968: Summer netting of bats at a cave in Pensylvania. *J. Mammal.*, **49**: 779–781.
Hanzal V. & Průcha M., 1988: Sezónní dynamika netopýřích společenstev na zimovištích Českého krasu v letech 1984–1986. *Lynx*, *n. s.*, **24**: 15–35.
Hanzal V. & Průcha M., 1992: Changes in the numbers of bats hibernating in the Bohemian karst during 1969–1987. Pp.: 71–74. In: Horáček I. & Vohralík V. (eds.): *Prague Studies in Mammalogy*. Charles Univ. Press, Praha, 248 pp.
Hanzal V. & Průcha M., 1996: Annual course of cave visitation by bats (Mammalia: Chiroptera) in the Bohemian karst (Czech Republic). *Acta Soc. Zool. Bohem.*, **60**: 25–30.
Horáček I., 1985: Population ecology of *Myotis myotis* in Central Bohemia (Mammalia: Chiroptera). *Acta Univ. Carol. – Biol.*, **1981**: 161–271.
Horáček I. (ed.), Hanák V., Benda P., Červený J., Hanzal V., Průcha M., Veselý J., Weinfurová D. & Zima J., 2001: Nejvýznamnější zimoviště netopýrů ve středních Čechách. *Vespertilio*, **5**: 121–145.
Horáček I. & Zima J., 1978: Net-revealed cave visitation and cave-dwelling on European bats. *Folia Zool.*, **27**: 135–148.
Kolektiv, 2001: Sčítání netopýrů v zimovištích ČR. *Vespertilio*, **5**: 3–330.
Kolektiv, 2002: Katalóg zimovísk netopierov SR. *Vespertilio*, **6**: 3–362.
Kolenati F. A., 1851: Zur Naturgeschichte der Fledermäuse in den Slouper Höhlen. *Lotos*, **1**: 41–43.
Koudelka M. & Reiter A., 2001: Netopýři zimující v Javoříčských jeskyních. *Vespertilio*, **5**: 155–162.
Kovařík M., 1997: Research and protection of *Rhinolophus hipposideros* in protected landscape area Moravian Karst during hibernation. Pp.: 85–87. In: Ohlendorf B. (ed.): *Tagungsband. Zur Situation der Hufeisennasen in Europa*. Arbeitskreis Fledermäuse Sachsen-Anhalt e. V. & IFA-Verlag GmbH, Stecklenberg-Berlin, 182 pp.
LaVal R. K. & LaVal M. L., 1980: *Ecological studies and management of Missouri bats, with emphasis on cave-dwelling species. Terrestrial Series No. 8*. Missouri Department of Conservation, Jefferson City, 93 pp.
Lučan R., 2000: Netopýři Hostýnských vrchů. *Vespertilio*, **4**: 111–116.
Nagel A. & Nagel R., 1987: Veränderungen des Bestandes Winterschlafender Fledermäuse im Winter 1982/83 in Württemberg. *Myotis*, **25**: 91–94.
Nagel A. & Nagel R., 1993: Activity of hibernating bats in their natural habitat recorded by automatic data logging. Pp.: 37–38. In: Palmeirim J. M., Rodriguez L. & Rabaça J. (eds.): *VI European Bat Research Symposium. Programme, Abstracts, List of participants*. European Bar Research Organization, Évora, Portugal, 64 pp.
Nagel A. & Nagel R., 1997: Nutzung eines Untertagequartieres durch die Kleine Hufeisennase (*Rhinolophus hipposideros*). Pp.: 97–108. In: Ohlendorf B. (ed.): *Tagungsband. Zur Situation der Hufeisennasen in Europa*. Arbeitskreis Fledermäuse Sachsen-Anhalt e. V. & IFA-Verlag GmbH, Stecklenberg-Berlin, 182 pp.
Reiter A., Hanák V., Benda P. & Obuch J., 1997: Savci Národního parku Podyjí. *Lynx*, *n. s.*, **28**: 5–141.

Rudolph B. U., 1990: Frühere Bestandsdichte und heutige Situation der Kleinen Hufeisennase *Rhinolophus hipposideros* in Nordbayern. *Myotis*, **28**: 101–108.
Řehák Z., 1995: *Letová aktivita netopýrů v Moravském krasu*. Dizertační práce. PřF MU Brno, 184 pp.
Řehák Z., 1997: Trendy ve vývoji početnosti netopýrů ve střední Evropě. *Vespertilio*, **2**: 81–96.
Řehák Z., 1998: Faunistický přehled netopýrů moravsko-slezské části Karpat (Česká republika) I. *Vespertilio*, **3**: 111–130.
Řehák Z. & Baroň I., 2001: Detectoring of flight activity of Lesser horseshoe bats, *Rhinolophus hipposideros*, at a cave entrance over a year. *Nietoperze*, **2**: 202–208.
Řehák Z. & Gaisler J., 1999: Long-term changes in the number of bats in the largest man-made hibernaculum of the Czech Republic. *Acta Chiropterol*., **1**: 113–123.
Řehák Z., Zukal J. & Kovařík M., 1994: Long- and short-term changes in the bat community of the Kateřinská cave (Moravian karst) – a fundamental assessment. *Folia Zool*., **43**: 425–436.
Skiba R., 1987: Bestandsentwicklung und Verhalten von Fledermäusen in einem Stollen des Westharzes. *Myotis*, **25**: 95–103.
Strelkov P. P., 1971: Ekologičeskije nabljudenija za zimnej spjačkoj letučich myšej (Chiroptera, Vespertilionidae) Leningradskoj oblasti. *Tr*. *Zool*. *Inst*. *AN SSSR*, **48**: 251–303.
Urbanczyk Z., 1992: Significance of the Nietoperek Reserve for central European population of *Myotis myotis* (Mammalia: Chiroptera). Pp.: 213–215. In: Horáček I. & Vohralík V. (eds.): *Prague Studies in Mammalogy*. Charles Univ. Press, Praha, 248 pp.
Valenciuc N., 1989: Dynamics of movements of bats inside some shelters. Pp.: 511–517. In: Hanák V., Horáček I. & Gaisler J. (eds.): *European Bat Research 1987*. Charles Univ. Press, Praha, 720 pp.
Wankel J., 1860: Beiträge zur Fauna der mährischer Höhlen. *Lotos*, **10**: 105–122.
Weideinger K., 1994: Bat communities of three small pseudokarstic caves in Eastern Bohemia (Czech Republic). *Folia Zool*., **43**: 455–464.
Weindinger K., 2001: Zimoviště netopýrů v oblasti Svitavské pahorkatiny. *Vespertilio*, **5**: 303–310.
Whitaker J. O. Jr. & Mumford R. E., 1971: Notes on a collection of bats taken by mist-netting at an Indiana cave. *Am*. *Midl*. *Natur*., **85**: 277–279.
Wilkinson L., 1990: SYSTAT: *The System for Statistics*. SYSTAT, Inc., Evanston.
Zima J., Kovařík M., Gaisler J., Řehák Z. & Zukal J., 1994: Dynamics of the number of bats hibernating in the Moravian karst in 1983 to 1992. *Folia Zool*., **43**: 109–119.
Zukal J. & Gaisler J., 1989: K výskytu a změnám početnosti netopýra severního, *Eptesicus nilssoni* (Keyserling et Blasius, 1839) v Československu. *Lynx*, *n*. *s*., **25**: 83–95.
Zukal J., Kovařík M., Řehák Z., Berková H., 2001: Početnost netopýrů zimujících ve dvou jeskyních v severní části Moravského krasu a její dlouhodobé změny. *Vespertilio*, **5**: 321–328.

Změny početnosti a struktura společenstva netopýrů

Bryja, J., Uhrin, M., Kaňuch, P., Bémová, P., Martínková, N. & Zukal, J. (2010) Mitochondrial DNA confirms low genetic variation of the greater mouse-eared bats, *Myotis myotis*, in Central Europe. Acta Chiropterologica 12, 73-81.

Acta Chiropterologica, 12(1): 73–81, 2010
PL ISSN 1508-1109 © Museum and Institute of Zoology PAS
doi: 10.3161/150811010X504608

Mitochondrial DNA confirms low genetic variation of the greater mouse-eared bats, *Myotis myotis*, in Central Europe

JOSEF BRYJA[1, 2, 7], MARCEL UHRIN[3, 4], PETER KAŇUCH[5], PETRA BÉMOVÁ[1], NATÁLIA MARTÍNKOVÁ[1, 6], and JAN ZUKAL[1, 2]

[1]*Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, CZ-603 65 Brno, Czech Republic*
[2]*Institute of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, CZ-637 01 Brno, Czech Republic*
[3]*Institute of Biology and Ecology, Faculty of Science, P. J. Šafárik University, Moyzesova 11, SK–040 01 Košice, Slovakia*
[4]*Department of Zoology, Faculty of Science, Charles University, Viničná 7, CZ–128 44 Praha 2, Czech Republic*
[5]*Institute of Forest Ecology, Slovak Academy of Sciences, Štúrova 2, SK-960 53 Zvolen, Slovakia*
[6]*Institute of Biostatistics and Analyses, Masaryk University, Kamenice 3, CZ-625 00 Brno, Czech Republic*
[7]*Corresponding author: E-mail: bryja@brno.cas.cz*

Recent data shows that range expansion of the greater mouse-eared bat *Myotis myotis* (Borkhausen, 1797) to Central Europe occurred mainly from the Iberian glacial refugium and in a lesser extent from South-eastern Europe. Here we present sequences of the mitochondrial control region obtained from 16 localities in the Czech Republic, Slovakia, and NW Romania. From the 97 sequences, 87 were identical with the haplotype H1, the most frequent one of haplogroup A occurring throughout Western Europe, and nine sequences (eight haplotypes) differed from H1 only by one substitution. This confirms decrease of genetic variability from south to north and colonisation of Central Europe from the Iberian Peninsula. However, we found a new haplotype, which is closely related to sequences from haplogroup D so far described in the nominative form of this species only from Greece and Bulgaria, which suggests two possible scenarios. First, colonization route from the Balkan refugium existed in this species as well, which is supported also by recently published analyses of historical DNA. Second, the Balkan haplotype entered Central Europe via interspecific hybridisation with *M. blythii*, a species, in which the haplogroup D is the most frequent in Europe and which is known to have colonised Europe from south-east.

Key words: genetic structure, mtDNA, control region, phylogeography, *Myotis myotis*

INTRODUCTION

Molecular data are widely used to assess recent and historical processes that formed genetic structure of contemporary species. Factors that have been shown to affect genetic architecture of populations include social systems or expansions and fragmentation of habitats (Avise, 2000; Beebee and Rowe, 2004). There is much interest in determining whether these factors are historical either caused by natural changes in the environment (glaciations — Taberlet *et al.*, 1998) or by activity of ancient people (deforestation, urbanisation — Horáček, 1984). The main difference is the age of the population process, such as population differentiation or expansion. Where older events are attributable to climatic and associated vegetation changes in Pleistocene glacial cycles, more recent events might be influenced by increasing human impact on environment in historical times. Genetic structure of populations that colonise new areas after range expansion is influenced either by genetic drift due to a founder effect that usually causes the decrease of genetic polymorphism (Ibrahim *et al*., 1996) and/or by genetic variation of original populations from which the expansion started (Avise, 2000).

In the last two decades, bats became one of the model groups for analysis of historical and contemporary factors affecting genetic structure of populations. Genetic studies of bat populations indicated that patterns of geographic genetic differentiation can be affected by a variety of factors — seasonal migration, geographical barriers, and past demographic processes (Burland and Wortington-Wilmer, 2001). Migratory species exhibited little genetic structure across the study range (McCracken *et al*., 1994; Petit and Mayer, 1999, 2000), while the gene flow in sedentary species was more restricted and

populations more genetically structured (Burland *et al*., 1999; Kerth *et al.*, 2000, 2002; Rivers *et al*., 2005). Species with female philopatry (the tendency to remain in or consistently return to the natal territory) might show strong substructure when examined with maternally inherited mitochondrial DNA, but this substructure generally disappears when biparentally inherited markers are used, owing to male-mediated gene flow (Petit and Mayer, 1999; Castella *et al*., 2001; Kerth *et al*., 2002). Phylogeographic studies in European bats usually confirm the most frequent scenario for colonization of Central Europe from southern refugia after the last glaciation and genetic variation of these bats decreases with northward range expansion (Ruedi and Castella, 2003; Hulva *et al.*, 2004; but see Petit *et al*., 1999).

The greater mouse-eared bat *Myotis myotis* (Borkhausen, 1797) is one of the most common and largest European bats. It is a non- or regionally migratory species endemic to Europe and the Levant (Mitchell-Jones *et al*., 1999; Castella *et al*., 2000; Hutterer *et al*., 2005). The current distribution of this species on its northern border is probably very recent. Southern populations (forming nursery colonies usually in warm caves) are considered to be old; e.g., in Spain, the species occurs in the fossil record since the Pleistocene. On the contrary, in Central Europe the species was absent in fossil records until late Holocene (Horáček, 1984; Postawa, 2004).

Until very recently (Bogdanowicz *et al*., 2009), genetic studies of *M. myotis* populations focussed mainly on individuals from the Alps and from the Mediterranean area. In the first study, Castella *et al*. (2001) showed unexpectedly high mitochondrial haplotype diversity in Alpine maternity colonies and a contrasting pattern of mitochondrial and nuclear population structure suggesting male-mediated gene flow and female philopatry. In the following phylogeographic study, Ruedi and Castella (2003) enlarged their sampling mainly into the western Mediterranean region, where they identified six haplogroups. All of them occur in the Mediterranean, suggesting the occurrence of glacial refugia in that area, with extraordinary high genetic diversity especially in Italy (Ruedi *et al*., 2008) and the Balkans (Ruedi and Castella, 2003). Nucleotide diversity of the mtDNA marker in particular colonies decreased significantly northwards, suggesting relatively recent colonization of more northern areas from southern refugia. However, sampling of populations in Central Europe (outside the Alps) was fragmentary. Ruedi and Castella (2003) included 20 individuals from a single nursing colony in Poland, where they found three different but very similar haplotypes from the most widespread haplogroup A. Finally, Bogdanowicz *et al*. (2009) recently analyzed greater mouse-eared bats from several localities in Central Europe and found again predominantly haplotypes from the clade A in *M. myotis myotis*.

In this study, we increased sampling in Central Europe and tried to answer two questions: (1) Is the mitochondrial diversity of Central European populations farther south as low as in Poland? (2) Are Central European *M. myotis* descendants of mothers originating only from the Iberian refugium as suggested by Ruedi and Castella (2003, but see Bogdanowicz *et al*., 2009)?

Material and Methods

Tissue Sampling and Amplification of the Mitochondrial Control Region

Altogether 15 nursery colonies of morphologically identified *M. myotis* and one swarming site (Fig. 1 and Table 1) were sampled within the area of Central Europe: Czech Republic (Moravian Karst and its surroundings, northern Bohemia), Slovakia (Borská nížina Lowland, Revúcka vrchovina Highlands, Slovak Karst), and NW Romania. The nurseries found in attics or towers of buildings provided 66 individuals. Additionally, we gathered 29 and two individuals in two cave nursery colonies and at one swarming site, respectively (Table 2). Tissues were taken from adult females collected directly from their roosting site using the non-destructive technique of sterile biopsy punch from the wing membrane (Worthington-Wilmer and Barratt, 1996; Weaver *et al*., 2009). The punches were stored in 96% ethanol at -20ºC).

DNA was extracted using DNeasy Tissue Kit (Qiagen, Halden, Germany) and phenol-chloroform extraction. The second hypervariable segment of the mitochondrial control region (HVII) of approximate length 1,000 base pairs (bp) was amplified with specific primers L16517 (Fumagalli *et al*., 1996) and sH651 (Castella *et al*., 2001) using the PCR protocol described in Castella *et al.* (2001). Successful PCR products were purified (PCR Purification Kit, Qiagen) and sequenced by BigDye Terminators ver. 1.1 sequencing chemistry (Applied Biosystems, Foster City, CA, USA) with the forward primer L16517. Sequencing reactions were analysed on ABI Prism 310 or 3130 Genetic Analyser (Applied Biosystems).

Sequence Analysis

Sequences were edited and aligned with published sequences in SeqScape v. 2.5 (Applied Biosystems). HVII sequences of *M. myotis* (haplotypes H1–H56 from Ruedi and Castella, 2003 and Ruedi *et al*., 2008, and new sequences H76–H81 reported herein) formed a 307 bp long alignment for phylogenetic tree reconstruction. Additional sequences of *M. myotis* found introgressed in European *M. blythii* (H58–H69 — Berthier *et al*., 2006) were added for network analysis. Haplotype frequencies were calculated from all available sequences (this study; Ruedi and Castella, 2003; Berthier *et al.*, 2006; Ruedi *et al.*, 2008). New haplotypes were numbered

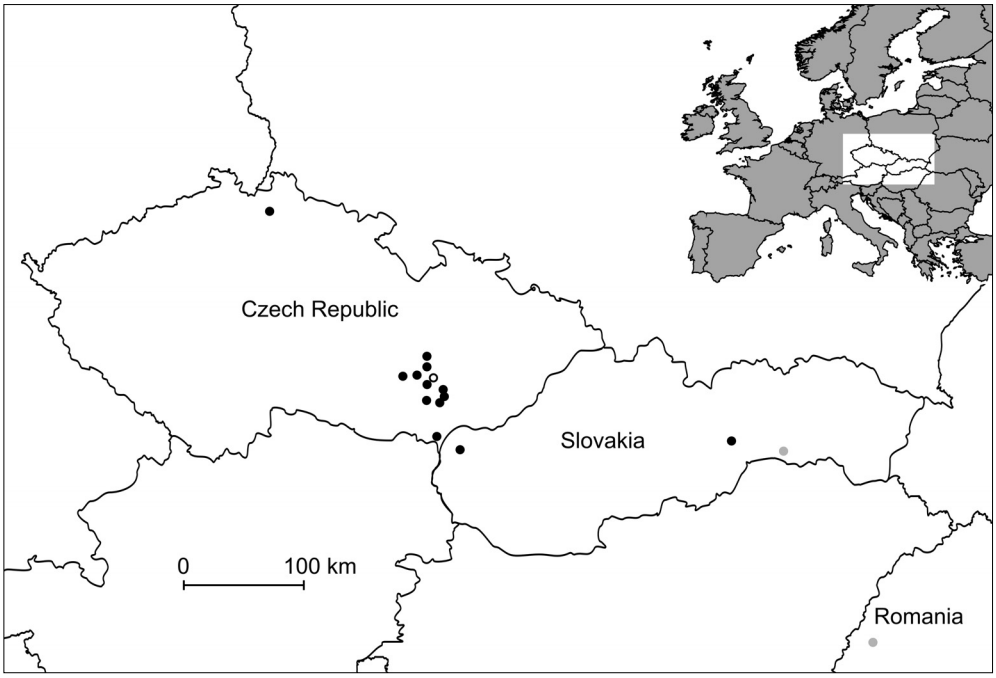


FIG. 1. Sampling sites in the Czech Republic, Slovakia, and NW Romania. ● — nursing colonies in house attics, ● — nursing colonies in caves, ○ — swarming site

consecutively with respect to previously published sequences (up to H75 in Berthier *et al*., 2006).

Optimal substitution model was estimated in Modeltest 3.7 (Posada and Crandall, 1998). The selected models differed between algorithms. We used the model indicated by Bayesian Information Criterion (BIC; Schwartz, 1978) because of its reliability (Posada and Buckley, 2004), and the selected model was the simplest thus minimising the risk of overparametrisation. The selected substitution model was Kimura-2-parameter model (K2P) with transition/transversion ratio equal to 25.206, rate heterogeneity ($\alpha = 0.6974$) and proportion of invariable sites ($I = 0.6462$). We eliminated proportion of invariable sites as this parameter cannot be estimated independently from rate heterogenetity, and the latter affects phylogenetic tree reconstruction more profoundly (Sullivan *et al.*, 1999; Mayrose *et al.*, 2005). Additionally, ML bootstrap was performed using the GTR + Γ model, and performance of both models was tested in BA. The differences were small, and we present node support from NJ (K2P + Γ), ML (GTR + Γ) and BA (K2P + Γ) analyses (Fig. 2).

TABLE 1. Distribution of haplotypes at sampled sites. All haplotypes except H1 (87 individuals) and H78 (two individuals) were found only in single individuals

Locality	Number of sequenced bats	Number of individuals with H1	Other haplotypes present at the site	Number of microsatellite variants
Kateřinská cave (CZ)[1]	2	2	–	2
Doubravník (CZ)[2]	5	5	–	4
Lysice (CZ)[2]	3	3	–	3
Boskovice (CZ)[2]	7	7	–	6
Břeclav (CZ)[2]	4	3	H32	4
Bučovice (CZ)[2]	3	3	–	3
Bohdalice (CZ)[2]	6	6	–	4
Račice (CZ)[2]	3	2	H81	2
Blansko (CZ)[2]	4	4	–	3
Borotín (CZ)[2]	2	2	–	1
Řečkovice (CZ)[2]	1	1	–	1
Český Dub (CZ)[2]	11	11	–	4
Rochovce (SK)[2]	14	11	H76, H79, H80	7
Drienovská cave (SK)[3]	7	7	–	6
Borský Mikuláš (SK)[2]	3	3	–	3
Betfia cave (RO)[3]	22	17	H10, H27, H77, H78	9
Total	97	87		12

Type of sampling locality: [1] — swarming site, [2] — nursing colony in a house attic, [3] — nursing colony in a cave

Distance (neighbour-joining, NJ) analysis was performed in HyPhy 2.0 (Kosakovsky Pond *et al.*, 2005) and Mega 4.1 (Tamura *et al.*, 2007) to maximise our ability to directly compare our results with previously published trees (Ruedi and Castella, 2003; Berthier *et al.*, 2006; Ruedi *et al.*, 2008). Maximum likelihood bootstrap analysis was calculated in RAxML 7.1.0 (Stamatakis, 2006) and Bayesian analysis (BA) in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). We used 10 initial rearrangement settings in the ML analysis. Bootstrap support was estimated from 10,000 replicates in the NJ and ML analyses. Two separate runs of ten Metropolis coupling Markov Chain Monte Carlo with one cold and nine heated chains were run for five million generations each sampled every 1,000th for the BA analysis. The Metropolis coupling parameters included chain temperature at 0.07 and two chain swaps were attempted every generation. The first 2,000 sampled trees were discarded as burn-in, and subsequent tree likelihoods were checked for convergence in Tracer 1.4 (Rambaut and Drummond, 2007). Gaps were treated as missing data in NJ, ML and BA analyses. Furthermore, median-joining (MJ) networks were constructed in Network 4.2 (Bandelt *et al*., 1999) using equal transition/transversion ratio. Haplotype frequencies were estimated from our data coupled with previously published information (Ruedi and Castella, 2003; Berthier *et al.*, 2006; Ruedi *et al.*, 2008). The sequences of *Myotis blythii* (Tomes, 1857) from Kirghizstan (haplotypes Kirg1, Kirg2, Kirg3; Ruedi *et al*., 2008) were used as outgroups to estimate the root of the *M. myotis* phylogenetic trees. The analyses were conducted on a computation cluster at the IVB AS CR, Brno.

Results

Distribution of Sequence Variability

We identified 10 different haplotypes among 307 bp long sequences of HVII in 97 *M. myotis* individuals (Tables 1 and 2). The most common haplotype corresponded to sequence H1 and occurred in 87 individuals (89.7%). Different haplotypes were mostly found only in single individuals from four localities (Table 1) and all but one of them differed by a single substitution from H1 (Table 2). New haplotype H81 differs by two transitions in positions 145 and 278 from the published H34 sequence from the haplogroup D (Table 2).

Phylogenetic Analysis

The alignment for phylogenetic tree reconstruction was 312 bp long, including gaps, and consisted of 62 *M. myotis* unique haplotypes and one (BA) to three (NJ, ML) *M. blythii* haplotypes as outgroups. Our comprehensive analysis showed similar topology to previous ones. However, not all haplogroups were supported (groups with bootstrap support ≥ 70 and/or Bayesian posterior probability ≥ 0.95 were considered). Haplogroups well supported in all analyses were E, F and G. Haplogroup B was only identified in 79% bootstrap replicates of the ML analysis, and groups A, C and D were never monophyletic in more than half of bootstrapped or posterior trees (Fig. 2).

Network analysis of a 307 bp long alignment including all *M. myotis* haplotypes with their respective frequency of occurrence also showed general concordance with previously recognised haplogroups (Fig. 3). Sequences found in Central

Table 2. Variable positions of the HVII haplotypes identified by sequencing 307 bp of the control region from individuals from Central Europe. The data from one Polish nursing colony by Ruedi and Castella (2003) are also included. Designation of haplotypes is according to Ruedi *et al*. (2008), newly described haplotypes are H76–H81 (GenBank Accession Nos. HM117842– HM117847). New haplotype H81 is the most similar to haplotype H34 that was described from Greece (Ruedi and Castella, 2003), shown here for comparison. Haplogroups were identified by Ruedi and Castella (2003)

Haplotype	Nucleotide position																		Haplogroup	Occurrence*
	1 0	5 5	9 1	9 5	1 2 1	1 3 0	1 4 5	1 5 8	1 6 0	1 8 8	1 9 0	1 9 7	2 0 9	2 1 9	2 3 3	2 6 0	2 7 8	2 8 7		
H1	G	A	T	G	A	G	A	A	A	G	G	T	A	G	A	G	A	T	A	CZ, F, PL, I, RO, SK, SP, SW
H10	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	A	RO, SP, SW
H27	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	A	RO, SP
H31	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	A	PL
H32	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	A	CZ, PL
H76	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	A	SK
H77	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	A	RO
H78	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	RO
H79	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	A	SK
H80	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	A	SK
H81	A	.	C	A	G	.	G	G	G	A	.	.	G	.	G	.	G	.	D	CZ
H34	A	.	C	A	G	.	.	G	G	A	.	.	G	.	G	.	.	.	D	GR

* — This study: CZ = Czech Republic, SK = Slovakia, RO = NW Romania; Ruedi and Castella (2003): F = France, GR = Greece, PL = Poland, SP = Spain, SW = Switzerland; Ruedi *et al.* (2008): I = N Italy (Bolzano)

Europe in this study appear to belong to two different haplogroups A and D. All new sequences but one belong to the group A: five are new (H76–H80; GenBank Accession numbers HM117842–HM117846), and three (H10, H27, and H32) were previously found. The sequence H81 (GenBank Accession number HM117847) is a newly described haplotype found only in the Račice colony (Czech Republic) that belongs to previously identified haplogroup D (Fig. 3).

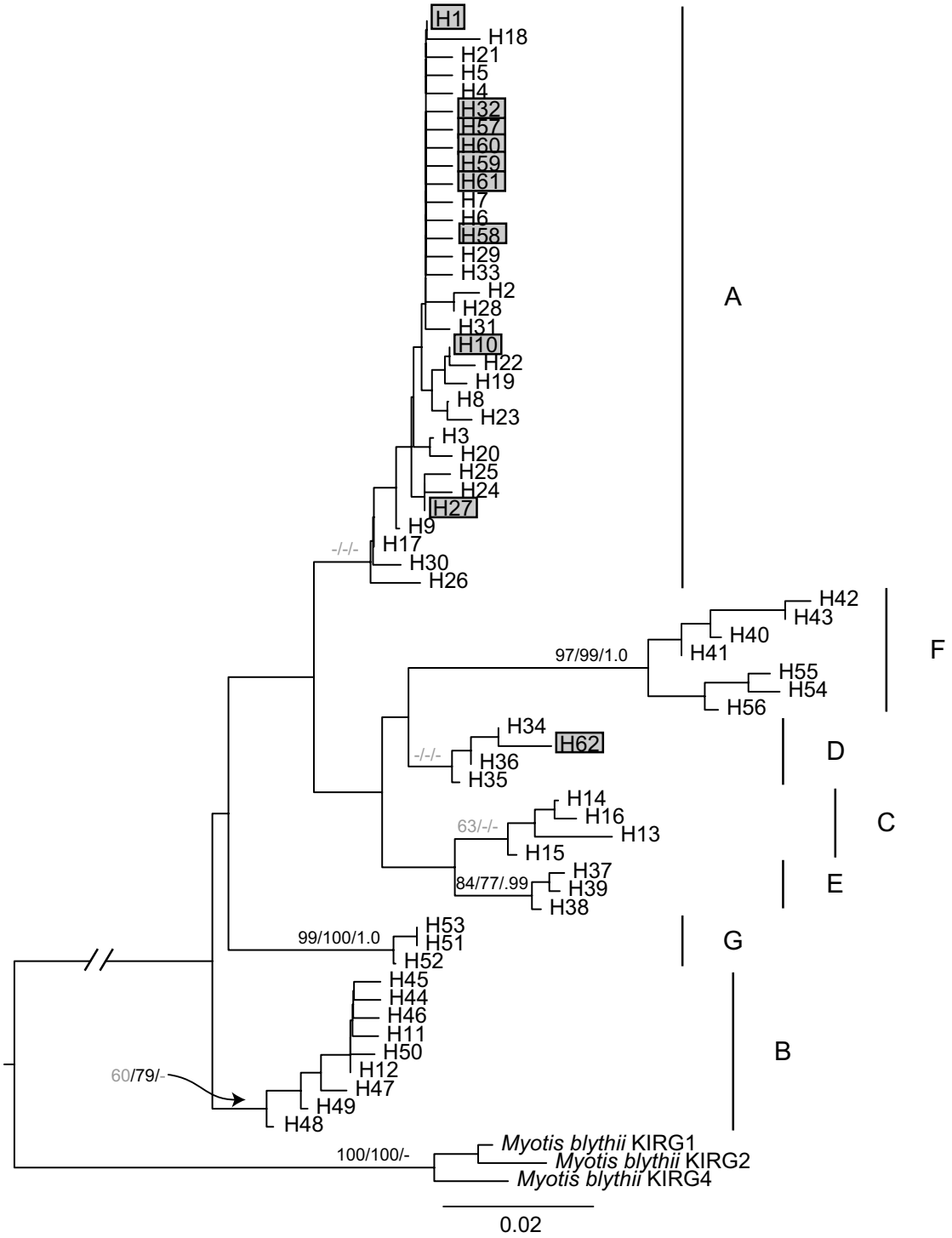


FIG. 2. Neighbor-joining phylogenetic tree using K2P + Γ substitution model of hypervariable segment II of mitochondrial control region haplotypes. Numbers above branches of previously recognised groups represent bootstrap support from 10,000 neighbour-joining and maximum likelihood bootstrap replicates and Bayesian posterior probability after chain convergence. Haplotypes found in Central Europe are highlighted in shaded boxes

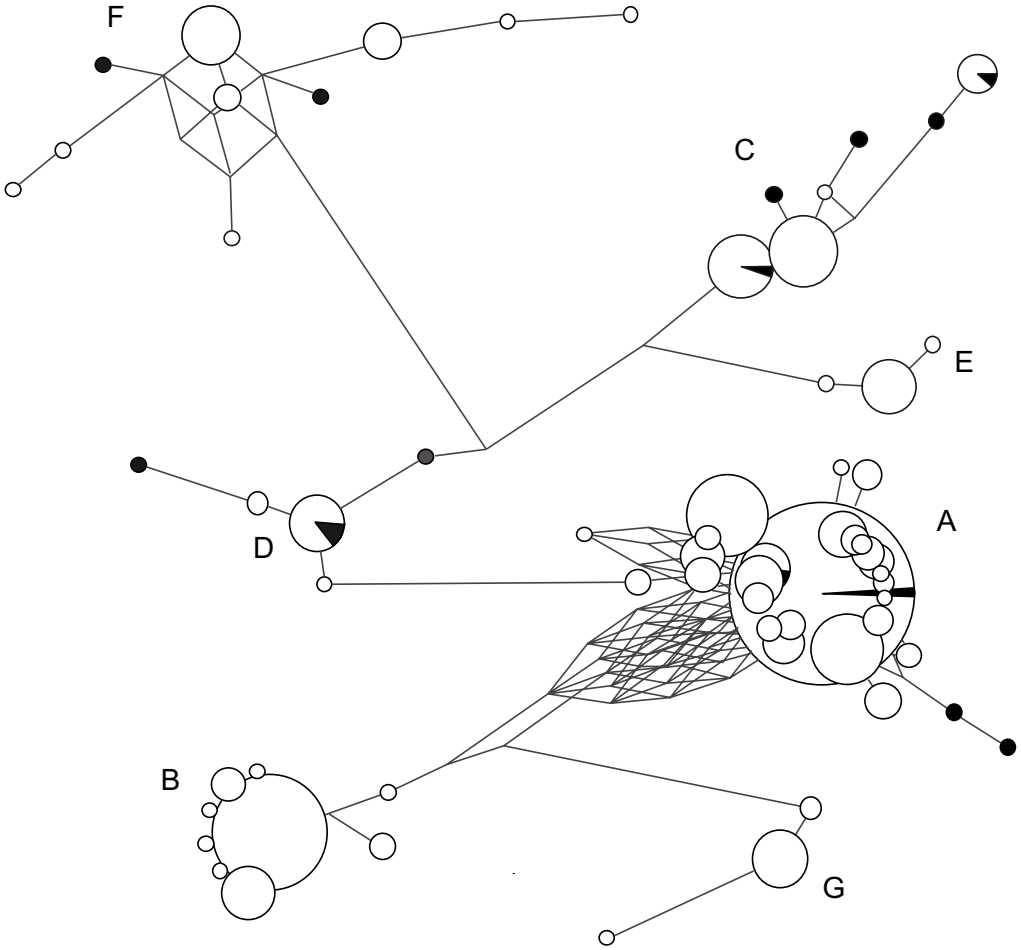


FIG. 3. Median-joining network of hypervariable segment II of mitochondrial control region haplotypes. Length of branches is proportional to number of substitutions along given branch, and circle size is proportional to haplotype frequency according to information in Ruedi and Castella (2003), Berthier *et al.* (2006), Ruedi *et al.* (2008) and this study. Haplotypes found in *M. blythii* are indicated in black, haplotype H81 in dark grey

DISCUSSION

Variability in the Control Region Sequences and Phylogeographic Implications

Sequences found in the Czech Republic, Slovakia, and NW Romania belong to two previously identified European haplogroups (Ruedi *et al*., 2008). Except haplotype H81, all individuals carried haplotypes from group A, and 89.7% of individuals had haplotype H1, which is the most common one in Europe (Ruedi and Castella, 2003). The greatest diversity of group A has been previously observed in western Mediterranean, suggesting the area of origin of this group in Iberian Peninsula (Castella *et al.,* 2001; Ruedi and Castella, 2003). Our data are consistent with this scenario. First, overall genetic mtDNA diversity in the studied region is low with predominance of sequences from group A similarly as in Poland (Ruedi and Castella, 2003; Bogdanowicz *et al*., 2009). Second, two haplotypes found in this study in NW Romania were found in Spain (H27) or in Spain and Switzerland (H10) to date. These facts strongly support Ruedi and Castella's (2003) notion that Central Europe was colonised relatively rapidly via western colonisation route (cf. Hewitt 2004).

However, identification of the new haplotype H81, which is most closely related to haplotypes from SE Balkan (group D), indicates an occurrence of further colonisation from another direction. Western and eastern colonisation routes into central Europe (the so-called 'Hedgehog paradigm' — Hewitt, 2000) are well described in other animal species (Taberlet *et al*., 1998), including bats (Hulva *et al*., 2004; Juste *et al*., 2004). However as we found a sequence from haplogroup D only in one individual, alternative explanations are more viable.

Instead of an indigenous colonization front of Balkan greater mouse-eared bats to Central Europe, it is theoretically possible that the female with H81 haplotype represents an occasional long-distance movement; i.e., reached the Czech Republic from the Balkans within its life-span. Greater mouse-eared bats are known to move the most frequently up to 50 km as evidenced by mark-recapture studies (Gaisler *et al*., 2003; Hutterer *et al*., 2005), but the longest distance recorded is 436 km (Hutterer *et al*., 2005). Based on the current data, it is impossible to distinguish whether presence of an individual of Balkan origin in Central Europe is evidence of colonization from southeast, in which case a genetic signature along the route should be traceable, or whether it is a record of occasional movement. Such differentiation requires additional sampling and analysis of bats along the possible colonization route between eastern Greece and the Czech Republic. Recent analyses of ancient DNA from Nietoperzowa Cave in southern Poland (Bogdanowicz *et al*., 2009) support the former hypothesis, because the authors provide evidence that another Balkan clade (haplogroup F) was present in Central Europe already 820 ± 25 years BP.

Alternative theoretical explanation could involve interspecific hybridisation with *M. blythii*, a species known to have colonised Europe from south-east (Benda and Horáček, 1995). *Myotis blythii* can hybridize with *M. myotis* (Bogdanowicz *et al*., 2009), and in continental Europe mtDNA of *M. blythii* is completely replaced by that of *M. myotis* (Berthier *et al*., 2006; but see different opinion in Bogdanowicz *et al*., 2009). Haplotype H81 belongs to haplogroup D, the same haplogroup, where multiple '*M. blythii*' sequences were found (Berthier *et al*., 2006; Bogdanowicz *et al*., 2009; Fig. 3 in this study). It is therefore possible to imagine that H81 was introgressed into *M. blythii* somewhere in the south-east part of *M. myotis* distribution and subsequently introduced to central European population of *M. myotis* through repeated hybridization with *M. blythii*. Mating of female *M. blythii* with male *M. myotis* would have led to the observed occurrence of this haplotype in central European *M. myotis*.

One of the conspicuous features of geographical distribution of haplotypes (see figure 1 in Ruedi and Castella, 2003) is its rough concordance between the distribution of some haplogroups and types of nurseries. In southern Europe, especially in the Balkans (with 'endemic' haplogroups), the nurseries are located in warm natural caves, while further north summer maternity colonies are usually located in buildings. The behavioural and physiological changes necessary for this shift in reproduction habits (Rodriguez *et al*., 2003) are usually attributed to rapid expansion of one successful haplogroup from south-western Europe (Ruedi and Castella, 2003). However, even though most nurseries in Central Europe inhabit buildings, there are nurseries of the greater mouse-eared bats established in caves as well, e.g. the Hranická propast cave in the Czech Republic (Řehák and Baroň, 2006), and the Drienovská jaskyňa cave and the Plavecká jaskyňa cave in Slovakia (Uhrin *et al.*, In press). In this study, we investigated mtDNA haplotypes from two nursery colonies found in caves. All 22 sequenced individuals from the Betfia cave in NW Romania as well as seven individuals from Drienovecká jaskyňa cave in Slovakia exhib-ited only haplotypes from group A. Pending necessity of more detailed research, it seems probable that this important variation in forming nurseries is not genetically based and is a result of phenotypic plasticity. At the same time it suggests that the populations of *M. myotis* had changed their roosting habits at least twice ('caves-attics-caves') during colonization of Central Europe from Iberian refugium.

Conclusion

We confirmed that *M. myotis* bats colonised Central Europe through the western colonisation route conforming to the previously suggested 'Bear Paradigm' of post-glacial colonisation. Already as far north as the Czech Republic and Slovakia, genetic variability of *M. myotis* is very low compared to the Alpine region. We found that individuals of Balkan origin played a minor role in populating the area.

Acknowledgements

We would like to thank to P. Benda, H. Berková, D. Horáček, K. J. Petrželková, and M. Pokorný for their help in sampling of studied animals, and P. Benda, N. Irwin, M. Ruedi, Z. Řehák, W. Bogdanowicz and three reviewers for their valuable comments on previous versions of manuscript. Research was supported by grants from the Czech Science Foundation (Nos. 206/01/1555, 206/09/0888), Ministry of Education of the Czech Republic (Research centre no. LC06073 and MSM 0021622416), Slovak Grant Agency VEGA (2/0130/08), and Slovak Research and Development Agency (APVV-18-032105). All handlings were conducted by experts provided with the appropriate permits issued by the Ministries of Environment of the Czech or Slovak Republics, respectively.

Literature Cited

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press, Cambridge, USA, 447 pp.

Bandelt, H. J., P. Forster, and A. Röhl. 1999. Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution, 16: 37–48.

Beebee, T. J. C., and G. Rowe. 2004. An introduction to molecular ecology. Oxford University Press, New York, 370 pp.

Benda P., and I. Horáček. 1995. Biometrics of *Myotis myotis* and *Myotis blythi*. Myotis, 32–33: 45–55.

Berthier, P., L. Excoffier, and M. Ruedi. 2006. Recurrent replacement of mtDNA and cryptic hybridization between two sibling bat species *Myotis myotis* and *Myotis blythii*. Proceedings of the Royal Society, 273B: 3101–3109.

Bogdanowicz, W., R. A. Van Den Bussche, M. Gajewska, T. Postawa, and M. Harutyunyan. 2009. Ancient and contemporary DNA sheds light on the history of mouse-eared bats in Europe and the Caucasus. Acta Chiropterologica, 11: 289–305.

Burland, T. M., and J. Worthington-Wilmer. 2001. Seeing in the dark: molecular approaches to the study of bat populations. Biological Reviews, 76: 389–409.

Burland, T. M., E. M. Barratt, M. A. Beaumont, and P. A. Racey. 1999. Population genetic structure and gene flow in a gleaning bat, *Plecotus auritus*. Proceedings of the Royal Society, 266B: 975–980.

Castella, V., M. Ruedi, L. Excoffier, C. Ibánez, R. Arlettaz, and J. Hausser. 2000. Is the Gibraltar Strait a barrier to gene flow for the bat *Myotis myotis* (Chiroptera: Vespertilionidae)? Molecular Ecology, 9: 1761–1772.

Castella, V., M. Ruedi, and L. Excoffier. 2001. Contrasted patterns of mitochondrial and nuclear structure among nursery colonies of the bat *Myotis myotis*. Journal of Evolutionary Biology, 14: 708–720.

Fumagalli, L., P. Taberlet, L. Favre, and J. Hausser. 1996. Origin and evolution of homologous repeated sequences in the mitochondrial DNA control region of shrews. Molecular Biology and Evolution, 13: 31–46.

Gaisler, J., V. Hanák, V. Hanzal, and V. Jarský. 2003. Výsledky kroužkování netopýrů v České republice a na Slovensku, 1948–2000. [Results of bat banding in the Czech Republic and Slovakia, 1948–2000]. Vespertilio, 7: 3–61. [in Czech with English summary].

Hewitt, G. M. 2000. The genetic legacy of the Quaternary ice ages. Nature, 405: 907–913.

Hewitt, G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society, 359B: 183–195.

Horáček, I. 1984. Remarks on causality of population decline in European bats. Myotis, 21–22: 138–147.

Hulva, P., I. Horáček, P. P. Strelkov, and P. Benda. 2004. Molecular architecture of *Pipistrellus pipistrellus*/*Pipistrellus pygmaeus* complex (Chiroptera: Vespertilionidae): further cryptic species and Mediterranean origin of the divergence. Molecular Phylogenetics and Evolution, 32: 1023–1035.

Hutterer, R., T. Ivanova, C. Meyer-Cords, and L. Rodrigues. 2005. Bat migrations in Europe. Naturschutz und Biologische Vielfalt, Federal Agency for Nature Conservation, Bonn, Heft 28, 162 pp.

Ibrahim, K. M., R. A. Nichols, and G. M. Hewitt. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansion. Heredity, 77: 282–291.

Juste, J., C. Ibáñez, J. Muñoz, D. Trujillo, P. Benda, A. Karataş, and M. Ruedi. 2004. Mitochondrial phylogeography of the long-eared bats (*Plecotus*) in the Mediterranean Palaearctic and Atlantic Islands. Molecular Phylogenetics and Evolution, 31: 1114–1126.

Kerth, G., F. Mayer, and B. König. 2000. Mitochondrial DNA (mtDNA) reveals that female Bechstein´s bats live in closed societies. Molecular Ecology, 9: 793–800.

Kerth, G., F. Mayer, and E. Petit. 2002. Extreme sex-biased dispersal in the communally breeding, nonmigratory Bechstein's bat (*Myotis bechsteinii*). Molecular Ecology, 11: 1491–1498.

Kosakovsky Pond, S. L., S. D. W. Frost, and S. V. Muse. 2005. HyPhy: hypothesis testing using phylogenies. Bioinformatics, 21: 676–679.

Mayrose, I., N. Friedman, and T. Pupko. 2005. A Gamma mixture model better accounts for among site rate heterogeneity. Bioinformatics, 21: 151–158.

McCracken, G. F., M. K. McCracken, and A. T. Vawter. 1994. Genetic structure in migratory populations of the bat *Tadarida brasiliensis mexicana*. Journal of Mammalogy, 75: 500–514.

Mitchell-Jones, A. J., G. Amori, W. Bogdanowicz, B. Krystufek, P. J. H. Reijnders, F. Spitzenberger, M. Stubbe, J. B. M. Thissen, V. Vohralík, and J. Zima (eds.). 1999. The atlas of European mammals. Academic Press, London, 484 pp.

Petit, E., and F. Mayer. 1999. Male dispersal in the noctule bat (*Nyctalus noctula*): where are the limits? Proceedings of the Royal Society, 266B: 1717–1722.

Petit, E., and F. Mayer. 2000. A population genetic analysis of migration: the case of the noctule bat (*Nyctalus noctula*). Molecular Ecology, 9: 683–690.

Petit, E., L. Excoffier, and F. Mayer. 1999. No evidence of bottleneck in the postglacial recolonization of Europe by the noctule bat (*Nyctalus noctula*). Evolution, 53: 1247–1258.

Posada, D., and T. R. Buckley. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology, 53: 793–808.

Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics, 14: 817–818.

Postawa, T. 2004. Changes in bat fauna during the Middle and Late Holocene as exemplified by thanatocoenoses dated with ^{14}C AMS from Kraków-Częstochowa Upland caves (Poland). Acta Chiropterologica, 6: 269–292.

Rambaut, A. and A. J. Drummond. 2007. Tracer v1.3, Available from http://beast.bio.ed.ac.uk/Tracer

Řehák, Z., and I. Baroň. 2006. Netopýři Hranické propasti. Pp. 37–38, *in* Hranická propast (M. Orálek, ed.). ČSOP, Valašské Meziříčí, 64 pp.

Rivers, N. M., R. K. Butlin, and J. D. Altringham. 2005. Genetic population structure of Natterer's bats explained by mating at swarming sites and philopatry. Molecular Ecology, 14: 4299–4312.

Rodriguez, L., A. Zahn, A. Rainho, and J. M. Palmeirim. 2003. Contrasting the roosting behaviour and phenology of an insectivorous bat (*Myotis myotis*) in its souhtern and northern distribution ranges. Mammalia, 67: 321–335.

Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES 3:

Bayesian phylogenetic inference under mixed models. Bioinformatics, 19: 1572–1574.

RUEDI, M., and V. CASTELLA. 2003. Genetic consequences of the ice ages on nurseries of the bat *Myotis myotis*: a mitochondrial and nuclear survey. Molecular Ecology, 12: 1527–1540.

RUEDI, M., S. WALTER, M. C. FISCHER, D. SCARAVELLI, L. EXCOFFIER, and G. HECKEL. 2008. Italy as a major Ice Age refuge area for the bat *Myotis myotis* (Chiroptera: Vespertilionidae) in Europe. Molecular Ecology, 17: 1801–1814.

SCHWARTZ, G. 1978. Estimating the dimension of a model. The Annals of Statistics, 6: 461–464.

STAMATAKIS, A. 2006 RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics, 22: 2688–2690.

SULLIVAN, J., D. SWOFFORD, and G. NAYLOR. 1999. The effect of taxon sampling on estimating rate heterogeneity parameters of maximum likelihood models. Molecular Biology and Evolution, 16: 1347–1356.

TABERLET, P., L. FUMAGALLI, A. G. WUST-SAUCY, and J. F. COSSON. 1998. Comparative phylogeography and postglacial colonization routes in Europe. Molecular Ecology, 7: 453–464.

TAMURA, K., J. DUDLEY, M. NEI, and S. KUMAR. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24: 1596–1599.

UHRIN, M., P. KAŇUCH, J. KRIŠTOFÍK, and L. PAULE. In press. Phenotypic plasticity in the greater mouse-eared bat in extremely different roost conditions. Acta Theriologica.

WEAVER, K. N., S. E. ALFANO, A. R. KRONQUIST, and D. M. REEDER. 2009. Healing rates of wing punch wounds in free-ranging little brown myotis (*Myotis lucifugus*). Acta Chiropterologica, 11: 220–223.

WORTHINGTON-WILMER, J., and E. BARRATT. 1996. A non-lethal metod of tissue sampling for genetic studies of chiropterans. Bat Research News, 37: 1–3.

Received 24 August 2009, accepted 30 March 2010

Výběr úkrytů a letová aktivita netopýrů

Gaisler, J., Zukal, J., Řehák, Z. & Homolka, M. (1998). Habitat preference and flight activity of bats in a city. Journal of Zoology (London) 244, 439-445.

J. Zool., Lond. (1998) **244**, 439–445

Habitat preference and flight activity of bats in a city

J. Gaisler[1]*, J. Zukal[2], Z. Rehak[1] and M. Homolka[2]

[1] Department of Zoology and Ecology, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic
[2] Institute of Landscape Ecology, Czech Academy of Sciences, Kvetna 8, CZ 603 65 Brno, Czech Republic

(Accepted 23 April 1997)

Abstract

Pipistrellus pipistrellus, Eptesicus serotinus, Nyctalus noctula, N. leisleri, Myotis daubentonii, M. myotis, unidentified small *Myotis* spp. and *Plecotus* spp. were recorded during bat detector transects within a central European city of 350,000 inhabitants. Bats were recorded in all seven habitat types under study, the levels of activity for each species and habitat type were significantly different. The relative activity of the whole bat community was highest in old outskirts (low density housing) and at the river, and lowest in the city centre and new housing estates (high density housing). Significant differences were found in the timing of bat activity during the night and the season. Within the first two hours after sunset, relative activity of *P. pipistrellus* and *E. serotinus* was highest in the first 0.5 h and decreased thereafter. In *N. noctula,* it was highest during the second and third 0.5 h and in *Myotis* spp. it was low in the first 0.5 h and increased till the end of monitoring. Flying bats were recorded from March till October; the lunar cycle had no significant effect on the amount of flight activity. The relative activity of *E. serotinus* was positively correlated with temperature. No significant correlation was found between the activity of bats and the number of trees and streetlamps per transect. Comparison with the results of an earlier visual census showed that more bats were recorded acoustically than visually except in the city centre. This is attributed to the effect of white streetlamps during the visual census. During the acoustic census, most white lamps were replaced by yellow lamps which biased the impact of lamps on bat traffic. Nevertheless, bat species known to benefit from white streetlamps remained the most common foragers within the city.

Key words: bat habitats, flight activity, city

INTRODUCTION

From 1965 to 1980, J. Hooper, a pioneer of field work with ultrasound receivers, monitored the echolocation calls of bats in the London area (Hooper, 1981). These and subsequent London bat detector records were later summarized by Mickleburgh (1987). Occasionally, this technique has been used in other European cities and towns but, in general, there are few bat detector records from urban habitats. Much more information, resulting from detector studies, has been obtained in woodland, farmland or riverine habitats (Baagoe, 1986; Furlonger *et al*., 1987; Jüdes, 1989; Ahlén & Gerell, 1989; Limpens *et al*., 1989; Negraeff & Brigham, 1995; Rydell & Racey, 1995; de Jong, 1995; Walsh & Harris, 1996*a*). In contrast, most records of bats in towns have come from captures and observations inside the buildings, from incidental records of dead or moribund bats, or from opportunistic nettings at potentially favourable sites (Gaisler & Bauerová, 1985–86; Klawitter, 1986; Sanchez, 1989; Spitzenberger, 1990; Haensel, 1992; Schober & Meyer, 1995).

In 1977, initial flying activity of bats was recorded visually on the territory of the city of Brno, with the co-operation of 28 previously trained observers (Gaisler, 1979). The observers walked along transects selected by themselves. For further analysis, the localities were grouped according to the prevailing environmental type, which was based on the classification published by an ornithologist (Hudec, 1976), who studied birds of the same town. The primary aim of the present study was to repeat the census, this time acoustically. We presumed that more bats, in terms of both species and individuals, would be recorded acoustically than visually and that detecting their echolocation calls would provide better insight into the knowledge of bat traffic in different urban habitats with respect to various environmental cues.

*All correspondence to: Dr J. Gaisler, Department of Zoology and Ecology, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

From 1990 to 1992, a nationwide survey of bats and their habitats was carried out in Britain, using bat detectors whilst walking preselected transects (Walsh, Harris & Hutson, 1995; Walsh & Harris, 1996*a, b*). Urban areas were included in the survey but they were most often residential areas of villages and small towns. Species identification was limited and 71% of the bat passes were unidentified. Results of this and other surveys and case studies were summarized by Racey (In press) in order to highlight the need of ecological data for the conservation of European bats. Thus the secondary goal of our paper was to complete the surveys made in Britain and elsewhere by supplying data on the activity of bats in urban habitats of a city while identifying the species as far as possible. The data are intended for better knowledge of bat biology in relation to their conservation in a particular habitat set (Racey, In press).

MATERIAL AND METHODS

Four bat detectors were used, D-980 Pettersson, SKYE, and 2 QMC Mini. We made acoustic recordings of bats within the town of Brno, Czech Republic (230 km^2, 350,000 inhabitants), from March till October 1993. The same habitat types were sampled under the same conditions as in 1977 (Gaisler, 1979). Habitat types included: A, the citycentre – historical centre of the town; B, old residential quarters – streets with blocks of buildings, mostly four–five-storeyed; C, old suburbs – streets of family houses with small gardens; D, villa quarters – scattered villas with large gardens; E, old outskirts – old warehouses, factory premises, open areas, trees; F, new housing estates – blocks of high rise (typically 8-storeyed) flats, lawns and newly planted trees; G – the river and its immediate surroundings.

Most observations started 10 min after sunset, on the nights of new and full moon (± 1 night), and lasted 30 min. In total, we covered 55 such transects labelled 'standard' which encompassed 1650 min of monitoring. Standard transects were used for all comparisons. In addition, 41 transects, 1230 min, were either performed at standard dates and localities but earlier (from sunset) or later at night, or they represented different dates and/or different localities. The data from all transects were pooled for the analysis of habitat preference and timing of bat activity during the night, except for 8 additional transects which were disregarded when analysing the habitat preference, since it was impossible to allocate the respective localities into any of the habitat types A to G.

In order to take into account different sensitivity and filtering capacity of our bat detectors (Forbes & Newhook, 1990; Walsh *et al.*, 1995), the observers changed places and detectors haphazardly and only the heterodyne mode was used. When possible, acoustic detections were coupled with visual observations, in order to improve the estimation of the number of flying bats. At the beginning of each transect, the detector was tuned at 40 kHz. Having heard a signal, the observer halted, tuned to the optimum frequency, recorded the duration of the time interval during which he heard the signals, rounded up to 1 sec, and estimated the number of flying bats. When bats were no longer heard, the observer continued walking until another registration or till the end of the 30-min period. Thus the length of individual transects differed (850–2490 m, average 1455.7 m); in total we walked *c*. 128 km. For each locality, the number of trees and streetlamps was recorded. The values of air temperature, relative humidity and air pressure were extracted from the data of the nearest meteorological station (Brno-Kroftova). Negative influence of climatic conditions, however, was *a priori* limited since we did not monitor bat activity under extreme situations such as very cold (temperature <10 °C), rainy or windy nights.

The level of bat activity was assessed primarily as in the paper by McAney & Fairley (1988), as the number of individual minutes in which bat ultrasound signals were detected. This number was converted into percentage of total number of monitoring minutes (per transect type, habitat, time interval, etc.). Secondarily, the number of flying bats was estimated, in order to obtain data comparable to those of the earlier visual census. The differences in bat activity were tested by contingency tables (χ^2 test) based on numbers of positive and negative minutes. To compare with the results of earlier visual census, a Mann–Whitney test was used. Possible correlation of bat activity, as revealed by different methods and with various environmental factors, was tested by a non-parametric Spearman correlation. Statistical analyses were performed according to Sokal & Rohlf (1981) and Wilkinson (1990).

RESULTS

General assessment of the level of bat activity

Six species and two species groups were recognized (Table 1). Based on both number of minutes and number of individuals observed, *Pipistrellus pipistrellus, Eptesicus serotinus* and *Nyctalus noctula* were most common. In all of them, distinct feeding buzzes gave evidence of their foraging activity. The ranks of remaining species and species groups were the same by both methods, except for *Myotis daubentonii* which was more common according to the number observed at additional transects. High numbers of recorded *M. daubentonii* resulted from visual observations of bats hunting close to the water level.

Habitat preference by bats flying in the city

Only the three most common species were recorded in all habitat types (Table 2). The levels of their activity in each habitat differed. In *P. pipistrellus,* this difference is relatively small though significant ($\chi^2 = 12.61$, $P = 0.05$),

Table 1. Comparison of bat call minutes recorded (min), bat call minutes in percentage of minutes of monitoring (%) and bat numbers estimated (*n*). Spearman one-tailed test shows significant correlation between the rank of species and species groups by bat call minutes and bat numbers ($r_s = 0.976$, $n = 8$ observations, $P<0.01$)

Species	Standard			Additional			All transects	
	min	%	*n*	min	%	*n*	min	*n*
P. pipistrellus	83	5.0	117	47	2.9	62	130	179
E. serotinus	84	5.1	136	18	1.1	19	102	155
N. noctula	45	2.7	52	28	1.7	32	73	84
Myotis spp.	13	0.8	15	21	1.3	22	34	37
M. daubentonii	8	0.5	10	8	0.5	65	16	75
N. leisleri	11	0.7	11	2	0.1	2	13	13
M. myotis	2	0.1	2	0	0.0	0	2	2
Plecotus spp.	0	0.0	0	2	0.1	2	2	2
Total	246	14.9	343	126	7.7	204	372	547

Table 2. Bat call minutes recorded in percentage of minutes of monitoring per habitat type: A, historical citycentre; B, old residential quarters; C, old suburbs; D, villa quarters; E, old outskirts; F, new housing estates; G, riverine habitats. For details see 'Material and methods'

Habitat	A	B	C	D	E	F	G
Monitoring min	150	450	540	630	300	330	240
P. pipistrellus	3.3	4.4	5.4	5.2	4.7	1.5	2.1
E. serotinus	3.3	2.9	4.1	3.0	9.0	4.2	0.8
N. noctula	0.7	2.9	2.6	1.1	2.7	3.0	7.1
Myotis spp.	0.0	0.9	0.9	1.9	1.0	0.0	3.3
M. daubentonii	0.0	0.2	0.2	0.0	0.7	0.0	5.0
N. leisleri	0.0	2.9	0.0	0.0	0.0	0.0	0.0
M. myotis	0.0	0.0	0.2	0.0	0.0	0.3	0.0
Plecotus spp.	0.0	0.0	0.0	0.3	0.0	0.0	0.0
Total	7.3	14.2	13.4	11.5	18.1	9.0	18.3
Number of species	3	6	6	5	5	4	3

which can be interpreted as a manifestation of a broad ecological niche with respect to urban habitats. A more detailed analysis shows that the citycentre (A), new housing estates (F) and riverine habitats (G), when coupled, are significantly less frequented than the remaining habitats ($P<0.001$).

In *E. serotinus,* the activity recorded per habitat type was significantly different ($\chi^2 = 29.92$, $P<0.001$). Preferred are the old outskirts (E), while riverine habitats (G) are significantly less frequented ($P<0.001$). Differences in activity at other habitats are insignificant. *N. noctula* also showed great differences of activity per habitat type ($\chi^2 = 26.63$, $P<0.001$). Riverine habitat is markedly preferred, and the citycentre (A) and villa quarters (D) are less frequented than the remaining habitats ($P = 0.006$). As expected, *M. daubentonii* was most often recorded at the river as well. Unidentified small *Myotis* species (most probably *M. emarginatus, M. mystacinus, M. brandtii* or *M. bechsteinii*, Gaisler & Bauerova 1985–86) were also frequently recorded close to the river, relatively often in villa quarters (D) and infrequently in four additional habitats. Both *Myotis* spp. and *M. daubentonii* were missing from the citycentre (A) and new housing estates (F). The sample of *Nyctalus leisleri, M. myotis* and *Plecotus* spp. (*P. austriacus* or *P. auritus*) is too small to draw any conclusions about their habitat preference.

Concerning all species lumped together, relative activity per habitat type was significantly different ($\chi^2 = 22.80$, $P = 0.001$). It was largest at old outskirts (E) and the river (G) and smallest in the citycentre (A) and new housing estates (F) (Table 2). Medium activity was recorded in the remaining habitats, namely, old residential quarters (B), old suburbs (C) and villa quarters (D). The differences within the habitats of high, low and medium activity, respectively, are insignificant. However, activity as recorded at medium frequented habitats differs significantly from that recorded at both preferred ($P = 0.003$) and not preferred habitats ($P = 0.01$). These data are not correlated with the number of species with the exception of the citycentre where the least number of species, as well as the least number of bat positive minutes, were recorded.

Comparison with the results of the earlier visual census

Three species were identified during the 1977 census but in only one of them, *E. serotinus,* can the identification be considered reliable (see **Discussion**). In the earlier paper, population density data were published (Gaisler, 1979), but original numbers of bats observed have been kept in the database. Bat numbers estimated at the standard transects were compared to these data. As expected, significantly more bats were recorded during the 1993 census (Table 3). Considering the numbers of bats per habitat type, in *E. serotinus* only the transects performed at old outskirts (E) revealed significantly more individuals recorded acoustically. In other species, significantly more bats were recorded acoustically than visually in all habitat types under study, except the citycentre (A). There the average number of bats estimated during the 1977 visual census was four times higher than that estimated during the 1993 acoustic census (Table 4).

Timing of bat activity during the first two hours after sunset

Three species and one species group were recorded during all four 0.5 h time intervals after sunset (Fig. 1). Levels of their flight activity were significantly different. In *P. pipistrellus* ($\chi^2 = 9.75$, $P = 0.021$) and *E. serotinus* ($\chi^2 = 17.30$, $P = 0.001$), the relative number of minutes recorded was highest during the first 0.5 h after sunset and decreased thereafter. In *N. noctula* ($\chi^2 = 12.18$, $P = 0.007$), it was highest during the second and third 0.5 h and in *Myotis* spp. ($\chi^2 = 11.41$, $P = 0.01$) it was low at the beginning and increased till the end of monitoring.

Table 3. Comparison of the average number of bats estimated per one transect during the 1977 visual and 1993 acoustic censuses, Mann–Whitney U-test

Transects	visual ($n = 318$)	acoustic ($n = 55$)	P
E. serotinus	1.0	2.5	0.002
Other species	1.1	3.8	<0.001
All bats	2.1	6.3	<0.001

Table 4. Difference between average numbers of bats estimated per habitat types during the visual and acoustic censuses, Mann–Whitney U-test. See Table 2 for identification of habitat types A to G

Habitat	A	B	C	D	E	F	G
E. serotinus							
visual	2.67	1.86	0.67	1.28	1.25	0.62	0.45
acoustic	2.25	1.90	2.00	1.88	9.50	0.88	0.50
P	n.s.	n.s.	n.s.	n.s.	0.002	n.s.	n.s.
Other species							
visual	4.00	1.44	0.70	1.72	1.58	0.51	0.86
acoustic	0.75	4.80	4.54	4.25	3.17	1.50	5.33
P	n.s.	0.016	<0.001	0.026	0.003	0.037	0.016
All bats							
visual	6.67	3.35	1.38	3.00	2.83	1.13	1.32
acoustic	3.00	6.70	6.54	6.13	12.67	2.38	5.83
P	n.s.	n.s.	0.001	0.026	0.003	0.037	0.016

Bat activity with respect to season

During the year, *P. pipistrellus* and *Myotis* spp. were recorded from March to September, *E. serotinus* from April to September and *N. noctula* from March to October. Throughout the year, significant fluctuations in the activity of most common species were recorded (Fig. 2). In *P. pipistrellus* ($\chi^2 = 70.88$, $P<0.001$), the peak of activity in late August and early September may reflect the emergence of weaned young. In *E. serotinus* ($\chi^2 = 114.53$, $P<0.001$) and *N. noctula* ($\chi^2 = 53.04$ $P<0.001$), the peaks in April and July, respectively, probably reflect increased activity at places with abundant prey during the nights of observation.

Bat activity with respect to other factors

In the three most common species, the relative number of minutes recorded during the nights of new, as compared to full, moons does not differ significantly (χ^2 test). Significant correlation of activity recorded with selected climatic factors (average values per transect) was found only in *E. serotinus*; its activity correlates positively with temperature and negatively with humidity ($r_s = 0.453$ and -0.473, respectively, $P<0.05$, $n = 27$). In all other cases concerning the number of trees and number of streetlamps, Spearman correlation analysis gave negative results. No correlation was found between the level of activity and the number of only white streetlamps either (see **Discussion**).

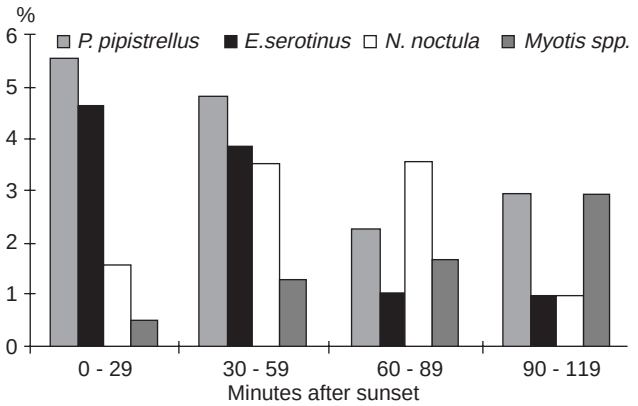


Fig. 1. Timing of activity in three species and one species group: bat call minutes recorded in percentage of minutes of monitoring per 0.5 h time intervals after sunset.

DISCUSSION

The composition and relative abundance of species, as revealed in this paper, are consistent with previous records of bats in Brno based on other methods. Within the built-up area nursery colonies of *E. serotinus, P. austriacus* and *M. myotis* were recorded (on the periphery in the last species) and mass hibernation and summer invasions of *P. pipistrellus* were observed (Gaisler & Bauerova, 1985–86). In 1990, a pregnant female *N. leisleri* was found and, in 1995, a mass hibernaculum of *N. noctula* was discovered (Gaisler, unpubl.). From among the common species, relative abundance of *Plecotus* bats was probably underestimated during the acoustic census due to their very weak signals (Ahlén, 1990). The three most common species, *P. pipistrellus, E. serotinus* and *N. noctula*, were often recorded in many other European cities and towns studied so far with respect to bats (Klawitter, 1986; Mickleburgh, 1987; Spitzenberger, 1990; Haensel, 1992; Schober & Meyer, 1995; Vernier, 1995; Walsh & Harris, 1996*a*) and appear as typical foragers in urban habitats.

E. serotinus is very conspicuous and easily identifiable visually when hunting (Klawitter & Vierhaus, 1975) and therefore it was recorded as the most common species during the earlier visual census (Gaisler, 1979). During that census, maximum numbers of serotines and of all bats observed were recorded in the citycentre. Negative correlation was found between the numbers of bats in each sampling locality and the distance of localities from the citycentre. Both regression lines representing the relationships from centre to 5 and 7 km, respectively, were statistically significant at the 0.01 level (Gaisler, 1979). No such relation was found in the 1993 bat detector sample and the number of bats recorded in the city centre was very low.

In 1977, all streetlamps gave a bluish-white light (mercury vapour lamps). As has been shown by numerous authors (Geggie & Fenton, 1985; Haffner & Stutz, 1985–86; Baagoe, 1986; Rydell, 1991, 1992; Blake *et al.*, 1994; Rydell & Racey, 1995), such lamps are

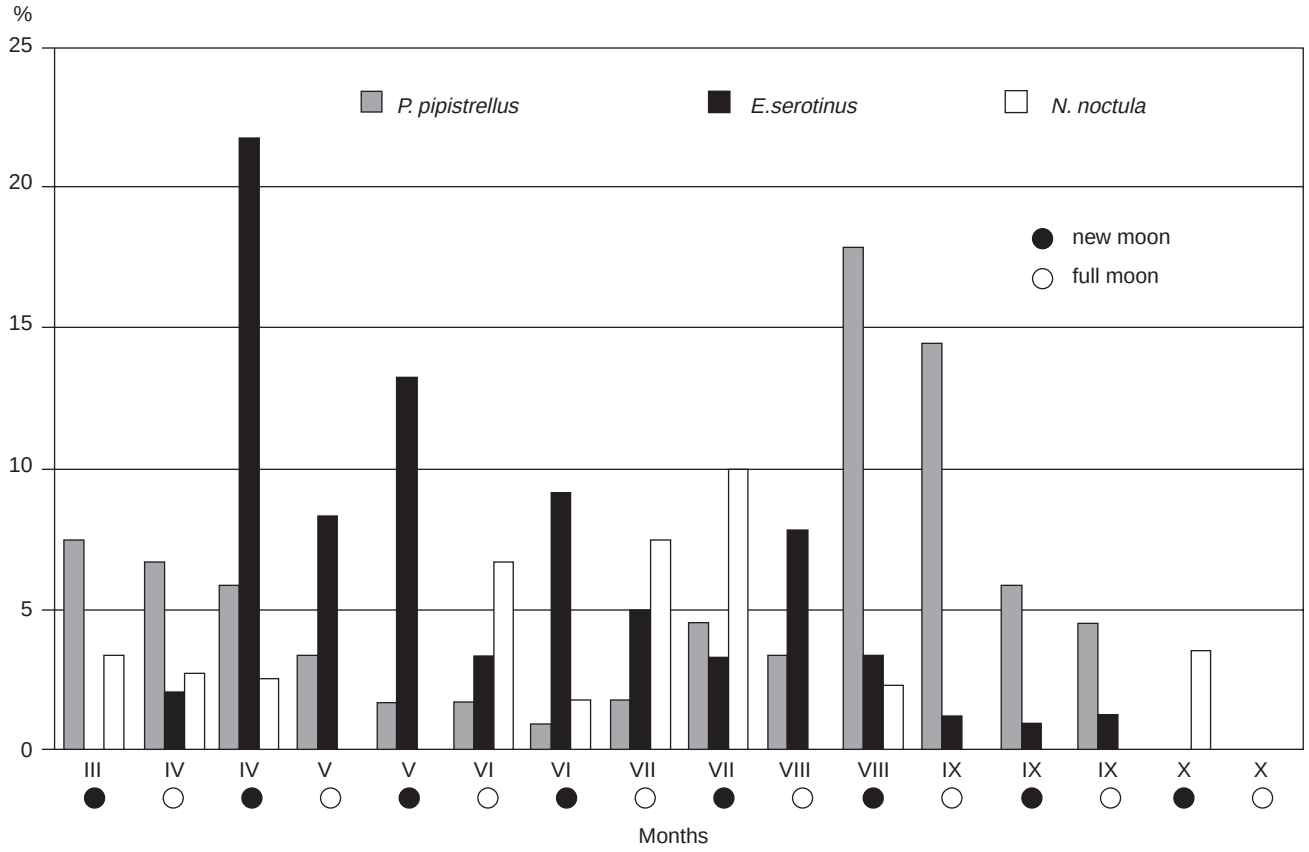


Fig. 2. Seasonal changes of activity in the three most common species: bat call minutes recorded in percentage of minutes of monitoring per night of new and full moon (23 March to 30 October).

attractive for certain bat species because they attract insects. In 1993 to 1995, low pressure sodium lamps were installed in the place of mercury lamps in Brno. Such lamps give yellow to orange light and are not attractive to insects and bats (Rydell & Racey, 1995). The first phase of exchanging streetlamps in Brno coincided with our bat detector survey. In October 1993, we counted 1 to 40 white streetlamps per locality, on average 14 or about one white to six yellow lamps. The decreasing number of white and increasing number of yellow lamps may have biased the impact of lamps on the bat traffic (Rydell, 1992). The lower number of bats estimated in the citycentre during the acoustic as compared to the visual census can be explained in this way.

All habitat types under study were frequented by flying bats which is in accordance with literary data that bats use a wide range of habitats (Baagoe, 1986; Furlonger, Dewar & Fenton, 1987; Rachwald, 1992; Jones & Rydell, 1994; de Jong, 1995; Walsh & Harris, 1996*a, b*; Racey, In press). In most studies, habitats associated with broadleaved woodland, water and vegetation corridors were found to be preferred, while open habitats were less frequented or avoided. Geggie & Fenton (1985) found no significant differences in the levels of activity of foraging *Eptesicus fuscus* over different urban habitats in Canada, but Rydell & Racey (1995) reported on significantly different densities of *E. nilssoni* and *Vespertilio murinus* in four different habitats of a small town in southern Sweden. These bats were most common in rural and residential areas away from the town centre. As a result of the national survey of bats in Britain, urban areas were found to be positively selected in three out of seven land class groups (Walsh & Harris, 1996*a*). The census, however, did not include centres of large cities. In our study, the citycentre was found to be avoided by all bats except *P. pipistrellus* and *E. serotinus*, with a rare occurrence of commuting *N. noctula*. This may be explained by low incidence of trees in the absence of the positive effect of white streetlamps. In contrast, riverine habitats with many trees were attractive for all common species except *E. serotinus*. The latter species was most common in old outskirts where the trees were also numerous. The number of trees per locality, however, had no positive effect on bat activity *per se*. Other factors which are difficult to quantify, such as the availability of roost sites in houses, may have influenced habitat use (Blake *et al*., 1994; Rydell & Racey, 1995; Walsh & Harris, 1996*a*; Racey, In press).

The observed differences in timing the overnight activity, namely the early emergence of *P. pipistrellus* and *E. serotinus* and later emergence of *Myotis* spp., correspond to literary data (Schober & Grimmberger, 1989; Jones & Rydell, 1994). *N. noctula* is another early emerging species with two peaks in activity, after sunset

and before sunrise (Rachwald, 1992). Its low abundance during the first 30 min as recorded in our study may have been due to the absence of summer shelters in the city. The noctules possibly came to forage in the town from their roosts in surrounding woods and were therefore delayed with respect to pipistrelles and serotines which roosted in the town. According to Rydell, Entwistle & Racey (1996), who studied the flight activity of *P. pipistrellus, M. daubentonii* and *Plecotus auritus* in north-east Scotland, timing of foraging flights of bats is related to insect activity and predation risk. They found that the activity of the first two species started earlier than in the last one. In our study, *M. daubentonii* was recorded as a relatively late emerging bat, the timing of its activity followed the same trend as in unidentified small *Myotis* spp. This implies possible local differences in timing activity of the same species.

Lunar cycle had no significant effect on the foraging activity of bats, which is in accordance with the data concerning American species *E. fuscus* and *Myotis lucifugus* (Geggie & Fenton, 1985; Negraeff & Brigham, 1995). Low temperature, rain and wind were shown to have negative influence on bat activity under certain conditions (Rydell, 1991; Blake *et al*., 1994; Negraeff & Brigham, 1995). In *N. noctula,* air temperature was found to determine the hunting strategy (Rachwald, 1992). In our study, only temperature appeared to have some effect on bat activity, but we did not work under rainy and windy weather. Negative correlation between the activity of *E. serotinus* and relative humidity merely resulted from the negative correlation between temperature and relative humidity ($P<0.01$) and we consider it a manifestation of the effect of temperature.

Our results tend to show that a 30 min walking transect about 1.5 km long is a good method of monitoring bat traffic in urban habitats with the aid of bat detectors. They also corroborate the findings that habitat is the primary predictor of bat abundance (Walsh & Harris, 1996*b*). Since urban habitats were shown to be frequented by flying and even foraging bats in summer, they represent a wide potential for further monitoring with respect to the conservation of bats.

Acknowledgements

We acknowledge that one of the QMC Mini bat detectors was kindly donated by the Bat Conservation International (Austin, TX, U.S.A.). The project was supported by the Grant 206/93/2241 (GA CR, Prague). Prof. P. A. Racey (Aberdeen, U.K.) and two anonymous referees read the manuscript critically and made useful suggestions.

REFERENCES

Ahlén, I. (1990). Identification of bats in flight. *Swed. Soc. Conserv. Nat., Stockh.* Item No. 0312: 1–50.

Ahlén, I. & Gerell, R. (1989). Distribution and status of bats in Sweden. In *European Bat Research 1987*: 319–326. Hanak, V., Horacek, I. & Gaisler, J. (Eds). Prague: Charles University Press.

Baagoe, H. J. (1986). Summer occurrence of *Vespertilio murinus* Linné-1758 and *Eptesicus serotinus* (Schreber-1780) (Chiroptera, Mammalia) on Zealand, Denmark, based on records of roosts and registration with bat detectors. *Ann. Naturhist. Mus. Wien* **88/89B**: 281–291.

Blake, D., Hutson, A. M., Racey, P. A., Rydell, J. & Speakman, J. R. (1994). Use of lamplit roads by foraging bats in southern England. *J. Zool. (Lond.)* **234**: 453–462.

de Jong, J. (1995). Habitat use and species richness of bats in a patchy landscape. *Acta Theriol.* **40**: 237–248.

Forbes, B. & Newhook, E. M. (1990). A comparison of the performance of three models of bat detectors. *J. Mammal.* **71**: 108–110.

Furlonger, C. L., Dewar, H. J. & Fenton, M. B. (1987). Habitat use by foraging insectivorous bats. *Can. J. Zool.* **65**: 284–288.

Gaisler, J. (1979). Results of bat census in a town (Mammalia: Chiroptera). *Vestn. Cesk. Spol. Zool.* **43**: 7–21.

Gaisler, J. & Bauerová, Z. (1985–1986). The life of bats in a city. *Myotis* **23–24**: 209–215.

Geggie, J. F. & Fenton, M. B. (1985). A comparison of foraging by *Eptesicus fuscus* (Chiroptera:Vespertilionidae) in urban and rural environments. *Can. J. Zool.* **63**: 263–267.

Haensel, J. (1992). In den Ostberliner Stadtbezirken nachgewiesene Fledermäuse – Abschlussbericht, insbesondere den Zeitraum 1980–1991 betreffend. *Nyctalus (N.F.), Berlin* **4**(4): 379–427.

Haffner, M. & Stutz, H. P. (1985–1986). Abundance of *Pipistrellus pipistrellus* and *Pipistrellus kuhlii* foraging at street-lamps. *Myotis* **23–24**: 167–172.

Hooper, J. H. D. (1981). The use of an ultrasonic receiver to obtain distribution data for pipistrelles and other bats within the London area. *Lond. Nat.* **60**: 47–63.

Hudec, K. (1976). Der Vogelbestand in der städtischen Umwelt von Brno (CSSR) und seine Veränderungen. *Acta Sci. Nat. Brno* **10**(11): 1–54.

Jones, G. & Rydell, J. (1994). Foraging strategy and predation risk as factors influencing emergence time in echolocating bats. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **346**: 445–455.

Jüdes, U. (1989). Erfassung von Fledermäusen im Freiland mittels Ultraschall-Detektor. *Myotis* **27**: 27–39.

Klawitter, J. (1986). Bestandsentwicklung, Gefährdung und Schutz der Fledermäuse in Berlin (West). *Berl. Naturschutzbl.* **30**: 74–85.

Klawitter, J. & Vierhaus, H. (1975). Feldkennzeichen fliegender Abendsegler, *Nyctalus noctula* (Schreber, 1774) und Breitflügelfledermäuse, *Eptesicus serotinus* (Schreber, 1774). *Säugetierkd.. Mitt.* **23**: 212–222.

Limpens, H. J. G. A., Helmer, W., Van Winden, A. & Mostert, K. (1989). Bats (Chiroptera) and linear landscape elements. *Lutra* **32**: 1–20.

McAney, C. M. & Fairley, J. S. (1988). Habitat preference and overnight and seasonal variation in the foraging activity of lesser horseshoe bats. *Acta Theriol.* **33**: 393–402.

Mickleburgh, S. (1987). Distribution and status of bats in the London area. *Lond. Nat.* **66**: 41–91.

Negraeff, O. E. & Brigham, R. M. (1995). The influence of moonlight on the activity of little brown bats (*Myotis lucifugus*). *Z. Säugetierkd.* **60**: 330–336.

Racey, P. (In press). The ecology of European bats in relation to their conservation. In *The biology and conservation of bats.* Kunz, T. H. & Racey, P. (Eds). Washington: Smithsonian Institution Press.

Rachwald, A. (1992). Habitat preference and activity of the noctule bat *Nyctalus noctula* in the Białowieza primeval forest. *Acta Theriol.* **37**: 413–422.

Rydell, J. (1991). Seasonal use of illuminated areas by foraging northern bats *Eptesicus nilssoni. Holarct. Ecol.* **14**: 203–207.

Rydell, J. (1992). Exploitation of insects around streetlamps by bats in Sweden. *Funct. Ecol.* **6**: 744–750.

Rydell, J., Entwistle, A. & Racey, P. A. (1996). Timing of foraging flights of three species of bats in relation to insect activity and predation risk. *Oikos* **76**: 243–252.

Rydell, J. & Racey, P. A. (1995). Street lamps and the feeding ecology of insectivorous bats. *Symp. zool. Soc. Lond.* No. 67: 291–307.

Sanchez, O. (1989). Bats of Mexico City. *Abstracts, Fifth Int. Ther. Congr. Rome* **2**: 975–979.

Schober, W. & Grimmberger, E. (1989). *A guide to bats of Britain and Europe*. London: Hamlyn.

Schober, W. & Meyer, M. (1995). Fledermausnachweise in Leipzig. *Veröff. Naturkund. Leipzig* **13**: 41–51.

Sokal, R. R. & Rohlf, F. J. (1981). *Biometry*. (2nd edn). New York: Freeman & Co.

Spitzenberger, F. (1990). *Die Fledermäuse Wiens*. Wien: J.+ V. Edition.

Vernier, E. (1995). Presence and distribution of bats in the town of Padova. *Myotis* **32–33**: 193–195.

Walsh, A. L. & Harris, S. (1996*a*). Foraging habitat preferences of vespertilionid bats in Britain. *J. Appl. Ecol.* **33**: 508–518.

Walsh, A. L. & Harris, S. (1996*b*). Factors determining the abundance of vespertilionid bats in Britain: geographical, land class and local habitat relationships. *J. Appl. Ecol.* **33**: 519–529.

Walsh, A. L., Harris, S. & Hutson, A. M. (1995). Abundance and habitat selection of foraging vespertilionid bats in Britain: a landscape-scale approach. *Symp. zool. Soc. Lond.* No. 67: 325–344.

Wilkinson, L. (1990). *SYSTAT: The system for statistics*. Evanston, IL: Systat Inc.

Výběr úkrytů a letová aktivita netopýrů

Kovařík, M., Zukal, J. & Řehák, Z. (2000) Aktivita netopýrů v Moravském krasu. Estavela 2, 5-6.

- Poškození nebo zničení jeskyně nebo její součásti (připomínám § 10 zákona o ochraně přírody a krajiny, kdy jsou za jeskyni považovány i závrty ponory, vývěry a další krasové jevy)
- pokácení dřeviny rostoucí mimo les

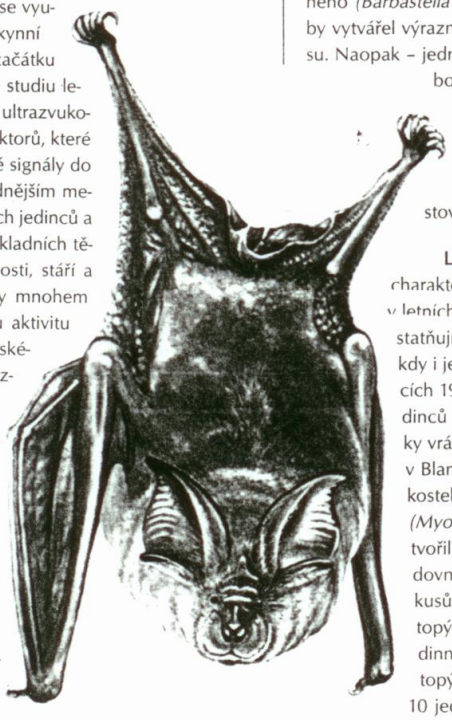
Pokutu fyzické osobě do 10.000,- Kč a právnické osobě do 1.000.000,- Kč lze např. uložit za :

- zničení zařízení určené k ochraně (např. uzávěry jeskyní), označení a vybavení chráněného území (veškeré značení rezervací, panely naučných stezek apod.)
- Pokutu fyzické osobě do 50.000,- Kč a právnické osobě do 1.000.000,- Kč lze např. uložit za :
- usmrcení zvláště chráněného živočicha kategorie kriticky nebo silně ohroženého druhu nebo tomu, kdo způsobí jeho úhyn zásahem do jeho životního prostředí (takto chráněno je např. 8 druhů netopýrů vč. vrápence malého a netopýra velkého)
- vykonávání zakázané činnosti (např. vstup bez povolení do NPR) nebo činnosti, ke kterým je vyžadován souhlas, bez tohoto souhlasu (např. vstupy do jeskyní v PR).

AKTIVITA NETOPÝRŮ V MORAVSKÉM KRASU

Miroslav Kovařík, Jan Zukal, Zdeněk Řehák, Správa CHKO Moravský kras

V předcházejících dvou článcích jsem se zabýval především jeskyněmi a netopýry. Ale tito zajímaví živočichové na území Moravského krasu využívají jeskyní především k zimnímu spánku. Pokud se poohlédneme po jejich způsobu života a zaměříme se na jeho aktivní část-mimohibernační období, zjistíme, že netopýři podléhají dvěma základním cyklům života. Jedná se o změny denního rytmu a dále změny sezónní. Výzkum netopýrů v mimohibernačním období nemá na území Moravského krasu tak dlouholeté tradice jako sledování netopýrů v zimním období, tedy při zimním spánku v jeskyních. Teprve až se zavedením odchytů létajících netopýrů do japonských nárazových sítí na začátku sedmdesátých let nastal obrat. K odchytu netopýrů se využívalo nejčastěji lokalit vázaných na jeskynní vchody, především velkých portálů. Od začátku let devadesátých se stále více využívá ke studiu letové aktivity netopýrů metody detekce ultrazvukových signálů pomocí ultrazvukových detektorů, které různým způsobem převádějí ultrazvukové signály do slyšitelné oblasti. Tak ke dvěma nejzákladnějším metodám výzkumu prostého sčítání zimujících jedinců a kroužkování spojeného se zjišťováním základních tělesných proporcí netopýrů, jejich hmotnosti, stáří a pohlaví přibyly další dvě, které umožnily mnohem podrobnější studie zaměřené na letovou aktivitu netopýrů. Na základě výzkumů z Moravského krasu lze sezónní aktivitu netopýrů rozdělit do několika ročních fází, které se od sebe významně liší.



Zimní období netopýři tráví v různých úkrytech a díky zimnímu spánku (hibernaci) je u nich aktivita minimální. Během zimního spánku se netopýři mohou probudit někdy nezávisle na vnějších podnětech nebo změnou vnějších podmínek, případně rušením. Při přerušení zimního spánku se netopýři často přesunují na jiné místo. Tyto přesuny jsou ve většině případů jsou vázány na stejnou jeskyni, jen někdy mohou změnit i jeskyni. Každé probuzení netopýrů znamená velkou energetickou ztrátu, proto pokud jsou několikrát vyrušeni ze zimního spánku, může dojít k jejich úhynu vyčerpáním. Podle výsledků z Kateřinské jeskyně patří k druhům s nejdelším obdobím zimního spánku netopýr brvitý *(Myotis emarginatus)* - kolem 190 dnů a mezi druhy s kratším obdobím spánku patří netopýr ušatý *(Plecotus auritus)* s odhadnutou délkou spánku kolem 110 dnů.

Jarní období (od konce března do konce května) začíná u netopýrů přelety ze zimovišť do přechodných úkrytů. Později samice postupně začínají vytvářet letní kolonie. Vzdálenost mezi letními a zimními úkryty se velice liší podle jednotlivých druhů. Mezi nejstálejší druhy můžeme řadit vrápence (rod *Rhinolophus*), netopýra ušatého a netopýra dlouhouchého (rod *Plecotus*). Další skupinou jsou druhy označované jako toulavé. Jedná se o většinu druhů rodu *Myotis*, a v krasu poměrně hojného netopýra černého *(Barbastella barbastellus)*. Mezi druhy migrujícími není žádný, který by vytvářel výrazné počty zimujících jedinců v jeskyních Moravského krasu. Naopak - jedná se o druhy, které v jeskyních zimují jen výjimečně nebo na jeskyně nejsou vázány. Lze uvést netopýra pestrého *(Vespertilio murinus)*, netopýra hvízdavého *(Pipistrellus pipistrellus)* a netopýra parkového *(Pipistrellus nathusii)*. Přelety mezi zimním a letním úkrytem tak mohou dosahovat vzdálenosti od několika desítek metrů po stovky kilometrů.

Letní období (od začátku června do konce července) je charakteristické rozmnožováním netopýrů. Samice rodí mláďata v letních koloniích. Mláďata rychle rostou a postupně se osamostatňují. Samci kolonie netvoří a jako denní úkryty využívají někdy i jeskyně. Mezi nejznámější letní kolonie prověřované v rocích 1992 - 1994 patří půda kostela ve Křtinách (kolem 200 jedinců netopýra velkého *(Myotis myotis)*, několik kusů až desítky vrápence malého *(Rhinolophus hipposideros)*, půda kostela v Blansku (100 - 200 netopýrů velkých *(Myotis myotis)*, půda kostela v Černé Hoře (300 - 700 jedinců netopýra velkého *(Myotis myotis)*. Vrápenec malý *(Rhinolophus hipposideros)* tvořil ještě kolonie na půdě stodoly a domu v areálu pily u Jedovnic (kolem 30 jedinců) a v kapličce v Mokré (více než 20 kusů). Z ostatních stojí za zmínku kolonie asi 30 jedinců netopýra hvízdavého *(Pipistrellus pipistrellus)* pod střechou rodinného domu v Jedovnicích. Na 4 lokalitách byl nalezen netopýr dlouhouchý *(Plecotus austriacus)*, ale počty nepřesáhly 10 jedinců. Pouze na jediné půdě byl objeven netopýr ušatý *(Plecotus auritus)* v počtu 5 a to na půdě zámku v Blansku.

Podzimní období (od začátku srpna do druhé poloviny listopadu) je spojeno s rozpadem letních kolonií samic s mláďaty a nastává doba podzimních přeletů. Jako na jaře dochází ke střídání přechodných úkrytů.

V tomto období je intenzita přeletů výraznější než na jaře a je spojena s vysokou pohlavní aktivitou. Podzimní fáze postupně končí přesunem netopýrů do zimních úkrytů. Jednotlivá období nemají vždy zcela ostrou hranici a liší se i u jednotlivých druhů.

DENNÍ RYTMUS

Netopýři patří mezi noční zvířata, při denním světle jsou vidět jen výjimečně. Úkryt opouští většina druhů po soumraku a přelétají do míst, kde loví hmyz. Během noci mohou přechodně využít noční úkryty a před rozedněním se vracejí z lovišť zpátky do denního úkrytu. Nejaktivnější jsou v první polovině noci. Samice, které kojí mláďata, se za nimi vracejí do místa denního úkrytu (letní kolonie) i během noci. Ve dne netopýři upadají do letargie, kterou ale nelze intenzitou srovnávat se zimním spánkem. Pro netopýry je důležité střídání biotopů, pestrost krajiny. To jim umožňuje se zaměřit při lovu na nejsnáze dostupnou potravu, nepatří tedy mezi potravní specialisty a složení potravy se mění během sezóny. Nejčastěji loví nad stojatými vodními hladinami, u břehových porostů, v zemědělské krajině u rozptýlené zeleně, u okraje lesů, na pasekách a podobně. Nevyhovuje jim jednotvárná jednoduchá krajina.

Výsledky výzkumů letové aktivity netopýrů v Moravském krasu z roků 1991 až 1994 jsou shrnuty v disertační práci Z. Řeháka (1995). Z těch nejzajímavějších upozorníme na následující:

Nově byly na území Moravského krasu zjištěny 2 druhy - netopýr stromový *(Nyctalus leisleri)* a netopýr parkový *(Pipistrellus nathusii)*. V České republice zde byl poprvé nalezen v období mimo zimního spánku netopýr pobřežní *(Myotis dasycneme)*. Netopýr pestrý *(Vespertilio murinus)* a netopýr východní *(Myotis blythi)* byly do těchto výzkumů známy pouze ze zimování v jeskyních Moravského krasu, jedná se tedy o první prokázání výskytu těchto druhů mimo zimní období. Netopýr pestrý byl v území prokázán po více než 100 letech. Kromě detektoringu byl tento druh prokázán i odchytem v budovách. První nález byl dne 9. 12. 1994 v budově Projektu ČKD Blansko a druhý nález byl v rodinné vilce v Adamově dne 17.10.1999. Pro oba případy je společné, že netopýři byli uvnitř kastlových oken. Další druh netopýr severní *(Eptesicus nilssoni)* byl zjištěn pouze pomocí detektoru.

ODCHYTY DO SÍTÍ U JESKYNNÍCH VCHODŮ

V severní části bylo zjištěno 15 druhů, ve střední části 13 druhů a v jižní části jen 11 druhů.

Sloupsko-šošůvské jeskyně

Zjištěno bylo15 druhů, daleko nejhojnější je netopýr velký *(Myotis myotis)*, který tvoří více než 50 % všech netopýrů. Pouze netopýr vodní *(Myotis daubentoni)* dosahuje ještě více než 10 % společenstva.

Hladomorna

Zjištěno bylo14 druhů, nejhojnější je netopýr velký *(Myotis myotis)*, který zde tvoří více než 30 % společenstva netopýrů. Významný podíl má i netopýr černý *(Barbastella barbastellus)*, který tvoří více než 20 %. Nad 10 % dosahují netopýr ušatý *(Plecotus auritus)* a netopýr vodní *(Myotis daubentoni)*.

Kateřinská jeskyně

Zjištěno bylo 13 druhů, nejhojnější jsou 2 druhy - netopýr velký *(Myotis myotis)* a netopýr řasnatý *(Myotis nattereri)* - kolem 20 %. O něco menších počtů dosahovaly druhy netopýr velkouchý *(Myotis bechsteini)* a netopýr vodní *(Myotis daubentoni)*. Vysoký podíl, který zaujímá ve společenstvu netopýr velkouchý, je unikátní v rámci Moravského krasu. Jedná se o daleko nejvyšší zjištěné počty tohoto druhu ze všech sledovaných lokalit (celkem zde bylo odchyceno 225 jedinců). Nad 10 % dosahoval ještě netopýr brvitý *(Myotis emarginatus)*.

Jeskyně Býčí skála

Zjištěno bylo 13 druhů, daleko nejhojnější je netopýr velký *(Myotis myotis)*, který tvoří téměř 40 % společenstva. Další 2 významné druhy (kolem 15 %) netopýr vodní *(Myotis daubentoni)* a netopýr řasnatý *(Myotis nattereri)*.

Ochozská jeskyně

Zjištěno bylo 9 druhů, daleko nejhojnější je vrápenec malý *(Rhinolophus hipposideros)*, který zaujímá téměř 50 % populace. Další významné druhy tvoří každý kolem 10 %. Jsou to netopýr řasnatý *(Myotis nattereri)*, netopýr velký *(Myotis myotis)* a netopýr vodní *(Myotis daubentoni)*.

Jeskyně Netopýrka

Zjištěno bylo 8 druhů, nejpočetnější je netopýr černý *(Barbastella barbastellus)*, který se podílí na celkovém počtu cca 35 %. Netopýr velký *(Myotis myotis)* tvoří 25 % a nad 10 % dosahuje ještě netopýr ušatý *(Plecotus auritus)*.

VÝSLEDKY SLEDOVÁNÍ NETOPÝRŮ DETEKTOREM - TRANSEKTY

Pole

Zjištěny pouze 3 druhy, nejpočetnější je netopýr rezavý *(Nyctalus noctula)*.

Louky

Zjištěno nejméně 5 druhů. Nejčastější je skupina netopýr velký *(Myotis myotis)*/ netopýr východní *(Myotis blythi)*. Detektorováním nelze tyto dva druhy bezpečně rozlišit.

Aleje, liniové porosty v otevřené krajině

Zjištěno nejméně 9 druhů. Nejčastější je skupina netopýr velký *(Myotis myotis)*/ netopýr východní *(Myotis blythi)*. Viz výše.

Zastavěná část obcí s pouličním osvětlením

Zjištěno nejméně 6 druhů. Nejčastější je netopýr večerní *(Eptesicus serotinus)*.

Skalní stěny, ostrožny, skalnaté žleby

Zjištěno nejméně 13 druhů. Nejhojnější je netopýr hvízdavý *(Pipistrellus pipistrellus)*.

Les

Zjištěno nejméně 9 druhů. Nejčastějšími je skupina netopýr ušatý *(Plecotus auritus)*/ netopýr dlouhouchý *(Plecotus austriacus)*. Detektorováním nelze tyto dva druhy bezpečně rozlišit.

Okraj lesa - ekoton

Zjištěno nejméně 8 druhů. Nejčastější je skupina netopýr velký *(Myotis myotis)*/ netopýr východní *(Myotis blythi)* viz výše a netopýr večerní *(Eptesicus serotinus)*.

Břeh potoka nebo říčky

Zjištěno nejméně 6 druhů. Daleko nejhojnější je netopýr vodní *(Myotis daubentoni)*.

Břeh rybníka

Zjištěno nejméně 8 druhů. Daleko nejhojnější je netopýr vodní *(Myotis daubentoni)*.

Z výsledků je patrné, že aktivita netopýrů je veliká. Tito drobní savci využívají schopnosti létat velice dokonale a tato možnost jim napomáhá úspěšnému přežívání. Vysokou energetickou náročnost létání kompenzují tím, že v klidové fázi upadají do různě hlubokých letargických stavů a tak snižují potřebu množství přijímané potravy.

Výběr úkrytů a letová aktivita netopýrů

Pokorný, M., Berková, H., Gaisler, J., Řehák, Z. & Zukal, J. (2003) Letní výskyt netopýrů v lidských stavbách v Moravském krasu a širším okolí. Vespertilio 7, 161-168.

Vespertilio 7: 161–168, 2003
ISBN 80-88850-20-7

Letní výskyt netopýrů v lidských stavbách v Moravském krasu a širším okolí

Martin POKORNÝ[1,2], Hana BERKOVÁ[1,2], Jiří GAISLER[2], Zdeněk ŘEHÁK[2] & Jan ZUKAL[1,2]

[1] *Ústav biologie obratlovců AV ČR, Květná 8, 603 65 Brno, Česko; pokorny@brno.cas.cz; zukal@brno.cas.cz; berkova@brno.cas.cz*
[2] *Katedra zoologie a ekologie, MU Brno, Kotlářská 2, 611 37 Brno, Česko; gaisler@sci.muni.cz; rehak@sci.muni.cz*

Summer occurrence of bats in buildings in the Moravian Karst and its wider surroundings. Bats hibernating in the Moravian Karst and its environs (Czech Republic) have been monitored for more than 40 years. However, the occurrence of bats in summer shelters of the same region has not been evaluated in details so far. In this paper, results of checks of potential summer shelters of bats in buildings, performed in 1992–2001, are presented, together with earlier unpublished data. In 1992–2001, 161 localities were checked comprising 222 lofts, attics and similar roof spaces of churches (145), castles (28) and other buildings (49). According to the presence of live bats or their fresh droppings, 50.5% of checks were positive. In 8.5% of cases bat cadavers or ancient droppings proved past presence of bats, 126 of checks were negative. In total, 9 bat species have been recorded in buildings within the non-hibernation period: *Rhinolophus hipposideros*, *Myotis myotis*, *M. mystacinus*, *M. emarginatus*, *M. daubentonii*, *Pipistrellus pipistrellus*, *Eptesicus serotinus*, *Plecotus auritus* and *P. austriacus*. The results have been compared with earlier data from the same territory and discussed with published information from other regions.

Bats, summer colonies, distribution, karstic area

Úvod

Moravský kras jako jedno z nejvýznamnějších zimovišť netopýrů v České republice patří k dlouhodobě a intenzivně sledovaným oblastem. Vizuální sčítání hibernujících netopýrů se na některých lokalitách provádí více než 45 let (Gaisler & Hanák 1972, 1973, Gaisler 1975, Bárta et al. 1981, Bauerová 1984, Vašátko et al. 1989, Gaisler & Řehák 2001, Zukal et al. 2001, Zima 2001) a jsou tak k dispozici data umožňující hodnotit dlouhodobé změny početnosti jednotlivých druhů (Bauerová et al. 1989, Zima et al. 1994, Řehák et al. 1994). Menší počet prací z této oblasti je založen na výsledcích odchytů netopýrů do sítí instalovaných před vchody do jeskyní, a to jak v letním období, tak v době pre- nebo posthibernační (Gaisler 1973, Bauerová & Zima 1988, Řehák 1995). Přestože vybraná data o pohybu netopýřích populací ve vztahu k Moravskému krasu byla publikována v souborných článcích o kroužkování (Hanák et al. 1962, Gaisler & Hanák 1969), zůstává stále řada otázek nezodpovězených, zejména do jakých vzdáleností a směrů létají netopýři ze zimovišť a kde tvoří letní kolonie.

Systematickým průzkumem letního výskytu netopýrů Na území CHKO Moravský kras se zabýval teprve Řehák (1995). Do té doby byl výskytu netopýrů v budovách na území Moravského krasu věnován jen jeden populární článek (Gaisler 1967), část území Moravského krasu a jeho širší okolí pak zahrnují zejména práce Gaislera, Vlašína a jejich spolupracovníků (Gaisler et al. 1988, 1989,

1990, Vlašín & Eleder 1991, Vlašín et al. 1993, 1995). Několik publikací je věnováno nálezům netopýrů na území města Brna, které byly umožněny jednak aplikací tradičních metod, jednak registrací echolokačních signálů netopýřími detektory (Gaisler 1979, Bauerová & Gaisler 1985, Gaisler & Bauerová 1986). Z literatury citované výše vyplývá, že na území Moravského krasu a v jeho okolí (oblast je vymezena níže v metodice) byl prokázán letní výskyt 19 druhů netopýrů, přičemž u 8 z nich (*Rhinolophus hipposideros*, *Myotis myotis*, *M. emarginatus*, *M. bechsteinii*, *Eptesicus serotinus*, *E. nilssonii*, *Nyctalus noctula* a *Plecotus austriacus*) byly nalezeny letní kolonie (kromě výše uvedených autorů ještě Zukal & Gaisler 1989). Cílem předloženého příspěvku je doplnit tento materiál o nová data i starší, ale dosud nepublikované nálezy

Metodika

Práce shrnuje dosud nepublikovaná faunistická data získaná autory z území CHKO Moravský kras a z jeho širšího okolí, které je vymezeno přibližně kruhem o poloměru 30 km od Kateřinské jeskyně zvolené za centrální bod sledované oblasti. Kontrolovaná plocha představuje cca 2800 km^2. Přehled je omezen na údaje získané systematickým průzkumem půdních prostor vhodných budov (zejména kostely a zámky) v období duben až září. Intenzívní průzkum probíhal na vlastním území CHKO v letech 1992–1994, v jeho okolí v roce 2001. Kromě toho byly vypsány nepublikované údaje z protokolů všech autorů, nejdelší časový úsek zahrnují protokoly J. Gaislera (od 17. 5. 1958, kdy byla kontrolována půda kostela v Adamově). Jména autorů nejsou u jednotlivých nálezů uváděna, výjimkou jsou data z pozůstalosti Dr. Zdeňky Bauerové, kdy je označen jejich původ. Pokud byla některá lokalita kontrolována vícekrát během téže sezóny, je publikován jen údaj z doby těsně před předpokládaným porodem mláďat. Použité zkratky: LK = letní kolonie, tj. reprodukční skupina samic, sdílející společný úkryt; ad = dospělý; juv = mládě.

Výsledky

V letech 1992 – 2001 bylo na 161 lokalitách zkontrolováno 222 podkrovních prostorů budov, z toho 145 kostelů, 28 zámků a 49 ostatních. Netopýři nebo jejich recentní přítomnost (čerstvý trus) byli zaznamenáni v 50,5 % objektů, v dalších 8,5 % případů byly nalezeny staré známky přítomnosti netopýrů (starý trus, kadaver), 126 kontrol bylo negativních.

Vrápenec malý (*Rhinolophus hipposideros*)

6366 Biskupice, půda zámku 1 ks 30. 7. 2001; Jaroměřice, půda kaple 1 ks 31. 7. 2001; Jaroměřice, půda kapličky (Kalvárie) 6 ks 31. 7. 2001; **6465** Kladoruby, sklepy hotelu 4 ks 13. 7. 1991; Křetín, půda zámku LK 60–80 ks 15. 6. 2001; Kunštát, půda zámku 1 ks 15. 6. 2001; **6467** Konice, půda zámku 1 ks 30. 7. 2001; Ptení, půda zámku LK 29 ks (ad+juv) 30.7.2001; **6565** Boskovice, půda Panského dvora 3 ks 25. 5. 2001; Boskovice, pod podlahou půdy zámku LK asi 50 ks 2. 6. 1961, asi 30 ks 30. 5. 1963, 3 ks 16. 8. 1977; Černá Hora, sklep zámku 3 ks 7. 5. 1963; 2 ks 24. 6. 1992, 4 ks 24. 5. 2001; Dlouhá Lhota, věž kostela LK 40–50 ks 14. 6. 2001; Lysice, půda zámku 1 ks 11. 8. 1976 (leg. Bauerová), LK 11 ks 24. 5. 2001; Rájec-Jestřebí, půda zámku LK 30–50 ks 19. 9. 1959, 8 ks 21. 5. 1963, 1 ks 21. 7. 1992, 1 ks 12. 6. 1994, 3 ks 27. 6. 2001; **6566** Sloup, věž kostela 2 ks 24. 6. 1992; **6568** Plumlov, půda zámku asi 20 ks 30. 5. 1963; **6664** Předklášteří, půda muzea LK asi 50 ks 27. 5. 1959, 6 ks 7. 6. 1963, 15 ks 9. 7. 1964; **6665** Kateřina, půda kostela 1 ks 11. 7. 1993; Újezd u Černé Hory, půda kostela 8 ks 14. 6. 2001; Vranov, půda kláštera 1 ks 23. 5. 2001; Vranov, půda kostela 7ks 16. 6. 1958, 1 ks 23. 5. 2001; **6666** Jedovnice, pila u Rudického propadání (půda stodoly) LK 25 ks (ad+juv) 25. 6. 1992, 29 ks (ad+juv) 11. 7. 1993, 8 ks 11. 6. 1994; Josefov, chata Švýcárna – půda domu 3ks 17. 5. 1958; **6764** Veveří, půda hradu LK asi 50 ks 12. 6. 1958; **6766** Adamov, půda kostela 3 ks 17. 5. 1958; Kanice, škola 1 ks 23. 6. 1992; Křtiny, půda kostela LK asi 20 ks (z toho 5 samic ad) 22. 5. 1958, asi 40 ks 13. 6. 1959, 14 ks 11. 6. 1983 (leg. Bauerová), 6 ks 27. 8. 1986, 35 ks (ad+juv) 22. 7. 1992, 9 ks 11. 6. 1994, 15 ks 28. 7. 2001, 14 ks 27. 6. 2002; **6767** Račice, půda budovy před zámkem LK asi 80 ks 3. 6. 1964; Račice, pod podlahou půdy zámku, 8 ks 14. 5. 2001; **6867** Slavkov, půda zámku LK 18 ks 14. 5. 2002.

Z literatury je známo 12 letních kolonií vrápence malého, dvě z nich (Štěpánov nad Svratkou a Mokrá-Horákov) zanikly v důsledku rekonstrukce objektu, na třech lokalitách (Plumlov, Pustiměř a Sokolnice-zámek) byly kontroly v roce 2001 negativní, nicméně přítomnost vrápenců na těchto lokalitách v budoucnosti nelze vyloučit, a u zbylých je statut neznámý. Nově bylo objeveno 7 kolonií o velikosti 10–80 ks, ve Křtinách jsou vrápenci minimálně po dobu 44 let.

Netopýr velký (*Myotis myotis*)

6364 Bohuňov, půda kostela 1 ks 28. 6. 2001; **6466** Borotín, půda zámku LK asi 500 ks (ad+juv) ks 4. 8. 2001, 443 ks 26. 6. 2002; Horní Štěpánov, půda kostela 1 ks 19. 7. 2001; Šebetov, půda zámku 1 ks 19. 7. 2001; **6468** Stařechovice, půda kostela 3 ks 28. 6. 2001; **6564** Doubravník, věž kostela LK asi 400 ks 22. 5. 2001, 425 ks 27. 6. 2002; Lomnice, půda zámku LK 26 ks 7. 6. 1963; **6565** Boskovice, půda zámku LK 64 ks 25. 5. 2001, 84 ks 27. 6. 2002; Černá Hora, půda kostela LK asi 200 ks 21. 5. 1963, 60 ks (ad+juv) 11. 8. 1976 (leg. Bauerová), asi 90 ks 21. 7. 1977 (leg. Bauerová), 350 ks 24. 6. 1992, 700 ks (ad+juv) 11. 7. 1993, asi 500 ks 12. 6. 1994, asi 500 ks (ad+juv) 28. 6. 1995, 1 ks 24. 5. 2001; Dlouhá Lhota, půda kostela 1 ks 14. 6. 2001; Doubravice nad Svitavou, půda kostela 1 ks 24. 5. 2001; Lysice, půda kostela LK asi 300 ks odchycena 1 samice 14. 6. 2001, 131 ks 27. 6. 2002; Rájec-Jestřebí, půda kostela LK 300–400 ks (ad+juv) 9. 8. 1958, asi 300 ks 19. 9. 1959, asi 80 ks 2. 6. 1961, 5 ks 7. 5. 1963, asi 100 ks 2. 7. 1964, 1 ks 21. 7. 1977; Svitávka, půda kostela 3 ks 27. 6. 2001; **6568** Plumlov, strop zámku LK neznámý počet 29. 6. 2001; **6664** Tišnov, půda a věž kostela LK 100–200 ks 2. 6. 1961; **6665** Blansko, půda kostela LK 300–400 ks 21. 5. 1963, asi 200 ks 16. 6. 1967, asi 80 ks 7. 4. 1973, asi 100 ks (ad+juv) 7. 7. 1977, 70 ks (ad+juv) 25. 6. 1983 (leg. Bauerová), 70 ks (ad+juv) 22. 6. 1992, asi 200 ks (ad+juv) 11. 7. 1993, 120 ks 21. 6. 1994, 160 ks (ad+juv) 28. 6. 1995, 280 ks 20. 7. 2000, asi 176 ks 23. 5. 2001, 213 ks 26. 6. 2002; **6666** Olomučany, věž kostela 1 ks 23. 6. 1992; **6668** Otaslavice, půda kostela LK 213 ks 29. 6. 2001, 267 ks 3. 7. 2002; Pustiměř, půda kostela 1 ks 25. 6. 2001; **6765** Brno-Komín, věž kostela LK asi 50 ks 9. 7. 2001; Brno-Řečkovice, věž kostela LK min. 20 ks 9. 7. 2001, asi 30 ks 27. 6. 2002; Lelekovice, půda kostela 1 ks (samec ad) 16. 6. 1958; **6766** Adamov, půda kostela 1 ks 17. 5. 1958; Křtiny, půda kostela LK min. 30 ks (9 samic ad) 22. 5. 1958, asi 50 ks 13. 6. 1959, asi 50 ks 27. 8. 1986, 180 ks 22. 7. 1992, 100 ks 11. 6. 1994, 550 ks 20. 7. 2000, asi 700 ks (ad+juv) 28. 7. 2001, asi 370 ks 27. 6. 2002; Pozořice, půda kostela LK asi 400 ks 21. 5. 2001, 590 ks 27. 6. 2002; **6767** Dědice, půda kostela LK asi 1300ks (ad+juv) 26. 7. 2001, 509 ks 27. 6. 2002; Drnovice u Vyškova, půda kostela 1 ks 26. 7. 2001; Komořany, půda kostela 1 ks 25. 6. 2001; Královopolské Vážany, půda kostela 1 ks 10. 7. 2001; Luleč, půda kostela sv. Martina 1 ks 26. 7. 2001; Luleč, půda kostela ve vesnici 2 ks 26. 7. 2001; Podbřežice, půda kostela 1 ks 12. 7. 2001; Račice, půda zámku LK asi 150 ks (ad+juv) 26. 7. 2001, 82 ks 26. 6. 2002; **6768** Bohdalice, půda budovy vedle zámku LK 387ks (ad+juv) 16. 7. 2001, 289 ks 25. 6. 2002; **6864** Rosice, půda kostela 1 ks 26. 6. 2001; Rosice, půda zámku LK asi 150 ks 27. 5. 1996, asi 300 ks 26. 5. 1997, asi 200 ks 27. 5. 1998, asi 200 ks 27. 5. 1999, asi 150 ks 28. 5. 2001, 164 ks 3. 7. 2002; **6865** Brno – Mendlovo nám., půda kostela 1 ks 21. 5. 1958; **6867** Slavkov, půda zámku LK 15–20 ks (ad+juv), 27. 7. 1990, 10 ks 10. 7. 2001, 1 ks 25. 6. 2002; **6868** Bučovice, půda zámku LK 20 ks 27. 7. 1990, 6 ks 22. 9. 1999, 237 ks 25. 6. 2002.

Nejčastěji zjištěný druh. Ze 14 publikovaných letních kolonií zanikly 3 díky rekonstrukci objektu (Brodek, Slavkov-zámek, Šlapanice), 3 lokality byly netopýry opuštěny (Černá Hora, Lelekovice, Rájec-Jestřebí), 4 kolonie dosud existují a stav zbylých 4 se nepodařilo ověřit. Na dvou nepublikovaných lokalitách ze 60. let minulého století (Tišnov a Lomnice) byly kontroly v roce 2001 negativní. V současné době je ve zkoumaném území ověřen výskyt 17 kolonií netopýra velkého o průměrné velikosti 245 (10–598) ks. V průběhu května 2002 byla početnost odhadnuta na 3700 dospělých samic, což je více než třikrát tolik jedinců, kolik bylo v posledním roce napočítáno na zimovištích celého CHKO Moravský kras, a to včetně samců (Kovařík, ústní sdělení).

Netopýr vousatý (*Myotis mystacinus*)

6764 Chudčice, za obložením chaty LK 25 ks (1 samice ad) 17. 6. 1989; **6865** Střelice, půda garáže u domu ul. Písečná č.572 5 ks 10. 5. 1993.

Uváděné údaje jsou prvními publikovanými doklady výskytu letních kolonií v oblasti, nicméně tento štěrbinový druh pravděpodobně uniká pozornosti.

Netopýr brvitý (*Myotis emarginatus*)

6466 Šebetov, půda zámku LK 13 ks (ad+juv) 19. 7. 2001; **6666** Olomučany, půda stodoly ve středu obce 1 ks 23. 6. 1992; **6766** Křtiny, půda kostela 1 samice ad 22. 5. 1958, 2 ks (samice ad) 13. 6. 1959, 1 samec juv 18. 9. 2002; **6864** Zastávka u Brna, půda býv. Rosických uhelných dolů LK asi 100 ks 26. 6. 1989.

V 60. letech existující kolonie v Račicích zanikla a byla nahrazena jedinci druhu *Myotis myotis*, stav druhé publikované kolonie v Rychtářově není znám, stejně jako kolonie v Zastávce u Brna.

Netopýr vodní (*Myotis daubentonii*)

6765 Ořešín, dům Odlehlá č. 17 LK 14 ks 30. 5. 1996.

Vzhledem k použité metodice nemohl být výskyt netopýra vodního adekvátně zmapován.

Netopýr hvízdavý (*Pipistrellus pipistrellus*) s. l.

6565 Rájec-Jestřebí, štěrbina pod střechou železniční stanice LK 20 ks 21. 7. 1977 (leg. Bauerová); **6666** Jedovnice, dům ul. Na kopci 519 LK 25 ks 21. 6. 1994.

Do přehledu nejsou zařazeny početné nálezy z takzvaných "invazí" netopýra hvízdavého do budov zejména v městě Brně, protože nejde o trvalé úkryty. Úkryt letní kolonie v Jedovnici zanikl ucpáním vletových otvorů.

Netopýr večerní (*Eptesicus serotinus*)

6366 Biskupice, půda kostela 1 ks 30. 7. 2001; **6466** Borotín, půda kostela 1 ks 4. 8. 2001; **6564** Dolní Čepí, půda kostela 2 ks 23. 5. 2001; **6565** Rájec-Jestřebí, půda zámku 20 ks 19. 9. 1959; **6568** Krumsín, půda kostela LK min. 18 ks 12. 7. 2001; **6664** Drásov, půda kostela LK min 16 ks 15. 6. 2001; **6665** Blansko, půda gymnázia 1 ks 22. 6. 1992; **6667** Krásensko, půda kostela 1 ks 19. 7. 2001; **6668** Želeč, půda kostela LK min. 12 ks 29. 6. 2001; **6766** Kanice, škola 1 ks 23. 6. 1992; **6767** Podbřežice, půda kostela 1 ks 12. 7. 2001; **6767** Račice, dům č.p. 253 LK 12 ks 15. 7. 1999; **6865** Střelice, půda domu na ul. Písečná č.572 LK asi 60 ks 7. 6. 1997, asi 35 ks 13. 6. 1998, 72 ks 15. 6. 2000; **6866** Podolí, půda kostela LK 10 ks z toho 1 samice ad 21. 5. 2001; **6866** Šaratice, půda kostela LK min. 16 ks 11. 7. 2001; **6867** Dražovice, půda kostela LK 20–30 ks 12. 7. 2001; **6867** Hodějice, věž kostela LK 45 ks (ad+juv) 12. 7. 2001; **6867** Slavkov, půda zámku LK 19 ks 25. 6. 2002.

Publikováno bylo 8 lokalit letních kolonií netopýra večerního, dvě z nich zanikly (Kuřim a Habrovany), dvě dosud existují (Dražovice a Slavkov-zámek), stav zbylých je neznámý. Nově se nám podařilo objevit 7 reprodukčních kolonií tvořených asi 210 samicemi, nicméně skutečný počet zvířat bude pravděpodobně vyšší.

Netopýr ušatý (*Plecotus auritus*)

6665 Blansko, půda zámku LK min. 4 ad, 1 juv 22. 6. 1992; Lipůvka, půda kostela 1 ks 14. 6. 2001.

Netopýr dlouhouchý (*Plecotus austriacus*)

6464 Sulíkov, půda kostela 4 ks 15. 6. 2001; **6466** Knínice u Boskovic, půda kostela LK 6 ks (ad+juv) 31. 7. 2001; **6564** Doubravník, půda kostela LK 25 ks 22. 5. 2001; Lomnice, půda kostela 2 ks 14. 6. 2001; **6565** Boskovice, půda zámku LK asi 20 ks 30. 5. 1963, 9 ks 16. 8. 1977, 8 ks 24. 5. 2001; Dlouhá Lhota, půda kostela LK 11 ks 14. 6. 2001; Rájec-Jestřebí, půda kostela 3 ks 9. 8. 1958, 5ks 7. 5. 1963, 6 ks 22. 6. 1992, 1 ks 12. 6. 1994; Rájec-Jestřebí, půda zámku asi 12 ks 19. 9. 1959; Sebranice, půda kostela 1 ks 15. 6. 2001; **6566** Sloup, půda kostela 6 ks 20. 8. 1983 (leg. Bauerová), 7 ks 24. 6. 1992, 4 ks 12. 6. 1994; Ždárná, půda kostela 5 ks 9. 5. 1997; **6663** Kuřimská Nová Ves, půda kostela LK 26 ks 21. 5. 2001; **6664** Předklášteří, půda muzea asi 20 ks 27. 5. 1959, 12 ks 7. 6. 1963; **6665** Bořitov, půda kostela LK asi 20 ks 14. 5. 1963; Újezd u Černé Hory, půda kostela LK 10 ks chycena 1 samice 14. 6. 2001; Vranov, půda kostela LK asi 6 ks (samice ad) 16. 6. 1958; **6666** Jedovnice, půda kostela LK 8 ks (ad+juv) 24. 6. 1992, 5 ks 11. 6. 1994; **6764** Domašov, půda kostela LK 7 ks chycena 1 samice

21. 5. 2001; Říčany, půda kostela 3 ks chycena 1 samice 21. 5. 2001; Veverské Knínice, půda kostela LK 28 ks 21. 5. 2001; **6766** Adamov, půda kostela LK 13 ks 17. 5. 1958**;** Křtiny, klášter u kostela 5 ks 25. 6. 1992; Křtiny, půda kostela 6ks (4 samice ad) 16. 6. 1958; **6865** Brno – Mendlovo nám., půda kostela 5 ks 21. 5. 1958; Moravany, půda kostela LK min. 5 ks chycena 1 samice 27. 6. 2001; **6867** Slavkov, půda zámku 6 ks 22. 9. 1958.

Druh tvořící jen malé letní kolonie, z 10 publikovaných nálezů jsme zkontrolovali čtyři lokality, všechny s negativním výsledkem. Recentně známo 8 letních kolonií.

***Plecotus* sp.**

6365 Deštná, půda kostela 5 ks 30. 7. 2001; **6464** Olešnice, půda kostela na náměstí 2 ks 28. 6. 2001; **6466** Vážany u Boskovic, půda kostela 1 ks 30. 7. 2001; **6467** Strážisko, půda kostela 1 ks 30. 7. 2001; **6468** Čechy pod Kosířem, půda kostela 2 ks 28. 6. 2001; **6564** Černvír, půda kostela 2 ks 22. 5. 2001; **6567** Stínava, půda kostela 3 ks 27. 6. 2001; **6568** Ohrozim, věž kostela 9 ks 28. 6. 2001; **6664** Tišnov, půda obecního úřadu 1 ks 21. 5. 2001; **6668** Pustiměř, půda kostela 3 ks 25. 6. 2001; **6765** Brno-Královo Pole, půda kostela na Božetěchově ul. 1 ks 8. 6. 1977; **6866** Blažovice, věž kostela 6 ks 17. 7. 2001; **6866** Újezd u Brna, půda kostela 1 ks 11. 7. 2001; **6966** Měnín, půda kostela 1 ks 11. 7. 2001.

Protože se vždy nepodařilo netopýry odchytit, nebylo možné ve výše uvedených případech určit druh – pravděpodobně se však jedná převážně o jedince druhu *Plecotus austriacus*.

Diskuse

Průzkumem půd vhodných budov se ve zkoumaném území podařilo prokázat přítomnost devíti druhů netopýrů, což činí 47 % druhů známých z letního období (Řehák 1995). Nejčastěji byly nalézány druhy vykazující silnou vazbu na lidské stavby tzn. *Myotis myotis*, *Rhinolophus hipposideros*, *Eptesicus serotinus* a *Plecotus austriacus*. Vzhledem k tomu, že nebyly kontrolovány ostatní možné úkryty (stromové dutiny, menší lesní stavby, štěrbiny na domech), nemohly být pravděpodobně zaznamenány typicky sylvikolní druhy *Nyctalus noctula*, *N. leisleri*, *Myotis bechsteinii* nebo *Barbastella barbastellus*. Tyto druhy se nicméně v oblasti vyskytují a pravděpodobně i rozmnožují. Stejně tak bylo použitou metodikou nesnadné zachytit druhy méně běžné jako *Myotis brandtii*, *M. nattereri*, *Pipistrellus nathusii* nebo *Vespertilio murinus*, či vysloveně vzácné (*Myotis dasycneme*). U všech námi zjištěných druhů byly nalezeny také jejich letní kolonie, u *Myotis mystacinus*, *M. daubentonii* a *Pipistrellus pipistrellus* jde o první publikované údaje z oblasti Moravského krasu a jeho okolí. Dříve známá letní kolonie *Eptesicus nilssonii* ve Ždárné (Zukal & Gaisler 1989) vykazovala značné kolísaní početnosti a její kontrola v roce 1991 byla negativní.

Popisované poměry jsou velmi podobné výsledkům získaným z podhůří Šumavy, kde Krátká & Krátký (1973, 1976, 1985) během osmi let zkontrolovali 280 budov a na 33 % z nich nalezli netopýry. Počet druhů (9) je stejný a i druhová struktura v jejich vzorku velmi podobná, data se však liší ve struktuře dominance jednotlivých druhů. Zatímco *Myotis myotis* je dominantním nalézaným druhem v lidských stavbách v obou oblastech, byl v datech ze Šumavy druhým nejpočetnějším druhem *Plecotus austriacus* a rovněž vysoké bylo zastoupení *Myotis nattereri* (chybí v našich datech). Naopak zde byla zaznamenána nízká dominance *Eptesicus serotinus*. Je otázkou, jestli je popsaný stav odrazem odlišného charakteru staveb (na Šumavě časté kostely s dřevěným obložením) nebo relativně vyšší nadmořskou výškou šumavských lokalit oproti Moravskému krasu.

Na území západního Slovenska našli Lehotská & Lehotský (1998) při srovnatelném počtu zkontrolovaných budov (202) netopýry na 47 % lokalit (tento výzkum 43 %). Druhové složení je prakticky totožné, s výjimkou přítomnosti *Rhinolophus ferrumequinum*, který byl v oblasti Moravského krasu nalezen výjimečně a pouze v zimním období (Gaisler & Hanák 1972, Bauerová & Zima

1998). Dalším rozdílem je nepřítomnost druhů *Myotis mystacinus* a *M. daubentonii*, u nichž se nám v okolí Moravského krasu podařilo doložit i letní kolonie. Tento rozdíl může být způsoben pouhým větším časovým rozpětím našich dat, protože nálezy obou zmíněných druhů jsou nepočetné, pocházejí z rodinných domů a mají tak spíše náhodný charakter.

Další zajímavou možnost srovnání poskytují data z východní části Slovenska (Danko et al. 2000). I přes vyšší počet zkontrolovaných objektů (315) a frekvenci výskytu netopýrů v nich (51 % pozitivních nálezů), je počet druhů nalezených na půdách budov (10) srovnatelný s poměry na Moravě. Podobné je i zastoupení nejpočetnějších druhů (*Myotis myotis*, *Eptesicus serotinus* a *Plecotus austriacus*). Významný rozdíl spočívá především v nízkém zastoupení nálezů *Rhinolophus hipposideros* ve vzorku z Východoslovenské nížiny, což je zřejmě dáno jeho celkovou absencí v regionu spíše než odlišnou úkrytovou preferencí. V rámci slovenského monitoringu je naopak častější zastoupení druhů *Myotis blythii* a *Myotis dasycneme*, jelikož jejich severní hranice výskytu zasahuje pouze okrajově na území ČR.

Další srovnatelné výzkumy byly prováděny na středozápadním (Obuch & Kadlečík 1997) a severovýchodním Slovensku (Hromada 1997). I přes jen přibližně třetinový počet zkontrolovaných objektů se relativní počet pozitivních kontrol pohybuje okolo 50% a velmi podobné jako v našem výzkumu je i zastoupení eudominantních (*Myotis myotis*, *Rhinolophus hipposideros*) a dominantních (*Plecotus austriacus*, resp. *Eptesicus serotinus*) druhů. Celkový počet zjištěných druhů je ovšem adekvátně nižší. Je možno konstatovat, že dominance výše zmíněných druhů je společná všem diskutovaným vzorkům a představuje tak všeobecný stav. Určitou výjimku představují jen data z východních Karpat (Matis et al. 2000), kde bylo i při malém počtu zkontrolovaných objektů nalezeno 10 druhů, přičemž nejčastější byly druhy *Plecotus auritus* a *Rhinolophus hipposideros*. Tento stav je zřejmě způsoben celkově vyšší různorodostí prostředí v kombinaci s vyšší nadmořskou výškou a malým zastoupením lidských staveb (nízký výskyt *Plecotus austriacus*, resp. přítomnost *Eptesicus nilssonii*).

Poděkování

Za pomoc při terénních pracích jsme zavázáni Martinu Gajdošíkovi, Evě Jánové, Miroslavu Kovaříkovi, Štěpánce Králové, Michaele Novákové, Kláře J. Petrželkové, Janě Rybaříkové, Davidu Staňkovi a Janu Zimovi. Výzkum byl zčásti podpořen grantem GA ČR 206/01/1555.

Literatura

Bárta Z., Červený J., Gaisler J., Hanák P., Hanák V., Horáček I., Hůrka L., Miles P., Nevrlý M., Rumler Z., Sklenář J. & Žalman J., 1981: Výsledky zimního sčítání netopýrů v Československu 1969–1979. *Sbor. Okr. Muz. v Mostě, Přír.*, **3**: 71–116.

Bauerová Z., 1984: Zur Fledermausfauna des Mährischen Karstes. *Nyctalus*, *N. F.*, **2**(1): 65–71.

Bauerová Z. & Gaisler J., 1985: Netopýr večerní a další netopýři ve městech. *Živa*, **33**: 230–231.

Bauerová Z. & Zima J., 1988: Seasonal changes in the visits to a cave by bats. *Folia Zool.*, **37**: 97–111.

Bauerová Z., Gaisler J., Kovařík M. & Zima J., 1989: Variation in numbers of hibernating bats in the Moravian Karst: results of visual censuses in 1983–1987. Pp.: 499–505. In: Hanák V., Horáček I. & Gaisler J. (eds.): *European Bats Research 1987*. Charles Univ. Press, Praha, 718 pp.

Danko Š., Uhrin M., Pjenčák P. & Matis Š., 2000: Netopiere Východoslovenskej roviny, Východoslovenskej pahorkatiny a Zemplínskych vrchov. *Vespertilio*, **4**: 37–58.

Gaisler J., 1967: Jak žijí netopýři v létě. *Živa*, **15**: 74–76.

Gaisler J., 1973: Netting as a possible approach to study bat activity. *Period. Biol.*, **75**: 129–134.

Gaisler J., 1975: A quantitative study of some populations of bats in Czechoslovakia (Mammalia: Chiroptera). *Acta Sci. Natur. Brno*, **9**(5): 1–44.

Gaisler J., 1979: Results of bat census in a town (Mammalia: Chiroptera). *Věst. Čs. Společ. Zool.*, **43**: 7–21.

Gaisler J. & Bauerová Z., 1986: The life of bats in a city. *Myotis*, **23–24**: 209–215.

Gaisler J. & Hanák V., 1969: Ergebnisse der zwanzigjährigen Beringung von Fledermäusen (Chiroptera) in der Tschechoslowakei: 1948–1967. *Acta Sci. Natur. Brno*, **3** (5): 1–33.

Gaisler J. & Hanák V., 1972: Netopýři podzemních prostorů v Československu. *Sbor. Západočes. Muz. v Plzni*, *Přír.*, **7**: 1–46.

Gaisler J. & Hanák V., 1973: Přehled netopýrů moravských jeskyň. *Čs. Kras*, **24**: 53–60.

Gaisler J. & Řehák Z., 2001: Netopýři zimující v jeskyních v údolí Říčky u Ochozu (Moravský kras). *Vespertilio*, **5**: 111–114.

Gaisler J., Bauerová Z., Vlašín M. & Chytil J., 1988: The bats of S-Moravian lowlands over thirty years: Rhinolophus and large *Myotis*. *Folia Zool.*, **37**: 1–16.

Gaisler J., Vlašín M. & Bauerová Z., 1989: The bats of S-Moravian lowlands over thirty years: small *Myotis*. *Folia Zool.*, **38**: 213–225.

Gaisler J., Chytil J. & Vlašín M., 1990: The bats of S-Moravian lowlands (Czechoslovakia) over thirty years. *Acta Sci. Natur. Brno*, **24**(9): 1–50.

Hanák V., Gaisler J. & Figala J., 1962: Results of bat-banding in Czechoslovakia, 1948–1960. *Acta Univ. Carol. – Biol.*, **1962**(1): 9–87.

Hromada M., 1997: Výsledky faunistického výskumu netopierov Ondavskej vrchoviny a Busova. *Vespertilio*, **2**:73–80.

Krátká D. & Krátký J., 1973: Letní výskyt netopýrů (Chiroptera) vázaných na lidské stavby na Šumavě I. Sušicko. *Zpr. Muz. Západočes. Kraje – Přír.*, *Plzeň*, **15**: 39–45.

Krátká D. & Krátký J., 1976: Letní výskyt netopýrů (Chiroptera) vázaných na lidské stavby na Šumavě II. Strážovská vrchovina. *Zpr. Muz. Západočes. Kraje – Přír.*, *Plzeň*, **19**: 63–67.

Krátká D. & Krátký J., 1985: Letní výskyt netopýrů (Chiroptera) vázaných na lidské stavby na Šumavě III. Prachaticko, Strakonicko, Českokrumlovsko . *Zpr. Muz. Západočes. Kraje – Přír.*, *Plzeň*, **30–31**: 69–78.

Lehotská B. & Lehotský R., 1998: Výskyt letných kolónií netopierov na území západného Slovenska. *Vespertilio*, **3**: 57–64.

Matis Š., Pjenčák P. & Danko Š., 2000: Netopiere Chránenej krajinnej oblasti Východné Karpaty a Národného parku Poloniny. *Vespertilio*, **4**:135–144.

Obuch J. & Kadlečík J., 1997: Letný výskyt netopierov v budovách Turca. *Vespertilio*, **2**: 51–58.

Řehák Z., 1995: *Letová aktivita netopýrů v Moravském krasu*. Dizertační práce, PřF MU Brno, 184 pp.

Řehák Z., Zukal J. & Kovařík M., 1994: Long- and short-term changes in the bat community of the Kateřinská cave (Moravian karst) – a fundamental assessment. *Folia Zool.*, **43**: 425–436.

Vašátko J., Gaisler J. & Bauerová Z., 1989: Živočišstvo se zvláštním zřetelem na bezobratlé a netopýry. *Studia Geograph.*, **82**: 75–93.

Vlašín M. & Eleder P., 1991: Rozšíření ochranářsky důležitých druhů savců v Jihomoravském kraji (I. část). *Vlastivěd. Sbor. Vysočiny*, *Odd. Přír. Věd*, **10**: 209–227.

Vlašín M., Eleder P. & Nečasová I., 1993: Rozšíření ochranářsky důležitých druhů savců v jihomoravském regionu (II. část). *Vlastivěd. Sbor. Vysočiny*, *Odd. Přír. Věd*, **11**: 273–295.

Vlašín M., Eleder P. & Málková I., 1995: Rozšíření ochranářsky důležitých druhů savců v jihomoravském regionu (3. část). *Vlastivěd. Sbor. Vysočiny*, *Odd. Přír. Věd*, **12**: 205–241.

Zima J., 2001: Zimoviště netopýrů ve střední části Moravského krasu. *Vespertilio*, **5**: 311–320.

Zima J., Kovařík M., Gaisler J., Řehák Z. & Zukal J., 1994: Dynamics of the number of bats hibernating in the Moravian karst in 1983 to 1992. *Folia Zool*., **43**: 109–119.

Zukal J. & Gaisler J., 1989: K výskytu a změnám početnosti netopýra severního, *Eptesicus nilssoni* (Keyserling et Blasius, 1839) v Československu. *Lynx*, *n. s*., **25**: 83–95.

Zukal J., Kovařík M., Řehák Z. & Berková H., 2001: Početnost netopýrů zimujících ve dvou jeskyních v severní části Moravského krasu a její dlouhodobé změny. *Vespertilio*, **5**: 321–328.

došlo 9. 6. 2003

Výběr úkrytů a letová aktivita netopýrů

Zukal, J., Berková, H. & Řehák, Z. (2005) Activity and shelter selection by *Myotis myotis* and *Rhinolophus hipposideros* hibernating in Kateřinská cave (Czech Republic). Mammalian Biology 70, 271-281.

Mammalian Biology
Zeitschrift für Säugetierkunde
www.elsevier.de/mambio

Original investigation

Activity and shelter selection by *Myotis myotis* and *Rhinolophus hipposideros* hibernating in the Kateřinská cave (Czech Republic)

By J. Zukal, Hana Berková and Z. Řehák

Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno und Department of Zoology and Ecology, Faculty of Science, Masaryk University, Brno, Czech Republic

Receipt of Ms. 21.4.2004
Acceptance of Ms. 9.3.2005

Abstract

In 1992–1993, the bat species *Myotis myotis* and *Rhinolophus hipposideros* hibernating in the Kateřinská cave were investigated by means of regular censuses without any handling and marking of the animals. Three basic parameters of their shelters were recorded (position in cave, type and relative height). In total, during 26 checks we registered 1141 findings of nine bat species. Movement activity, expressed as percentage of new findings during a particular visit, was registered during the whole winter season. Its level fluctuated in different ways and the hibernation period of *R. hipposideros* could be divided into three different parts, while the level of *M. myotis* movement activity was relatively high during all season. The shelter selection of *R. hipposideros* was not dependent on the part of cave where the bats were hibernating, and it did not change during the season. Hibernating specimens of *R. hipposideros* most frequently used exposed places, in which they were always hanging free. *Myotis myotis* was registered in all types of shelter with one exception. *Rhinolophus hipposideros* used mainly the middle part of the cave at a distance between 121 and 180 m from the entrance. The most preferred part of the cave by *M. myotis* was a small segment of Corridor (between 21 and 30 m), i.e., the entrance part of the cave. *Rhinolophus hipposideros* is a highly specialized species which prefers parts of the cave with very stable microclimatic conditions and, on the contrary, *M. myotis* appears to be indifferent to all parameters studied, and it uses the shelters indiscriminately.

Key words: Bats, shelter selection, activity, hibernation

Introduction

With its more than 1100 caves, the Moravian Karst is among the most important regions for the hibernation of bats in Europe. Even when the first data on bats originate from the half of the 19th century, systematic research was initiated there by Gaisler as late as 1957. Some caves in the southern part of the Moravian Karst are hibernaculas which have been monitored for the longest time in central Europe (Řehák 1997). Attention has mainly been given to the monitoring of long-term changes in bat communities (Zima et al.

1616-5047/$ - see front matter
doi:10.1016/j.mambio.2005.03.003

1994). Studies of bat ecology during hibernation are very rare and are mostly concerned with the thermopreferendum of bats (Gaisler 1970; Bauerová and Zima 1988).
The aim of the present study is to evaluate the selection of different places with specific parameters by two most abundant bat species in a natural cave and the level of their movement activity. We tested the hypothesis that shelter selection by bats depends on the internal characteristics of cave and, therefore, it differs in the particular parts of the cave and changes in the course of a year.

Material and methods

Research was carried out in a natural limestone cave (Kateřinská Cave) situated in the eastern part of the Czech Republic. The total length of the cave is about 300 m and it has only one entrance which is closed by an iron gate with a vertical hole in its upper part (Řehák et al. 1994). The cave can be divided into a dynamic and a static part by the differences in temperature and airflow. The approximately 50 m long outer part (Corridor) is dynamic from the microclimatic point of view, as the air temperature in this part of the cave is influenced by the changes in ambient temperature (Figs. 1 and 2). The main inner part of the cave consists of three large domes (Main dome, Dome of the Witch and Dome of Chaos) which are considered as microclimatically stable parts. They are characterized by relatively stable temperatures, ranging between 6.3 and 8.8 °C in the course of the year (Fig. 2). Two small and inaccessible parts were not checked regularly as there were no hibernating bats, and thus these parts were excluded from the monitoring (Fig. 1). Except for 2 months (December and January) when only the employees of the Agency for Nature Conservation and Landscape Protection of the Czech Republic were permitted to enter the cave, tourists visited the cave.
We used the visual census method without any handling and marking of animals (Zima et al. 1994), as one of the main requirements of our research was to avoid any disturbance of the hibernating bats. Air temperature was measured by the digital thermometer DT-20 during the census of hibernating bats and it was left at least 5 min at each point. We registered the following characters for each bat or their cluster (i.e., group of two or more specimens in close contact): (1) Species, number of specimens and their clusters. Small species of *Myotis* spp. were recorded as *M.* sp. if we were not able to determine them exactly.

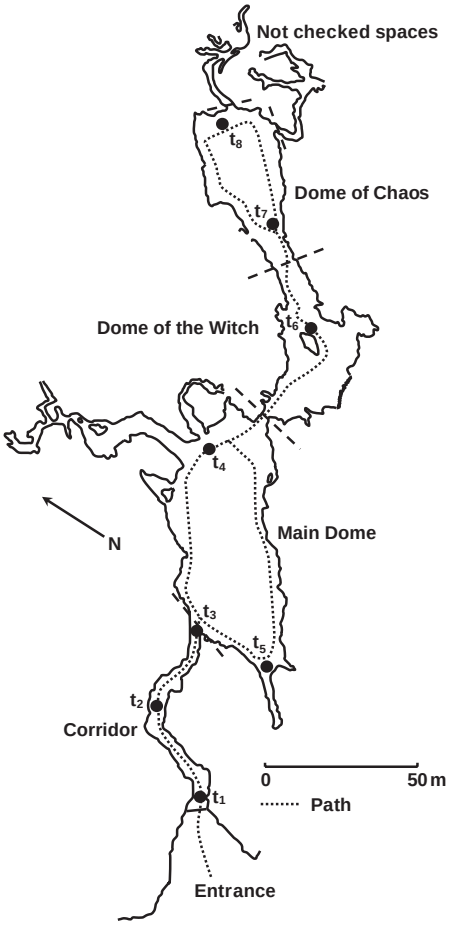


Fig. 1. Division of the Kateřinská Cave into four basic parts. Explanations: $t_{1..8}$–points of air temperature measurement.

(2) Position of a shelter in the cave, with accurately plotting it in a map. The exact position of a hibernating bat with information whether it was a new finding or the same specimen and/or cluster was registered again during the subsequent visit. The relative distance of the bat shelter from the cave entrance (the iron gate with an exit hole) was subsequently ascertained from the cave map. For this purpose, the position of the iron gate was considered to be the center point from which concentric circles were drawn at distances of 10 m. (3) Type of shelter. Eight types of shelters were distinguished, viz., the bat hanging free on the ceiling (i.e., a slope $<45°$) (FC); on the wall (i.e., a slope $>45°$) (FW); in the chimney (FCh); the bat

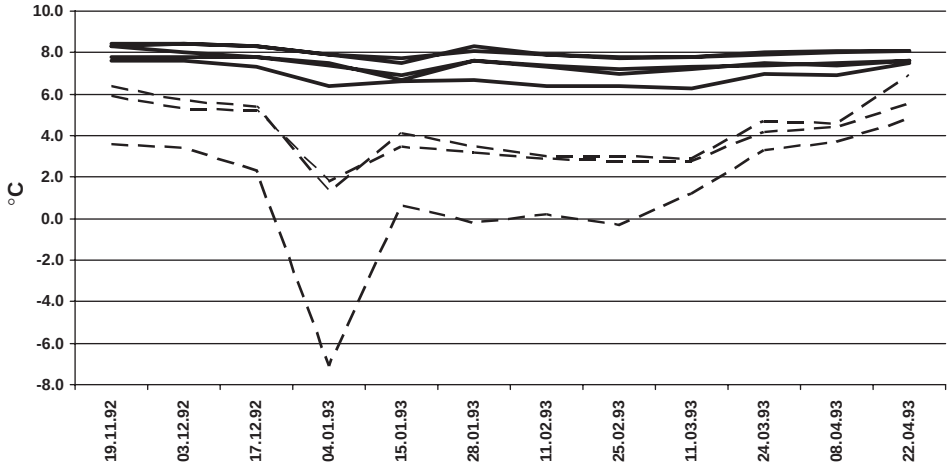


Fig. 2. Course of air temperature changes during the season. Explanations: dashed lines – outer parts of cave (t_1–t_3), solid lines – inner parts of cave (t_4–t_8), $t_{1..8}$ – points of air temperature measurement (see Fig. 1).

hanging in contact with the ceiling (CC); the wall (CW); or the chimney (CCh); and the bat hibernating in a horizontal (HC); or a vertical crevice (VC) (one of the dimensions of a shelter was 7 cm at least). These selected types of shelter were pooled by the degree of exposition reflecting the variability of microclimatic parameters to exposed (FC, FW, CC, CW), semi-exposed (FCh and CCh), and hidden (HC and VC). (4) Relative height above the floor. Four categories of relative height were established, based on the morphological diversity of the Kateřinská Cave, viz., <1, 1–3, 3–5 and >5 m.

From October 10, 1992 to October 8, 1993, we carried out 26 checks approximately once every 2 weeks. In total, we registered 1141 findings of nine bat species with the high predominance of *M. myotis* and *Rhinolophus hipposideros* and therefore we analyzed only the activity and shelter selection of these two bat species. The sibling species *M. blythii* was never found in the cave during the hibernation. The results of visits between November 19, 1992 and April 22, 1993, when at least five specimens of the species under study were registered, i.e., 903 findings during 12 checks, were used for statistical analysis. *Myotis myotis* found in front of the iron gate (3.1% of all findings) and the only finding of *R. hipposideros* in the Corridor were excluded from the analysis, except for the data on the position and the type of their shelter.

Movement activity is expressed as a percentage of new findings during particular visit, i.e., a percentage of bats which were not registered at the same place during the previous visit, Based on its analysis, the whole study period was divided into three different parts: (1) Early hibernation (from November 19 to December 16) (2) Hibernation (from December 17 to March 11) and (3) Late hibernation (from March 12 to April 22). Only the first new findings of bats in particular parts of the cave were calculated for the assessment of differences in shelter selection. On the contrary, all findings registered during any particular visit were used in the analysis of temporal changes of bat shelter selection.

All statistical analyses were performed with Statistica for Windows 6.0, according to Sokal and Rohlf (1981). Contingency tables were used to assess the differences in the selection of various shelter characteristics. The correlation between movement activities of the two bat species under study was tested by Spearman's correlation coefficient.

Results

Abundance and movement activity of bats in hibernaculum

In total, we registered 1141 findings of nine bat species, viz., *R. hipposideros*, *M. myotis*, *M. nattereri*, *M. emarginatus*, *M. mystacinus*, *M. daubentonii*, *M. dasycneme*, *Plecotus auritus* and *Barbastella barbastellus*. Nevertheless, the findings of *M. myotis* and *R. hipposideros* accounted for a major part of the data obtained (81.9%).

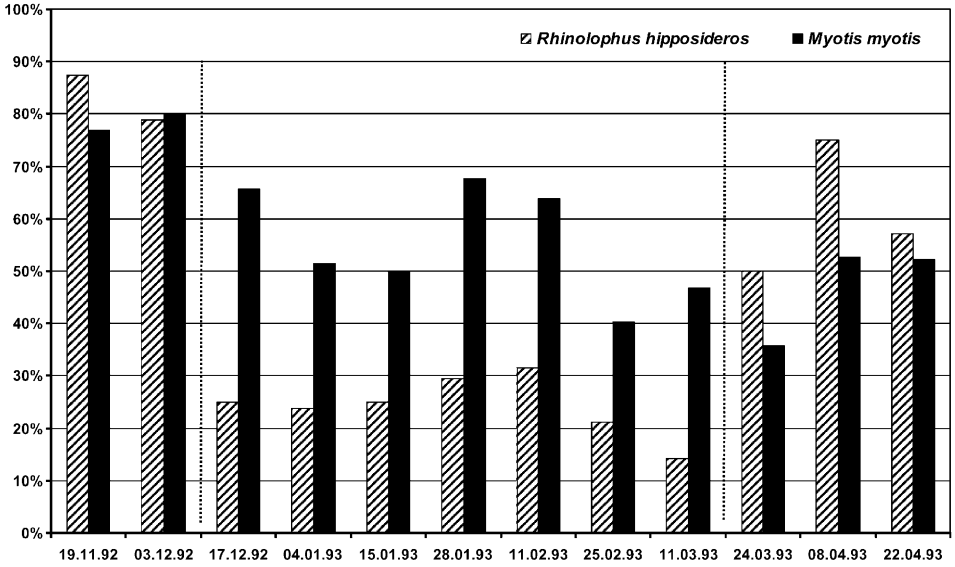


Fig. 3. Changes in the level of movement activity. Explanations: dotted line – the dividing of separate parts of season.

On the basis of the activity level the hibernation period of *R. hipposideros* can be divided into three different parts (Fig. 3). During the first one ("early hibernation") the number of bats considerably increased and this increase was correlated with the high level of movement activity. The number of hibernating *R. hipposideros* was subsequently stabilized (ca. 20 specimens) and only occasional movements were registered ("hibernation"). Since the middle of March the abundance has dropped and the bats continuously left the hibernaculum ("late hibernation"). This decrease in the abundance of bats correlated again with the increase in their movement activity. There was a significant difference in the level of movement activity in the successive periods (χ^2, DF = 2, $P < 0.001$).

The level of *M. myotis* movement activity was relatively high during all season and the "deep hibernation" period with decreased activity could not be clearly separated. In contrast to *R. hipposideros*, the total number of bats of this species was increasing gradually since October. The peak was recorded at the beginning of April (112 individuals). The highest increase in abundance was registered in the clusters formed in Corridor. Unfortunately, we could not assess the exact number of new bats in clusters by the visual method of census without disturbing the bats but only the change in the total number of hibernating bats, i.e., the difference between the previous and present numbers. Nevertheless, the movement activity of *M. myotis* and *R. hipposideros* was significantly correlated ($r_S = 0.60$, $P = 0.040$, $N = 12$), therefore the hibernation period was divided in the same way for both species (Fig. 3).

Shelter selection and its changes during the season

The shelter selection of *R. hipposideros* was not dependent on the part of the cave where the bats were hibernating (χ^2, DF = 4, $P = 0.609$) and it did not change during the season (χ^2, DF = 22, $P = 0.642$). Hibernating *R. hipposideros* most frequently used exposed places (FC, FW), where they were always hanging free (Fig. 4). The only individual *R. hipposideros* found in the Corridor (22.4.1993) was also hanging free on the wall of the cave at the height of 3–5 m above the floor.

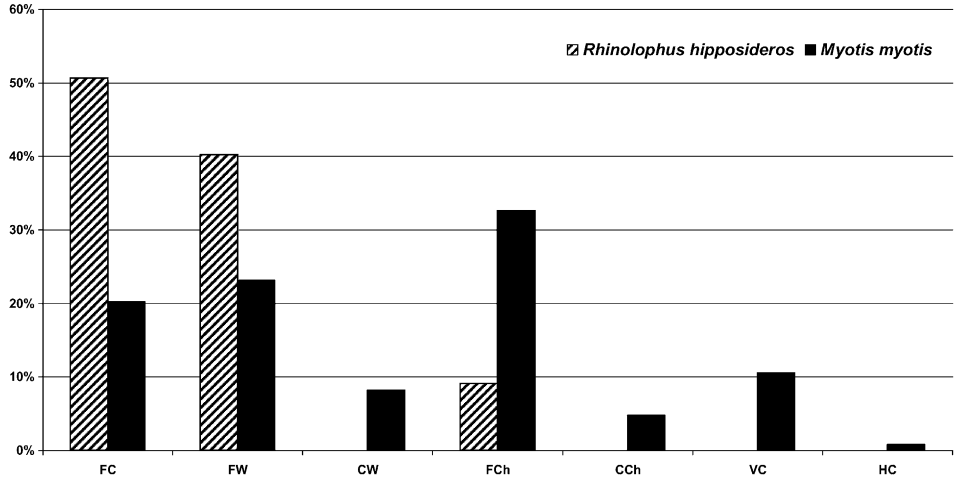


Fig. 4. Shelter-type selection by bat species under study. Abbreviations: the bat hanging free on the ceiling (FC); on the wall (FW); in the chimney (FCh); the bat hanging in contact with the ceiling (CC); the wall (CW); or the chimney (CCh); and the bat hibernating in a horizontal (HC); or a vertical crevice (VC).

Myotis myotis was registered in all types of shelters except for CC (Fig. 4). In general, the types where the bats hibernated without contact with the rock wall predominated. No significant differences were found in the use of the three types of shelter exposition during successive hibernation periods (χ^2, DF = 2, $P = 0.299$ for early hibernation; DF = 12, $P = 0.901$ for deep hibernation and DF = 4, $P = 0.090$ for late hibernation). However, a difference was observed between Corridor and Domes in the selection of these three shelter types (χ^2, DF = 2, $P < 0.001$). Most of the bats hibernating in the Corridor were hanging free on the ceiling and walls but the ratio of exposed vs. semi-exposed shelters used was equal in the Domes (Table 1). The differences in the selection of shelter types in the three Domes were not statistically significant (χ^2, DF = 4, $P = 0.102$) but more detailed analysis found differences only in the position of exposed shelter types (χ^2, DF = 2, $P < 0.001$). In the larger spaces (Main dome and Dome of Chaos) the bats were more frequently hanging on the ceiling (approx. 35% of findings) than on the wall (approx. 10% of findings). This ratio was contrary in the Dome of the Witch, which corresponds with the high diversity of this dome and thereby larger total area of cave walls.

Changes in the selection of shelter height above the floor

The distribution of shelter heights used by *M. myotis* was different in the Corridor and in the inner parts of the cave (χ^2, DF = 2, $P < 0.001$). The positions > 5 m above the floor predominated in the Domes, which corresponds with the generally higher offer of this height category in the largest spaces of the cave (Table 1). On the contrary, *R. hipposideros* more often used lower positions of shelters both in the Domes and in the Corridor (χ^2, DF = 6, $P = 0.008$), and it is hibernating even at the height < 1 m above the floor.

Seasonal variability (Fig. 5) in the preferred height of shelter was minimal for *R. hipposideros* and the predominance of particular height categories did not reach more than 50% (χ^2, DF = 33, $P = 0.999$). In relation to the increasing number of *M. myotis* in the Corridor (the lowest part of the cave), the percentage of bats using the height > 5 m has decreased from the middle of February and

Table 1. Type and height of shelter used by *Rhinolophus hipposideros* and *Myotis myotis* in the different parts of the cave

Part of the cave	Type of shelter			Height above the floor				Number of individuals
	Exposed (%)	Semi-exposed (%)	Hidden (%)	<1 (%)	1–3 (%)	3–5 (%)	>5 (%)	
R. hipposideros								
Corridor	100.0	0.0	0.0	0.0	0.0	100.0	0.0	1
Main dome	89.5	10.5	0.0	15.8	21.1	5.2	57.9	19
Dome of the Witch	92.5	7.5	0.0	2.5	40.0	25.0	32.5	40
Dome of Chaos	88.9	11.1	0.0	11.1	77.8	11.1	0.0	9
M. myotis								
Corridor	60.7	24.3	15.0	0.0	35.0	59.3	5.7	140
Main dome	48.1	49.4	2.6	0.0	5.2	15.6	79.2	77
Dome of the Witch	46.5	43.4	10.1	0.0	6.2	17.8	76.0	129
Dome of Chaos	45.0	30.0	25.0	0.0	0.0	5.0	95.0	20

their percentage in height categories 3–5 m and 1–3 m increased (χ^2, DF = 22, $P < 0.001$). These differences were mainly caused by the changes in preferred shelter height during the late hibernation period (χ^2, DF = 4, $P = 0.001$), which are also linked with the high movement activity of *M. myotis*.

Relative distance of shelter from the cave entrance

The distribution of relative distances of all new bat findings (a cluster was counted only as one selected place) was different in both species under study (χ^2, DF = 24, $P < 0.001$). *Rhinolophus hipposideros* used mainly the middle part of the cave with the distance between 121 and 180 m from the entrance (Dome of the Witch) where 59.4% of its findings were registered. In other parts of the cave, this species was found only occasionally (Fig. 6). The most preferred part of the cave by *M. myotis* was a small segment of Corridor (between 21 and 30 m), where a fourth part of all new findings of this species was registered. Similarly, to *R. hipposideros*, the area of the "Dome of the Witch" was also important for the hibernation of *M. myotis* (36.5% of findings). Nevertheless, *M. myotis* were recorded practically in all spaces of the cave.

Discussion

Abundance and movement activity of bats in hibernaculum

Rhinolophus hipposideros and *M. myotis* predominate in the majority of the Moravian Karst caves during the winter season (Bauerová and Zima 1988; Zima et al. 1994; Řehák et al. 1994; Kovařík 1997). *Rhinolophus hipposideros* exhibited a stable abundance of hibernating community from the beginning of December to mid-March (cf. Daan and Wichers 1968) when the level of its movement activity was the lowest. On the contrary, the previous and successive periods are characterized by high movement activity in the cave that was influenced by the continuous arrival and/or departure of bats in the hibernaculum. The same model of movement activity with its low level during "deep hibernation" was also confirmed by the detection of ultrasound signals (Nagel and Nagel 1997; Řehák and Baroň 2001) and Harmata (2000) noted that "deep hibernation" of this bat species began in the period between

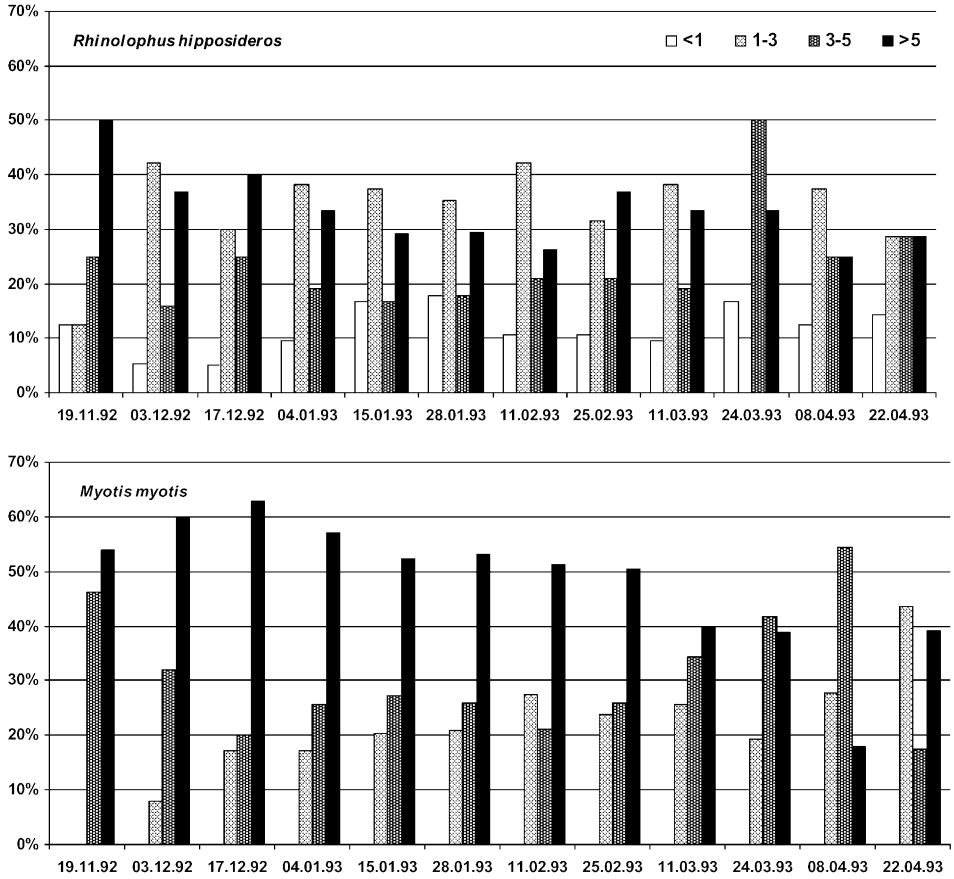


Fig. 5. Seasonal changes in used height of shelter.

November 15 and 20 and lasted until February of the next year. During climatically suitable days, even in the middle of winter, some awakened horseshoe bats left the cave and returned to it after a short time (Harmata 2000; Řehák and Baroň 2001).

General trend in *M. myotis* abundance changes (continual growth with rapid emergence) was mainly influenced by the changes in the outer part of the cave (Corridor). The flight activity of bats in the entrance hole of the cave is almost nil during the winter period (Řehák et al. 1994). Therefore, the considerable increase in *M. myotis* abundance is more likely due to movements within the cave from the inaccessible parts towards the entrance than to the immigration from neighboring shelters. Maximum abundance of this species at the end of the hibernation period and its shift towards the entrance part of the caves was also reported for other European hibarnacula (Nagel and Nagel 1987; Hanzal and Průcha 1988, 1996; Fuszara et al. 1996).

The movement activity of *M. myotis* could not be clearly divided into the successive periods based on its level, as done for *R. hipposideros*. High activity was also confirmed by Průcha and Hanzal (1989), who registered very short mean duration of the hibernation period when newly found bats were included in the analysis (7.1 and 9.1 days for *M. myotis* in different years). Nagel and Nagel (1994) reported the movement activity of *M. myotis* within the

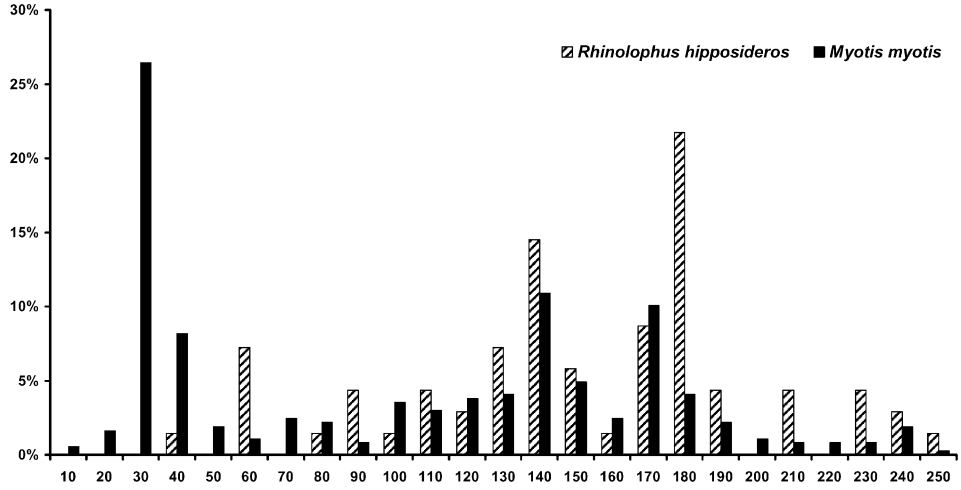


Fig. 6. Shelter preference in different distances from the cave entrance.

hibernaculum as increasing from mid-February till the end of April. This relatively high activity is associated with the use of shelters with varying microclimate during the winter period, similarly as in other *Myotis* species (Daan 1973; Hanzal and Průcha 1988). In case of *M. myotis* the cave air temperature does not influence winter flight activity, but the activity increases with a decrease in barometric pressure (Nagel and Nagel 1994).

Shelter preference

Shelter selection differs in various parts of the hibernation period, depending on the changes of microclimatic conditions in the hibernaculum, and it is also species specific (Daan 1973; Altringham 1996). Saint Girons et al. (1969) and Brosset and Poillet (1985) suggest that the changes in *R. ferrumequinum* shelters during winter do not correspond to the changes in environment, as the temperature does not fluctuate in deep parts of caves used by horseshoe bats, but it corresponds only to the phases of annual cycle, physiological status and behavior of studied bats.

In general, bats use their hibernation sites with constant air temperatures during the middle of the hibernation period. They can probably reduce their energy consumption by the selection of these parts during the period of absolute lack of food. The dynamic parts of the hibernacula are occupied by hibernating bats during the beginning and the end of the hibernation period (Baagøe et al., 1988; Baroň and Řehák 1997). This behavior can be related to foraging activity. As the air temperature in outer dynamic parts reflects ambient temperature changes outside the cave, the bats can awake from lethargy when the conditions are suitable for foraging (Whitaker and Rissler 1993). However, the moving of the bats into the dynamic cave parts can also be associated with the tendency to avoid dry circumstances, i.e., with water loss compensation (Daan and Wichers 1968; Dorgelo and Punt 1969). Nevertheless, it should be noted that mainly the temporal changes in cave use could be biased because only a limited part of the hibernating population can be found in any particular period (Řehák 1997; Řehák and Gaisler 1999).

Rhinolophus hipposideros hibernated in the Kateřinská Cave at unprotected places, always hanging free, and a similar observation was published by Dorgelo and Punt (1969). Daan and Wichers (1968) registered the presence of this species in exposed and less frequently in semi-exposed places, almost

always in the rear of an artificial limestone cave system. Moreover, *R. hipposideros* was using almost absolutely the inner parts of the Kateřinská Cave (Domes) (with exception of one individual found in the Corridor), similarly as in the limestone cavern in Poland where 68.6% of the hibernating bats were found in its deepest part (Harmata 2000) and in the pseudokarstic sandstone crevice-type caves in Northern Moravia (Baroň and Řehák 1997).

The seasonal dynamic in the use of particular parts of the cave was characterized for *M. myotis* by declining percentages of this species in the Domes (inner parts) and increasing abundance of bats in the Corridor from mid-November to the beginning of April. A similar tendency was also registered for *M. myotis* by Dorgelo and Punt (1969) and Hanzal and Průcha (1988). The increasing number of bats during winter period was most probably caused by the movements of hibernating bats from highly protected shelters and/or places which are not accessible for people (high chimneys, etc.) to more exposed places. The increase could not be influenced by immigration of bats from neighboring shelters, as the flight activity at the entrance of the Kateřinská Cave (netting) was practically nil during winter months (Řehák et al. 1994). The mentioned type of movement behavior was also reported in some other species of *Myotis* (Daan and Wichers 1968; Daan 1973; Degn 1987; Baagøe et al., 1988). *Myotis myotis* hibernated mostly exposed, hanging free on the ceiling, free on the wall or free in the chimney. Nevertheless, they were sometimes found in crevices too (Bezem et al. 1964; Hanzal and Průcha 1988). Lesiński (1986) recorded this bat species hibernating in forts near Warszawa, mainly in ventilation shafts, i.e., in protected type of shelter, and less frequently in crevices and exposed places. Daan and Wichers (1968) observed that *M. myotis* were using highly protected places in the entrance section of a limestone cave, similarly as did Hanzal and Průcha (1988) who noted that *M. myotis* selected rather protected (in crevices deeper than 10 cm) or semi-protected (in crevices up to 10 cm) places after having moved into the colder parts of hibernacula. On the other hand, in the Kateřinská Cave the preference of exposed places (ceiling, wall) was higher in the Corridor (60%) than in the Domes (45%).

Bezem et al. (1964) also registered the height where the hibernating bats were hanging, the relation between shelter type and height above the floor, and the relative height of site (height of hibernating bat divided by corresponding height of corridor). They did not find any statistically significant correlation of these parameters and consequently they classified *M. myotis* as a species using the highest located shelters and, on the contrary, *R. hipposideros* using the lowest location of shelters. There was no significant correlation between the type of shelter and its height in both *M. myotis* and *R. hipposideros* hibernating in Kateřinská Cave. Harmata (2000) noted that the height of hanging *R. hipposideros* did not change during hibernation. The horseshoe bats were hibernating mostly at height between 1 and 3 m but often they used very low lying shelters (20–30 cm above the floor).

Generally, the range of temperatures detected during hibernation of both species under study overlaps in wide extent (*R. hipposideros* 2.0–13.0 °C and *M. myotis* −4.0 to 12.0 °C, Webb et al. 1996) but both species differ in the time of arrival in the hibernaculum, development of abundance changes, level of internal migration and shelter preference in the caves. These differences should reflect the life history and body size of bat species under study. The cave temperatures selected by bats in a cold climate are negatively correlated with their body weight (McNab 1974) and the clusters of bats select even colder temperatures. Small non-clustering *R. hipposideros* (5–9 g) developed specific style of hibernation, i.e., hanging free with wing membrane covering all the body. It is capable of hibernating at higher air temperature but it needs the stability of microclimate that is ensured by use of inner parts of caves. *Myotis myotis*, a large species (20–35 g), is able to hibernate in clusters within colder parts of cave close to the entrance. The clustering behavior and large weight determinate also its ability to have a high movement activity during the hibernation period (McNab 1974).

Acknowledgements

The authors wish to thank all colleagues who participated in the field works. We are also much obliged to Prof. Jiří Gaisler, Dr. Sc., for kindly providing inaccessible literature and valuable comments on the manuscript, and RNDr. Miroslav Kovařík, for long-term help and cooperation in the field. The work was supported by a grant of The Ministry of Education of the Czech Republic No. 143100010 and a grant of Grant Agency of the Czech Republic No. 206/01/1555.

Zusammenfassung

Aktivität und Ruheplatzwahl von winterschlafenden *Myotis myotis* und *Rhinolophus hipposideros* in der Kateřinská Höhle (Tschechien)

In 1992–1993 wurden die in der Kateřinská Höhle überwinternden Individuen von *Myotis myotis* und *Rhinolophus hipposideros* untersucht. Die Untersuchung bestand aus einer regelmäßigen Zählung, bei der die Tiere nicht berührt bzw. markiert wurden. Es wurden drei grundlegende Parameter ihrer Hangplätze aufgenommen (Lage innerhalb der Höhle, Typ und relative Höhe). Insgesamt wurden im Laufe von 26 Kontrollgängen 1141 Einzelbeobachtungen von neun Fledermausarten erfaßt. Bewegungstätigkeit, ausgedrückt als prozentueller Anteil der Neufunde während eines bestimmten Kontrollgangs, wurde während der gesamten Wintersaison registriert. Ihr Niveau schwankte unterschiedlich, wobei die Überwinterungsperiode von *R. hipposideros* in drei unterschiedliche Abschnitte aufgeteilt werden konnte, während das Niveau der Bewegungsaktivität von *M. myotis* im Verlauf der Saison ständig relativ hoch blieb. Die Ruheplatzwahl von *R. hipposideros* war unabhängig von dem Höhlenteil in dem die Fledermäuse überwinterten und unterlag im Saisonverlauf keinen Änderungen. Überwinternde Exemplare von *R. hipposideros* nutzten vor allem exponierte Plätze an denen sie immer frei hingen. *Myotis myotis* wurde an allen Hangplatztypen bis auf einen festgestellt. *Rhinolophus hipposideros* nutzte vor allem den mittleren Höhlenteil in einer Entfernung von 121 bis 180 m vom Höhleneingang. Von *M. myotis* wurde ein kurzer Abschnitt des Höhlenganges (zwischen 21 und 30 m), d.h. nahe dem Eingang, am meisten bevorzugt. *Rhinolophus hipposideros* ist eine hochspezialisierte Art, welche Höhlenteile mit sehr stabilen mikroklimatischen Bedingungen bevorzugt, während *M. myotis* den erfaßten Parametern gegenüber als völlig indifferent erscheint.

References

Altringham, J. D. (1996): Bats: biology and behaviour. New York: Oxford University Press.

Baagøe, H.; Degn, H. J.; Nielsen, P. (1988): Departure dynamics of *Myotis daubentonii* (Chiroptera) leaving a large hibernaculum. Vidensk. Meddr dansk Naturh. Foren. **147,** 7–24.

Baroň, I.; Řehák, Z. (1997): Contribution to the hibernation of lesser horseshoe bats (*Rhinolophus hipposideros*) in the Vsetín region. Zprav. Okr. Vlastivěd. Muzea ve Vsetíně **1997,** 3–12 (in Czech).

Bauerová, Z.; Zima, J. (1988): Seasonal changes in visits to a cave by bats. Folia Zool. **37,** 97–111.

Bezem, J. J.; Sluiter, J. W.; Van Heerdt, P. F. (1964): Some characteristics of the hibernating locations of various species of bats in south Limburg. II. Proc. Koninkl. Nederl. Akademie van Wetenschappen, Ser. C **67,** 337–350.

Brosset, A.; Poillet, A. (1985): Structure d'une population hibernante de grands rhinolophes *Rhinolophus ferrumequinum* dans l'est de la France. Mammalia **49,** 221–233.

Daan, S. (1973): Activity during natural hibernation in three species of vespertilionid bats. Netherlands J. Zool. **23,** 1–71.

Daan, S.; Wichers, H. J. (1968): Habitat selection of bats hibernating in a limestone cave. Z. Säugetierkd. **33,** 262–287.

Degn, H. J. (1987): Bat counts in Mønsted limestone cave during the year. Myotis **25,** 85–90.

Dorgelo, J.; Punt, A. (1969): Abundance and "internal migration" of hibernating bats in an artificial limestone cave ("Sibbergroeve"). Lynx (n.s.) **10,** 101–125.

Fuszara, E.; Kowalski, M.; Lesinski, G.; Cygan, J. P. (1996): Hibernation of bats in underground shelters of central and northeastern Poland. Bonn. Zool. Beitr. **46**, 349–38.

Gaisler, J. (1970): Remarks on the thermopreferendum of palearctic bats in their natural habitats. Bijdr. Dierk. (Amsterdam) **40,** 33–35.

Hanzal, V.; Průcha, M. (1988): Seasonal dynamics of bat communities at winter shelters of Český kras (Central Bohemia) during 1984–1986. Lynx (n.s.) **24,** 15–35 (in Czech).

Hanzal, V.; Průcha, M. (1996): Annual course of cave visitation by bats (Mammalia: Chiroptera) in the Bohemian Karst (Czech Republic). Acta Soc. Zool. Bohem. **60,** 25–30.

Harmata, W. (2000): Phenological dynamics of hibernating lesser horseshoe bat *Rhinolophus hipposideros* (Bechst.) (Chiroptera, Rhinolophidae) in a limestone cavern. Studia Chiropterol. **1,** 13–28 (in Polish).

Kovařík, M. (1997). Research and protection of *Rhinolophus hipposideros* in protected landscape area Moravian Karst during hibernation. In: Zur Situation der Hufeisennasen in Europa. Ed. by Arbeitskreisirs Fledermäuse Sachsen-Anhalt e.V. Berlin: IFA Verlag. Pp. 85 – 87.

Lesiński, G. (1986): Ecology of bats hibernating underground in central Poland. Acta Theriol. **31,** 507–521.

McNab, B. K. (1974): The behavior of temperate cave bats in a subtropical environment. Ecology **55,** 943–958.

Nagel, A.; Nagel, R. (1987): Veränderungen des Bestandes winterschlafender Fledermäuse im Winter 1982/83 in Württemberg. Myotis **25,** 91–94.

Nagel, A.; Nagel, R. (1994): Activity of hibernating bats in their natural habitat recorded by automatic data logging. Bat Res. News **35,** 37.

Nagel, A., Nagel, R. (1997). Nutzung eines Untertagequartieres durch die Kleine Hufeisennase (*Rhinolophus hipposideros*). In: Zur Situation der Hufeisennasen in Europa. Ed. by Arbeitskreisirs Fledermäuse Sachsen-Anhalt e.V. Berlin: IFA Verlag. Pp. 97 – 108.

Průcha, M.; Hanzal, V. (1989): Some aspects of hibernation of bats wintering in the Bohemian karst (Central Bohemia, Czechoslovakia). Acta Univ. Carol. Biol. **33,** 315–333.

Řehák, Z. (1997): Long-term changes in the size of bat populations in Central Europe. Vespertilio **2,** 81–96 (in Czech).

Řehák, Z.; Baroň, I. (2001): Detectoring of flight activity of Lesser horseshoe bats, *Rhinolophus hipposideros*, at a cave entrance over a year. Nietoperze **2,** 202–208.

Řehák, Z.; Gaisler, J. (1999): Long-term changes in the number of bats in the largest man-made hibernaculum of the Czech Republic. Acta Chiropterol. **1,** 113–123.

Řehák, Z.; Zukal, J.; Kovařík, M. (1994): Long- and short-term changes in the bat community of the Kateřinská cave (Moravian karst)—fundamental assessment. Folia Zool. **43,** 425–436.

Saint Girons, H.; Brosset, A.; Saint Girons, M. C. (1969): Contribution a la connaissance du cycle annuel de la chauve-souris *Rhinolophus ferrumequinum* (Schreber, 1774). Mammalia **33,** 357–374.

Sokal, R. R.; Rohlf, F. J. (1981): Biometry. New York: Freeman and Co.

Webb, P. I.; Speakman, J. R.; Racey, P. A. (1996): How hot is a hibernaculum? A review of the temperatures at which bats hibernate. Can. J. Zool. **74,** 761–765.

Whitaker Jr., J. O.; Rissler, L. J. (1993): Do bats feed in winter? Am. Midl. Nat. **129,** 200–203.

Zima, J.; Kovařík, M.; Gaisler, J.; ák, Z.; Zukal, J. (1994): Dynamics of the number of bats hibernating in the Moravan karst in 1983 to 1992. Folia Zool. **43,** 109–119.

Authors' addresses:
Jan Zukal and Hana Berková, Institute of Vertebrate Biology Academy of Sciences of the Czech Republic, Kvetna 8, 603 65 Brno, Czech Republic
(e-mail: zukal@brno.cas.cz)
Zdeněk Řehák, Department of Zoology and Ecology, Faculty of Science, Masaryk University Brno, Kotlářská 2, 611 37 Brno, Czech Republic

Výběr úkrytů a letová aktivita netopýrů

Zukal, J. & Řehák, Z. (2006) Flight activity and habitat preference of bats in a karstic area, as revealed by bat detectors. Folia Zoologica 55, 273-281.

Folia Zool. – 55(3): 273–281 (2006)

Flight activity and habitat preference of bats in a karstic area, as revealed by bat detectors

Jan ZUKAL[1,2] and Zdeněk ŘEHÁK[1]

[1] *Institute of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic; e-mail: zukal@brno.cas.cz, rehak@sci.muni.cz*

[2] *Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic; e-mail: zukal@brno.cas.cz*

Received 17 February 2006; Accepted 25 August 2006

A b s t r a c t . The flight activity of bats was studied at 21 localities in the Moravian Karst (Czech Republic). From April to October, bat detectors were used to record echolocation calls of bats on line transects during the first half of the night. Nine habitats were distinguished. In total, 666 minutes of the presence of flying bats and at least 16 bat species were registered during 3387 transect minutes. *Myotis daubentonii* was the most numerous species (46.2%) The number of bat species was the highest in rocky habitats (13 species), and the lowest in agrocoenoses (3 species). The greatest intensity of flight activity of the bat community was observed over ponds (35.0 min+/h) and streams (26.6 min+/h). With respect to habitat preference, *M. mystacinus/brandtii*, *M. myotis/blythii*, *Eptesicus serotinus*, *Nyctalus noctula*, *Pipistrellus pipistrellus,* and *Plecotus auritus/austriacus* appear to be eurytopic and *M. daubentonii*, *M. nattereri* and *M. emarginatus* to be stenotopic species.

Key words: Moravian Karst, echolocation calls, bat community, detectoring, line transect

Introduction

During the past decade, an increased number of articles was published on the habitat preference and activity of bat communities (W a l s h & H a r r i s 1996, v o n Z a h n & M a y e r 1997, G a i s l e r et al. 1998). This is due to the expansion of ultrasound detector use in field research, including studies in such highly diversified landscapes as karstic regions. While the use of bat detectors has become established the standard methods of carrying out research in bat activity (A h l é n & B a a g o e 1999) it has its intrinsic technical (different types of signal transformation) and ecological (whispering bat species, direction of signals etc.) constraints which could more or less influence the study results (H a y e s 2000, G a n n o n et al. 2003). The most frequently designs used in field research include the transect method (G a i s l e r & K o l i b á č 1992, W a l s h & H a r r i s 1996, V e r b o o m 1998), which was adopted from ornithology, and/or the point method (W a l s h & M a y l e 1991, R a c h w a l d 1992, R y d e l l et al. 1994).

Bats forage in various types of habitats, from forest habitats up to villages, and the spatial distribution of bat activity is mainly determined by the distribution of their prey (R y d e l l 1992). Habitat selection is also influenced by the ability of various bat species to exploit these habitats, depending on their structure or their accessibility (d e J o n g 1995, V e r b o o m 1998, K u s c h et al. 2004). Among insectivorous bats, the following five foraging strategies based on wing morphology and structure of echolocation calls (N o r b e r g & R a y n e r 1987, F e n t o n 1990) can be identified: fast and slow aerial hawking, flycatching, trawling, and gleaning. Many bat species can use more than one

foraging technique. Most central European bat species forage by aerial hawking, often with the addition of one or more other techniques (N o r b e r g & R a y n e r 1987).

The bat fauna of the Moravian Karst is characterized by high density and diversity. So far, this phenomenon has only been explained by the large number of caves in this area (ca. 1200), but they are used by bats mainly as hibernacula (Z i m a et al. 1994). Nevertheless, there is a mosaic of various habitats under different anthropogenic impact and this fact permits the presence of a rich bat community even during the non-hibernating period.

The purpose of this study was to obtain data on the flight activity of bats during the non-hibernating period in various habitats of the karstic area under study. The landscape of the Moravian Karst is subject to management as part of the agricultural, hydrological and recreational demands of tourism. By assessing the relative importance of the particular habitats for bat distribution, it should be possible to improve the landscape management system in favour of the bats.

Material and Methods

The flight activity of bats was studied in 21 localities in all parts of the Moravian Karst area (Central Moravia, Czech Republic) (Table 1). The size of this karstic area is 85 km^2. Nine habitat types were distinguished for the comparison of bat activity: fields (fi), meadows (m), linear landscape elements (l), villages (v), rocks (r), forests (fo), edges of forest (e), streams (s), and ponds (p).

Bat detectors (Pettersson Elektronik, D100 and D980) were used to record echolocation calls of bats on the line transects. The transects were mostly carried out during the first half of the night from April to October of 1992–1994. The number of minutes during which a particular species or two sibling species was registered, related to 1 hour of transect (min+/h), was used as the measure of bat activity (M c A n e y & F a i r l e y 1988).

Table 1. Summary of monitoring activity in particular habitats.

Habitats	Number of monitoring minutes (min)	Number of positive minutes (min+)	Dominance of positive minutes (%)
rocks	615	79	11,9
forests	515	52	7,8
linear landscape elements	484	38	5,7
streams	470	206	30,9
edges of forest	382	39	5,9
villages	359	67	10,1
ponds	238	163	24,5
fields	176	8	1,2
meadows	148	14	2,1
Sum	3387	666	100,0

With regard to the difficult determination of some of the bat species in the field, the transects were conducted simultaneously by two researchers using two detectors. Where appropriate, we made records for comparison with reference records (A h l é n 1987). Foraging behaviour was also included in the process of species identification when we tried to catch flying bats in the beam of a halogen lamp.

In order to group bat species that use similar foraging habitats, we performed a cluster analysis using the complete linkage method (Euclidean distance) (Z a r 1984). The same type of clustering (complete linkage) was also applied to the matrix of Renkonen's index of dominance similarity (dominance of particular bat species) to compare the habitat types under study. In order to provide a comprehensive account of bat species-habitat association (bat species recorded for over 10 min+ in the total sample of 11 hours), the data on total bat activity in various habitats were subjected to canonical correspondence analysis.

Results

In total, 666 minutes of bat activity were registered by bat detectors during 3387 transect minutes (68 monitoring nights). At least 16 bat species were recorded, viz., *Rhinolophus hipposideros* (Rh), *Myotis mystacinus/brandtii* (Ms), *M. emarginatus* (Me), *M. nattereri* (Mn), *M. bechsteinii* (Mb), *M. myotis/blythii* (Mm), *M. daubentonii* (Md), *Vespertilio murinus* (Vm), *Eptesicus nilssonii* (En), *E. serotinus* (Es), *Nyctalus leisleri* (Nl), *N. noctula* (Nn), *Pipistrellus pipistrellus* (Pp), *P. nathusii* (Pn), *Barbastella barbastellus* (Bb), *Plecotus auritus/austriacus* (Pa). In field work, the two species of *Pipistrellus* i.e. *P. pipistrellus* and *P. pygmaeus* were not distinguished but later analyses revealed the presence of both species in the area under study, with the absolute predominance of *P. pipistrellus*.

The greatest intensity of flight activity of bats was observed over ponds (41.1 min+/h) and streams (26.3 min+/h) (Fig. 1). The interior of villages was another important habitat showing high flight activity of flying bats, above all, near streetlamps (11.2 min+/h). However, agrocoenoses lacking patches of trees or shrubs were poorly used by bats (2.7 min+/h).

The greatest intensity of flight activity was recorded in *M. daubentonii* (52.1 min+/h), especially at watersides (Fig. 2). Further species with relatively high activity included *Pipistrellus pipistrellus* (13.3 min+/h) and *Eptesicus serotinus* (11.6 min+/h), which are able to exploit a wider variety of habitats as foraging sites. On the other hand, *E. nilssonii* and *Vespertilio murinus* represent very rare faunistic records of these bat species in the area.

Cluster analysis divided habitat types into four groups (Fig. 3). The first group consists of water habitats, showing the specific structure of the bat community which was dominated by *M. daubentonii*. Fields, being highly influenced anthropogenic habitat types, are used by bats only for sporadic passes. Like the villages, this habitat is specific and separated. However the villages are providing roosts and foraging sites for synanthropic bat species. Cluttered and semi-cluttered habitats with similar bat communities (mainly forest-dwelling species) formed the last group.

Comparisons of the flight activity of nine common bat species or species pairs (over 1% of total sample) in particular habitats separated into three clusters (Fig. 4). *E. serotinus* differs from other bat species in its greatest ability to exploit man-made environments (e.g. villages). Further two clusters comprise groups of bat species. *M. emarginatus*, *M. mystacinus/brandtii*, *M. myotis/blythii* and *Plecotus auritus/austriacus* forage in cluttered and semi-cluttered habitats. Finally, *M. daubentonii*, *M. nattereri*, *Nyctalus noctula* and *P. pipistrellus* show high levels of flight activity at watersides.

The habitat preference of nine common species or species pairs (over 10 min+ in the total sample) was assessed by canonical analysis (Fig. 5). The total level of flight activity in particular habitats was used for this analysis. The highest preference of a single habitat type is apparent for the following species: *M. daubentonii* (watersides, i.e. streams and ponds), *M. mystacinus/brandtii* (forest), *E. serotinus* (villages) and *N. noctula* (open habitats, i.e. fields). *M. myotis/blythii* shows relatively close affinity to narrow meadow belts and linear

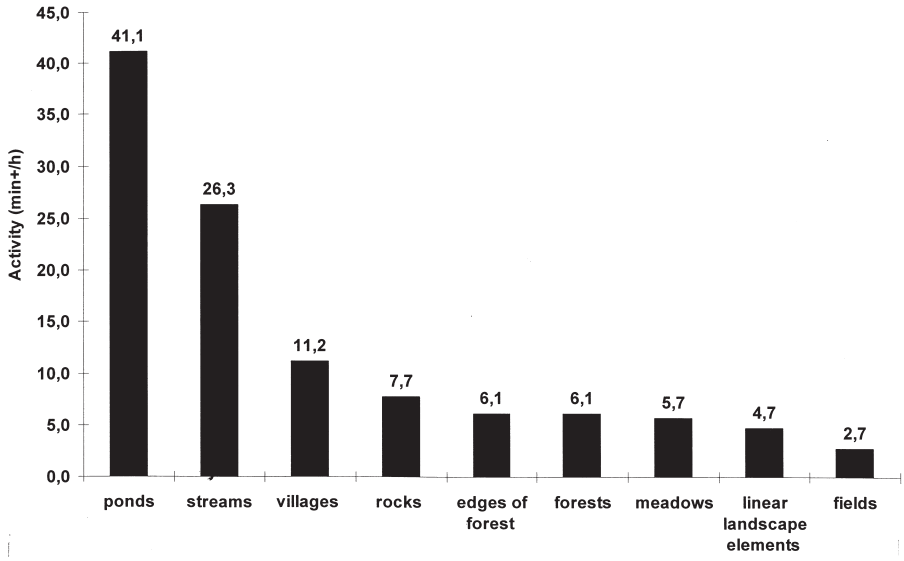


Fig. 1. Total intensity of bat flight activity (all species) in particular habitat types.

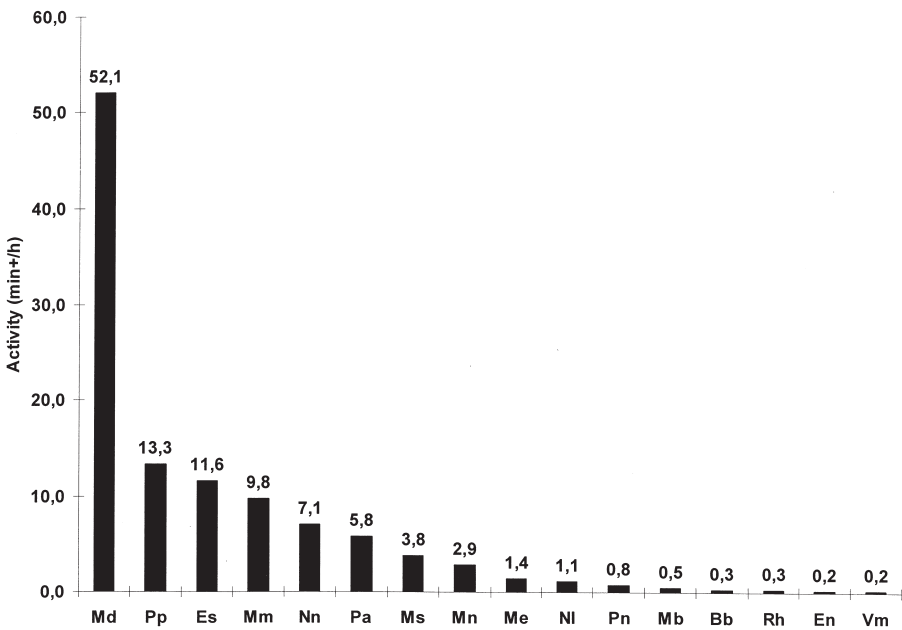


Fig. 2. Total intensity of the flight activity of particular bat species in the area under study. For abbreviation of bat species see chapter Results.

landscape elements. These two habitats may be considered semi-cluttered spaces for flying bats. Four species (*M. emarginatus, P. auritus/austriacus*, *M. nattereri,* and *P. pipistrellus*) show no strong habitat preference. Nevertheless, each of these bat species shows somewhat different requirements for foraging habitat.

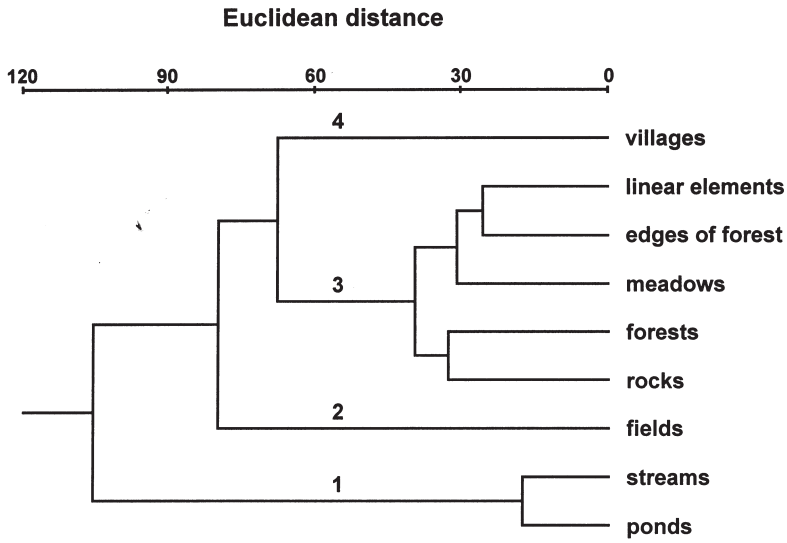


Fig. 3. Comparison of habitat types according to the dominance of particular bat species (cluster analysis – complete linkage).

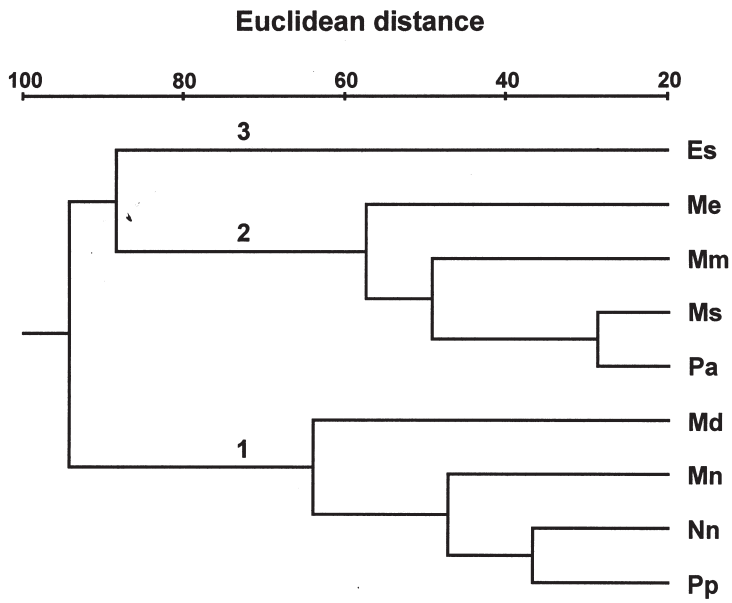


Fig. 4. Comparison of the nine common bat species or pair of species according to the distribution (in %) of their total flight activity in the particular habitats (cluster analysis – complete linkage). For abbreviation of bat species see chapter Results.

Discussion

The use of bat detectors, like any other research method, has its internal constraints. A major problem in bat community research is posed by correct species determination of echolocating bats (Walsh & Harris 1996, Gannon et al. 2003). It is influenced by many different

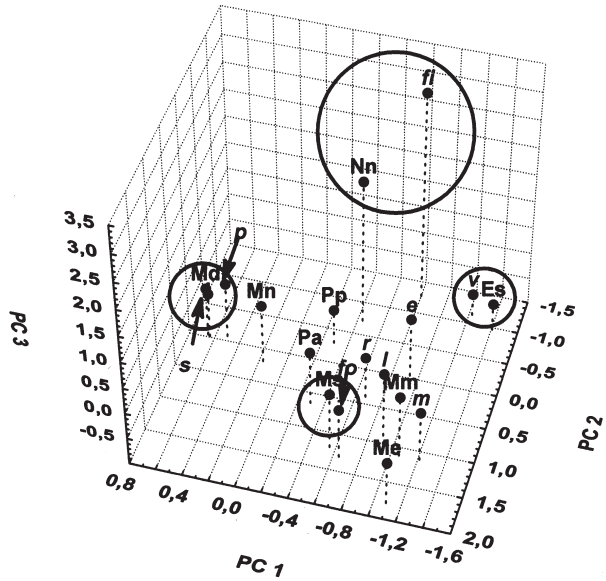


Fig. 5. Habitat preference of nine common species or couple of species according to the total flight activity (results of canonical analysis). For abbreviation of bat species see chapter Results.

factors, such as the duration of bat calls, presence of "whispering bat species", and experience of the researcher. Nevertheless, bat-detectors, mist nets and harp traps are the only useful research methods for study of the activity of whole bat communities, and detectors are the most widespread method used at present. The authors tried to minimize the impact of technical and ecological constraints on the results of study in various ways, e.g. by using the same types of bat detectors, grouping species with similar echolocation calls, using reference records, as well as long-term experience in using bat-detectors in the field. A less complicated situation is found in investigating the flight activity of only one bat species (Rachwald 1992, Catto et al. 1996, Robinson & Stebbings 1997) and/or the activity of a group of selected and readily distinguishable species (von Zahn & Maier 1997, Gaisler et al. 1998, Bartonička & Zukal 2003, Kusch et al. 2004).

Bats forage in various habitat types. Nevertheless, their preference for particular habitats depends on the presence of suitable food resources. The foraging activity of bats is affected by the distribution of prey not by the type of the habitat used (Brigham et al. 1992, Racey & Swift 1985, Warren et al. 2000, Kusch et al. 2004). Roosting requirements (number and distance of potential shelters) may also influence habitat utilization (Geggie & Fenton 1985). Many bat species forage only in the surroundings of their shelters. However, a habitat offering abundant food has no useful roosts in its vicinity the bats will seldom utilize it. On the contrary, if bats have the possibility of selecting from a large number of roosts they will choose those the closest to the food resources (Rydell 1989, Kusch et al. 2004). Bats prefer mainly diversified habitats and, on the contrary, their density is significantly lower in open landscape and/or inside dense forests (McAney & Fairley 1988, Kusch et al. 2004).

A preference for foraging in a single habitat type is very rare in insectivorous bats, as in general they use more habitat types, depending on actual food supply (Brigham et al. 1992), and they move between them during the night (Verboom & Spoelstra 1999).

The most important foraging areas include all types of water bodies, from small streams and ponds to larger rivers, canals and lakes, as confirmed by our results. Their attractiveness is due to the great biomass of insects swarming over the water surface. From this point of view, stagnant water bodies are preferred to running ones (Frenckell & Barclay 1987, Mackey & Barclay 1989). Riparian vegetation is also important, mainly where watercourses run through open landscape (Rydell et al. 1994, Zahn & Maier 1997). In open agricultural landscape, patches of woods and linear elements (e.g. windbreaks) are also highly important as reservoirs of insects. Similar habitat types are only suitable where the bats can obtain enough food in agrocoenoses (Gaisler & Kolibáč 1992). In the opposite, this type of habitats was utilised by bats minimally in variable area of the Moravian Karst. Lowland deciduous and mixed forests are preferred among forest habitats. In such forests, open forest edges and clearings are favourite foraging areas of bats (Mayle 1990, Limpens & Bongers 1991, Walsh & Mayle 1991, Rachwald 1992). The extent to which bats make use of man-made structures is expressed in their habitat preference. A number of bat species forage in villages mainly in the vicinity of streetlamps where a high density of insects was registered (cf. Furlonger et al. 1987, Rydell 1992).

Different bat species can utilize different foraging strategies, which also indirectly influences the selection of foraging habitats. There is a clear relationship between a bat functional design, i.e. flight morphology and structure of echolocation calls, and its commuting and foraging behaviour including the structure of used habitats (Fenton 1990, Verboom 1998). Wing morphology confers mechanical and energetical constraints on flight speed and manoeuvrability (Norberg & Rayner 1987). Echolocation signals have evolved to optimize foraging efficiency of various bat species in particular habitats.

M. daubentonii shows a strong association with watersides and its activity is extremely high there. It is a typical trawler, gaffing prey from the water surface or aerially hawking insects over the smooth water surface (Warren et al. 2000). Turbulent rapid streams are used rather more for commuting flights than for foraging, as this species often forages in groups and creates group foraging territories. *M. daubentonii* has a considerable overlap in the structure of calls with *M. nattereri* and there might be some misidentifications. Nevertheless, *M. nattereri* was detected mainly foraging higher over the water surface in the vicinity of the riparian vegetation (deJong 1995). Its activity would also be underestimated due to silent calls and/or if the calls had a poor signal to noise ratio when the calls of *M. nattereri* are masked by the loud signals of *M. daubentonii* foraging groups.

M. mystacinus/brandtii were the species most frequent recorded in forests, as in Sweden where *M. brandtii* used the coniferous forest more than expected and the deciduous woodland in proportion to its area (de Jong 1995). Generally, flight activity in forests is very low. *M. mystacinus/brandtii* also forage in the vicinity of water courses (von Zahn & Maier 1997) and they may be underestimated here, as with *M. nattereri*. The presence of both species i.e. *M. mystacinus* and *M. brandtii* in the region under study was documented by netting (Řehák et al. 1994). Together with *M. mystacinus/brandtii*, individuals of the genus *Plecotus* were often recorded in the forests. Most probably, these were to *P. auritus*, which prefers forest habitats (de Jong 1995) whereas records from gardens within villages probably pertain to the sibling species *P. austriacus* (Bauerová 1982). Similarly as in other pairs of sibling species, their echolocation calls cannot be differentiated in the field.

A relatively low activity was registered for *M. emarginatus*, a species foraging in cluttered and semi-cluttered habitats. This species behaved as a typical gleaner using edges of forests with a well-developed shrub layer adjacent to vertical rock walls surrounded by

shrubs. In these habitats, *M. emarginatus* will probably glean small arthropods, especially spiders (Bauerová 1986).

The species included in the *M. myotis/blythii* pair differ significantly in habitat preference and thus also in different prey selection – terrestrial (e.g. carabid beetles) vs. grass-dwelling (mostly bush crickets) (Arlettaz et al. 1997). Higher activity registered over the meadows should indicate the presence of *M. blythii* in the Moravian Karst. However, in the present study such sites were mostly narrow meadow belts surrounded by woods. In addition, only search calls were recorded there, and thus these bats may have been commuting specimens of *M. myotis*. During the past three years, telemetry has revealed that even *M. myotis* will forage in the open habitats of the Moravian Karst, including fields (Pokorný, Berková & Zukal, unpublished data). In addition, *M. blythii* is a very rare species in the area under study, recorded sporadically in the caves during hibernation and/or at cave entrances during autumn migrations (Řehák et al. 1994).

E. serotinus is able to exploit a wide range of habitats foraging mainly over streetlamps, in gardens as well as over ponds (Catto et al. 1996). As a typical semisynantropic species, *E. serotinus* differs from other bat species recorded in the Moravian Karst by the highest ability to exploit man-made environment (Verboom 1998). Only *N. noctula* is able to forage in similar habitats. Nevertheless, this species regularly used open habitats and was recorded flying very high over fields. At twilight, *N. noctula* often preys on swarming insects over the ponds but, later on, it appears in villages, catching prey in the vicinity of streetlamps (Rachwald 1992). This movement is influenced by the dwindling abundance of insects due to the falling ambient temperature in the natural habitats.

P. pipistrellus seems to be highly adaptive in foraging habitat preference in comparison with other bat species showing similar foraging strategies (Warren et al. 2000). *P. pipistrellus* forages both over water bodies and in riparian vegetation and, together with *E. serotinus* and *N. noctula*, use the parts of villages illuminated by streetlamps (Rydell 1992). Nevertheless, their activity is very low in very dense as well as in entirely open habitats (von Zahn & Maier 1997, Verboom & Spoelstra 1999).

Acknowledgement

The authors wish to thank Martin Pokorný for his help in field work. The technical support for the study was provided by grant No. MSM 0021622416.

LITERATURE

Ahlén I. 1987: European bat sounds transformed by ultrasound detectors – 25 species flying in natural habitats. *Tape. Univ. Agr. Sci. Uppsala.*

Ahlén I. & Baagoe H.J. 1999: Use of ultrasound detectors for bat studies in Europe: experiences from field identification, surveys, and monitoring, *Acta Chiropterologica 1: 137–150.*

Arlettaz R., Perrin N. & Hausser J. 1997: Trophic resource partitioning and competition between the two sibling bat species *Myotis myotis* and *Myotis blythii*. *J. Anim. Ecol. 66: 897–911.*

Bartonička T. & Zukal J. 2003: Flight activity and habitat use of four bat species in a small town revealed by bat detectors. *Folia Zool. 52: 155–166.*

Bauerová Z. 1982: Contribution to the trophic ecology of the grey long-eared bat, *Plecotus austriacus*. *Folia Zool. 31: 113–122.*

Bauerová Z. 1986: Contribution to the trophic bionomics of *Myotis emarginatus*. *Folia Zool. 35: 305–310.*

Brigham R.M., Aldridge H.D.J.N. & Mackey R.L. 1992: Variation in habitat use and prey selection by Yuma bats, *Myotis yumanensis*. *J. Mammal. 73: 640–645.*

Catto C.M.C., Hutson A.M., Racey P.A. & Stephenson P.J. 1996: Foraging behaviour and habitat use of the serotine bat (*Eptesicus serotinus*) in southern England. *J. Zool., London 238: 623–633.*

Fenton M.B. 1990: The foraging behaviour and ecology of animal-eating bats. *Can. J. Zool. 68: 411–422.*

von Frenckell B. & Barclay R.M.R. 1987: Bat activity over calm and turbulent water. *Can. J. Zool. 65: 219–222.*

Furlonger C.L., Dewar H.J. & Fenton M.B. 1987: Habitat use by foraging insectivorous bats. *Can. J. Zool. 65: 284–288.*

Gaisler J. & Kolibáč J. 1992: Summer occurrence of bats in agrocoenoses. *Folia Zool. 41: 19–27.*

Gaisler J., Zukal J., Řehák Z. & Homolka M. 1998: Habitat preference and flight activity of bats in a city. *J. Zool., London 244: 439–445.*

Gannon W. L., Sherwin R. E. & Haymond S. 2003: On the Importance of Articulating Assumptions When Conducting Acoustic Studies of Habitat Use by Bats. *Wildlife Society Bulletin 31: 45–61.*

Geggie J. F. & Fenton M. B. 1985: A comparison of foraging by *Eptesicus fuscus* (Chiroptera: Vespertilionidae) in urban and rural environments. *Can. J. Zool. 63: 263–267.*

Hayes J.P. 2000: Assumptions and practical considerations in the design and interpretation of echolocation-monitoring studies. *Acta Chiropterologica 2: 225–236.*

deJong J. 1995: Habitat use and species richness of bats in a patchy landscape. *Acta Theriol. 40: 237–248.*

Jüdes U. 1989: Erfassung von Fledermäusen im Freiland Mittels Ultraschall-detektor. *Myotis 27: 27–40.*

Kusch J., Weber C., Idelberger S. & Koob T. 2004: Foraging habitat preferences of bats in relation to food supply and spatial vegetation structures in a western European low mountain range forest. *Folia Zool. 53: 113–128.*

Limpens H. J. G. A. & Bongers W. 1991: Bats in Dutch forest. *Myotis 29: 129–136.*

Mackey R.L. & Barclay R.M.R. 1989: The influence of physical clutter and noise on the activity of bats over water. *Can. J. Zool. 67: 1167–1170.*

Mayle B.A. 1990: A biological basis for bat conservation in British woodlands – a review. *Mammal Review 20: 159–195.*

McAnney M.C. & Fairley J.S. 1988: Habitat preference and overnight and seasonal variation in the foraging activity of lesser horseshoe bats. *Acta Theriol. 33: 393–402.*

Norberg U.M. & Rayner J.M. 1987: Ecological morphology and flight in bats: wing adaptation, flight performance, foraging strategy and echolocation. *Phil. Trans. R. Soc. Lond. B 316: 335–427.*

Racey P.A. & Swift S.M. 1985: Feeding ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during pregnancy and lactation. I. Foraging behaviour. *J. Anim. Ecol. 54: 205–215.*

Rachwald A. 1992: Habitat preference and activity of the noctule bat *Nyctalus noctula* in the Bialowieza Primeval Forest. *Acta Theriol. 37: 413–422.*

Robinson M.F. & Stebbings R.E. 1997: Home range and habitat use by the serotine bat, *Eptesicus serotinus*, in England. *J. Zool., Lond. 243: 117–136.*

Rydell J. 1989: Feeding activity of the northern bat *Eptesicus nilssoni* during pregnancy and lactation. *Oecologia 80: 562–565.*

Rydell J. 1992: Exploitation of insects around street lamps by bats in Sweden. *Functional Ecology 6: 705–744.*

Rydell J., Bushby A., Cosgrove C. & Racey P.A. 1994: Habitat use by bats along rivers in North East Scotland. *Folia Zool. 43: 417–424.*

Řehák Z., Zukal J. & Kovařík M. 1994: Long- and short-term changes in the bat community of the Kateřinská cave (Moravian Karst) – a fundamental assessment. *Folia Zool. 43: 425–436.*

Verboom B. 1998: The use of edge habitats by commuting and foraging bats. *Dissertation Thesis, DLO Institute for Forestry and Nature Research (IBN-DLO), Wageningen, 121 pp.*

Verboom B. & Spoelstra K. 1999: Effects of food abundance and wind on the use of tree lines by an insectivorous bat, *Pipistrellus pipistrellus*. *Can. J. Zool. 77: 1393–1401.*

Walsh A.L. & Harris S. 1996: Foraging habitat preferences of vespertilionid bats in Britain. *Journal of Applied Ecology 33: 508–518.*

Walsh A.L. & Mayle B.A. 1991: Bat activity in different habitat in a mixed lowland woodland. *Myotis 24: 97–104.*

Warren R.D., Waters D.A., Altringham J.D. & Bullock D.J. 2000: The distribution of Daubenton's bats (*Myotis dabentonii*) and pipistrelle bats (*Pipistrellus pipistrellus*) (Vespertilionidae) in relation to small-scale variation in riverine habitat. *Biological Conservation 92: 85–91.*

von Zahn A. & Maier S. 1997: Jagdaktivität von Fledermausen an Bächen und Teichen. *Z. Säugetierkd. 62: 1–11.*

Zar J. H. 1984: Biostatistical analysis. *Simon and Schuster, Englewood Cliffs, New Jersey, USA.*

Zima J., Kovařík M., Gaisler J., Řehák Z. & Zukal J. 1994: Dynamics of the number of bats hibernating in the Moravian karst in 1983 to 1992. *Folia Zool. 43: 109–119.*

Výběr úkrytů a letová aktivita netopýrů

Zukal, J., Berková, H., Řehák, Z. & Kovařík, M. (2010) Activity and ecological parameters of bat hibernation in caves of the Moravian karst. Pp. 313-314 In: Horáček et al. A Tribute to Bats. Lesnické práce s.r.o., Praha.

Activity and ecological parameters of bat hibernation in caves of the Moravian karst

Jan Zukal, Hana Berková, Zdeněk Řehák and Miroslav Kovařík

CAS Institute of Vertebrate Zoology Brno, Czech Republic

The ecology and behaviour of temperate zone microchiropteran bats are fundamentally affected by seasonal changes in day length and associated climatic variables, which become more pronounced at increasing latitudes. These changes require flexible behavioural adjustments of their circadian as well as circannual activity patterns.

A characteristic feature of the annual cycle of insectivorous temperate zone bats is hibernation, as an optimal adaptation to a prolonged fall in temperature and reduction in prey availability. Selection of suitable hibernation site is crucial for overwinter survival and caves and mines are the most common type of hibernacula.

Our research conducted during last 15 years in several model caves in the central part of Moravian karst was aimed to various aspects of bat hibernation including the variation of flight activity of bats at the entrance of cave (seasonal and overnight) and different factors which influence it, the selection of places with specific parameters within a hibernaculum and the level of bat movement activity during hibernation period, the thermo-preferendum of various bat species etc.

Two major model species are studied i.e. mouse-eared bat *Myotis myotis* and lesser horseshoe bat *Rhinolophus hipposideros* by means of various research methods including visual census without any handling and marking of animals, as one of the main requirements of our research is to avoid any disturbance of the hibernating bats, automatic recording of activity with a double infrared-light logging system, census by night-vision scope, measurement of thermal parameters with non-contact thermometer.

The results published in a series of separate papers (see below) can be summarized as follows:

Based on the major pattern of the bat flight activity, five distinct periods can be distinguishes. All the periods showed a non-random temporal distribution and a concentration of flight activity around specific time and its level was influenced by climatic factors. However, the effect of individual variables and their contribution to variability in activity levels changed during the year.

(1) Hibernation period (half November – beginning of March), with very low activity. During hibernation, average temperature (T_{avg}) and the range of daily temperature ($T_{dif\ Max\text{-}Min}$) were the best predictors of the general level of activity. The percentage of nights on which activity occurred increased with increasing temperature. Activity occurred even at temperatures <0°C (T_{min} = -13.2°C). The recordings were all positive at $T_{max} \geq 6.2$°C. The activity within corresponding temperature groups was significantly lower during hibernation than during late hibernation. (2) Late hibernation (March – mid April), with intensive departure during the first quarter of the night. Flight activity was positively affected by T_{avg}, and negatively by minimal temperature of the preceding day. (3) Departure period (mid April – beginning of June), with emergence activity in the first quarter, and a small number of bats entering the cave in the fourth part of the night. The peak of activity was in the second part of the night. A significant positive relationship was found between total daily activity and T_{avg} and P_{avg} (mean barometric pressure). Rainfall in the preceding day caused drop in activity levels. (4) Summer period (beginning of June – mid/ end July), with low activity. Activity increased as $T_{dif\ Max\text{-}Min}$ increased and was suppressed by rainfall in the preceding day. In contrast, rainfall in the study day caused increase in activity. Differences were also apparent in the course of the night. (5) Autumn period (late July – half November), with very high activity and increasing number of bats entering the cave. The peak of activity was around midnight. The activity was positively related to T_{avg}, P_{avg} and amount of rainfall in the study day.

Hibernation is usually interrupted by periodical arousals. Such arousals may concern drinking, feeding (in mild periods), or even mating but switching hibernation site (i.e. leaving of used site) was not registered. Movement activity of bats inside of hibernaculum, expressed as percentage of new findings during a particular visit, was registered during the whole winter season.

Its level fluctuated in different ways and the hibernation period of *R. hipposideros* could be divided into three different parts (early, deep and late hibernation), while the level of *M. myotis* movement activity was relatively high during all season.

The shelter selection of *R. hipposideros* did not change during the season and it was not dependent on the part of cave where the bats were hibernating. Hibernating specimens of *R. hipposideros* most frequently used exposed places, in which they were always hanging free. *M. myotis* was registered in all types of shelter. High vulnerability of *R. hipposideros* to human activities was not registered as the specimens (continuously increasing number during the last 8 years) were able to hibernate next to frequently used footpath in the cave visited by speleotherapy patients. However, *R. hipposideros* is a highly specialized species which prefers parts of the cave with very stable microclimate conditions (stable temperature and humidity with minimal air flow) and, on the contrary, *M. myotis* appears to be indifferent to all parameters studied, and it uses the shelters indiscriminately.

The results of comparison of *Myotis myotis* hibernation in two natural caves indicate that the bats are using various strategies of hibernation (level of movement activity, preference of different types of shelters) in caves with different microclimatic profile (dynamic vs. stable). Additionally, the level of clustering behaviour is different (number, stability and size of clusters of hibernating bats). Used strategies are always tend to the same target i.e. use of roost place with maximum stable microclimate during the late part of hibernation period. High fidelity of bats to the particular underground shelter also suggests that accepted strategy of hibernation limits the bats in consecutive use of hibernacula.

References

Zukal J., Kovařík M., Řehák Z. & Berková H., 2001: Početnost netopýrů zimujících ve dvou jeskyních v severní části Moravského krasu a její dlouhodobé změny. Vespertilio 5: 321–328.

Pokorný M., Berková H., Gaisler J., Řehák Z. & Zukal J., 2003: Letní výskyt netopýrů v lidských stavbách v Moravském krasu a širším okolí. Vespertilio 7: 161–168.

Zukal J., Řehák Z. & Kovařík M., 2003: Netopýři Sloupsko - šošůvských jeskyní (Moravský kras). Lynx (Praha) n.s., 34: 205–220.

Berková H. & Zukal J., 2004: Sezónní změny letové aktivity netopýrů u vchodu do jeskyně zjištěné pomocí automatického monitorovacího systému. Vespertilio 8: 45–54.

Zukal J., Berková H. & Řehák Z., 2005: Activity and shelter selection by Myotis myotis and Rhinolophus hipposideros hibernating in the Kateřinská cave (Czech Republic). Mammalian Biology 70 (5): 271–281.

Berková H. & Zukal J., 2006: Flight activity of bats at the entrance of a natural cave. Acta Chiropterologica 8 (1): 187–195.

Zukal J. & Řehák Z., 2006: Flight activity and habitat preference of bats in a karstic area, as revealed by bat detectors. Folia Zoologica, 55(3): 273–281.

Berková H. & Zukal J., 2010: Cave visitation by temperate zone bats: effects of climatic factors. Journal of Zoology, 280: 387–395.

Výběr úkrytů a letová aktivita netopýrů

Berková H., Pokorný, M. & Zukal, J. (2014) Selection of Buildings as Maternity Roosts by Greater Mouse-eared Bats (*Myotis myotis*). Journal of Mammalogy 95, 1011-1017.

Journal of Mammalogy, 95(5):1011–1017, 2014

Selection of buildings as maternity roosts by greater mouse-eared bats (*Myotis myotis*)

Hana Berková,* Martin Pokorný, and Jan Zukal

Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, 60365 Brno, Czech Republic (HB, JZ)
Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic (MP, JZ)

* *Correspondent: berkova@brno.cas.cz*

Loss of roost sites in buildings represents the major threat to *Myotis myotis* populations in the Czech Republic. To identify features that may determine roost selection by *M. myotis*, we compared a range of structural and habitat variables for 17 maternity roosts and 17 unoccupied, but potentially suitable, buildings in the Moravian Karst (Czech Republic). Roosts and control sites were mainly in churches and chateaus and all were detached from the surrounding buildings and uninsulated. The only difference between habitat surrounding roost and control buildings was that roost buildings had relatively lower amounts of hedges as linear connective features. Our results suggest that bats do not select building features from among suitable detached and uninsulated churches and chateaus and that bats tend to select building roosts that are not connected to woodland by hedges. Protection of roosts is an important conservation issue for female *M. myotis* and suitable roost sites such as detached and uninsulated buildings that are not connected to woodland by hedges are important maternity roosting resources.

Key words: Chiroptera, habitat, linear landscape elements, maternity roosts, Moravian Karst, *Myotis myotis*

DOI: 10.1644/13-MAMM-A-102

During the summer, female greater mouse-eared bats (*Myotis myotis*) form nursery colonies, where they give birth and nurse their offspring. In the southern part of their European range, nurseries are found in underground cave roosts (Pandurska 1998; Benda et al. 2003; Rodrigues et al. 2003). In Central Europe, however, nursery colonies are typically found in buildings, especially those with large roof spaces such as church attics and castles (Horáček 1981; Gebhard and Ott 1985; Gaisler et al. 1988, 1990; Bilo 1990; Rudolph and Liegl 1990; Zahn 1999; Pokorný et al. 2003; Hanák and Anděra 2006).

Alterations to roost sites in buildings (mainly due to reconstruction of roof and attic spaces) can affect the survival of local bat populations (Hutson et al. 2001) and has been recognized as one of the major threats to *M. myotis* populations in the Czech Republic (Horáček and Uhrin 2010). The widespread use of buildings by bats in Central Europe and the increasing number of human–bat interactions suggest that an improved understanding of roost characteristics required by female *M. myotis* during the reproductive period is necessary for their conservation. Moreover, *M. myotis* is the European bat species with the highest white nose syndrome (WNS) prevalence (Pikula et al. 2012; Wibbelt et al. 2013) and maternity roosts should play an important role in WNS transmission (Puechmaille et al. 2011).

The availability of suitable roosting sites is a key factor in determining the distribution and limiting population size of bat species (Humphrey and Cope 1976). Various features may have an important role in the selection and use of a building roost site by bats. This may include specific structural attributes of buildings, such as number of exit points (Williams and Brittingham 1997), exit point size and height from the ground (Neubaum et al. 2007), the microclimate within the roost (Zahn 1999), an absence of insulation (Moussy 2011), as well as surrounding habitat characteristics and proximity of suitable foraging areas (Tuttle 1976), distance to similar roosts (Neubaum et al. 2007), level of disturbance (Rudolph and Liegl 1990), and/or risk of predation (Jenkins et al. 1998; Petrželková and Zukal 2003). As such factors have a key role in understanding the biology of *M. myotis*, it is important to assess the extent to which selection of roost sites depends upon

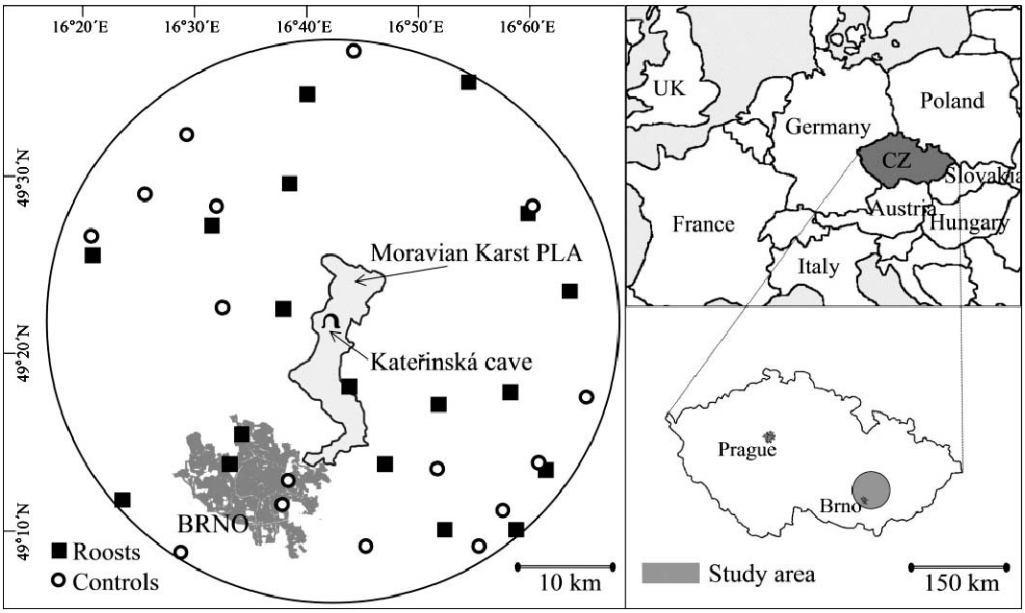


Fig. 1.—Study area and location of *Myotis myotis* maternity roosts and control buildings in the Moravian Karst Protected Landscape Area (PLA) and its surroundings, Czech Republic in 2001 and 2002. The right map shows the location of the study area in the context of Central Europe and the Czech Republic. The left map shows the distribution of *M. myotis* maternity roosts (■) and control buildings (○) within the study area.

characteristics of the buildings themselves or those of the adjacent landscape.

Several previous studies have investigated features of roosts occupied by female *M. myotis* (Stutz and Haffner 1983–1984; Rudolph and Liegl 1990; Zahn et al. 2006), but none has compared occupied and unoccupied buildings. The goal of this study was to investigate roosting preferences of female *M. myotis* by comparing construction features and local habitat characteristics at occupied and unoccupied buildings. We hypothesized that female *M. myotis* are selective in their choice of roost site. Although *M. myotis* have been widely reported as using spacious church attics and castles, we predicted that it is not just the type of building that is important but also its construction and location (a combination of the habitat features around the building). Specifically, we investigated which variables distinguish between occupied and unoccupied buildings.

Materials and Methods

Study area.—This study was undertaken in the Moravian Karst Protected Landscape Area and its surroundings, the largest karst region of the Czech Republic, which contains more than 1,000 natural limestone caves, many including hibernacula. We surveyed an area of 2,826 km^2 within a radius of 30 km around the Kateřinská Cave (49°21′N, 16°42′E), one of the most important bat hibernacula in the region (Zima et al. 1994). The study area was comprised mainly of arable land 42.5%; other cultivation patterns, pastures, and shrubs 10.1%; coniferous woodland 18.5%; mixed woodland 13.3%; broad-leaved woodland 6.6%, and artificial (urbanized) areas 8.6% (Fig. 1).

Methods.—Between May and August 2001, we checked 187 buildings of various types within a radius of 30 km around the Kateřinská Cave for occurrence of bat colonies. The 187 buildings were selected because they appeared suitable for *M. myotis* maternity colonies, and were comprised of 143 churches, 23 chateaus, and 21 other building types. We identified 17 maternity roosts of *M. myotis* (Fig. 1). Ten of these were found during our survey and 7 were known before 2001 (Pokorný et al. 2003). During the maternity season (between 14 May and 12 June) in 2002, we visited the roosts and control buildings again to confirm continued presence/absence of the bats and to assess approximate colony size.

We then randomly selected 17 buildings from the 170 that we surveyed but in which we found no bats. We measured a suite of structural attributes and surrounding habitat of the buildings. All variables we measured are listed in Table 1. Building characteristics were measured on site during the checks in 2002. Habitat characteristics were measured using a combination of CORINE land cover (c.f. Bossard et al. 2000) and aerial photographs and geographic information system (GIS) software ArcGIS (ESRI 2010).

Statistical analysis.—We examined differences in the frequency of structural variables between roost and control buildings using contingency tables. We used Spearman's correlation coefficient to assess the relationship between colony size and habitat variables for the 17 roost sites. To obtain a better understanding of multidimensionality in the adjacent habitat data, we carried out principal components analysis on the standardized habitat characteristics. To avoid pseudoreplication, 2 of the control buildings were eliminated from the habitat analysis as they were close to known roosts.

Table 1.—Building and habitat variables of *Myotis myotis* maternity roosts and control buildings in the Moravian Karst Protected Landscape Area and its surroundings, Czech Republic. Building characteristics were measured on site in 2002. Habitat characteristics were measured using a combination of CORINE land cover and aerial photographs and ArcGIS software.

Variables	Description
Building	
Type of building	Church, chateau, other
Reconstruction during last 10 years	Yes or no
Relative height of building[a]	Dwarfing surrounding houses or not
Isolation of building	Detached or nondetached
Exact location of the colony	Loft, church tower, or other
Orientation of broad sides of roof	N-S, W-E, NE-SW, NW-SE, or no orientation[b]
Construction material used for roof	Tile, shingle, or metal
Insulation	Yes or no
Height of the loft	< 5 m or > 5 m
Habitation by humans	Inhabited[c] or uninhabited
Surrounding habitat	
Distance to nearest open water	Meters
Distance to nearest block of woodland	Meters
Tree lines to woodland	Percentage of trees in the linear element connecting the building to the nearest woodland
Hedge lines to woodland	Percentage of hedges in the linear element connecting the building to the nearest woodland
Artificial areas including impervious surfaces of urban fabric	Percent cover within 3.5 km of the building
Arable land	Percent cover within 3.5 km of the building
Pastures, vineyards, orchards, meadows, and shrubs	Percent cover within 3.5 km of the building
Coniferous woodland	Percent cover within 3.5 km of the building
Broad-leaved and mixed woodland	Percent cover within 3.5 km of the building

[a] Compared with surrounding houses.

[b] Towers or dome-shaped roofs.

[c] Inhabited = buildings in continual use during the day.

Statistical differences between the roosts and control sites were assessed by comparing the values of the principal components (PC1–PC4) using the Mann–Whitney *U*-test and the *F*-test (to compare standard deviations of roost and control buildings—Manly 1994; Di Ciaccio 2012). We performed post hoc comparisons on any variables that had high weights (> 0.4) on significantly different PC axes using a Mann–Whitney *U*-test with Bonferroni adjustment ($P \leq 0.01$). All statistical analyses were undertaken using STATISTICA 12 (StatSoft, Inc. 2013).

Results

We recorded 3,962 *M. myotis* individuals at 15 localities between 14 May and 12 June 2002. At 2 roosts, bats could not be counted accurately as they were inaccessible (Řečkovice—estimated at 20 individuals; Plumlov—estimation impossible due to presence of fissures in which bats were hidden). Of the 17 sites included in the survey, 5 colonies (including Plumlov) were classified as small ($n < 100$ adults), 6 as medium ($n =$ 101–300 adults), and 6 as large ($n > 300$ adults).

Nine of the roosts were in churches, 7 in chateaus, and 1 in an old parish house. Roosting sites were usually located in lofts (76.5%). Three sites were in church towers, and in 1 roost bats were found hidden in fissures in the wall inside the building. Thirteen of the control buildings were churches, 3 chateaus, and 1 school. All buildings were detached, with most of them (roosts 88.2%, controls 64.7%) being higher than the surrounding houses and uninhabited (both roosts and controls 88.2%). During the last 10 years, 64.7% of roost buildings and 41.2% of control buildings were reconstructed. The roofs of 50.0% of the 14 occupied buildings (3 church towers not included) had their broad sides oriented east–west, 28.6% north–south, and 7.1% northwest–southeast. The roofs of 70.6% of controls were oriented east–west and 17.6% northeast–southwest. The roofs of 14.3% of the roost buildings and 11.8% of controls were dome shaped with no orientation. The most common roof construction materials were ceramic tiles (roosts 64.7%, controls 82.4%) and metal (roosts 29.4%). Roof spaces of all buildings were uninsulated. The space available under the roof was higher than 5 m in 64.3% of roosts and 64.7% of controls. There were no significant differences in structural features between roosts and control buildings (Table 2).

Table 2.—Comparison of structural variables at 17 *Myotis myotis* maternity roosts and 17 control buildings in the Moravian Karst Protected Landscape Area and its surroundings, Czech Republic.

Variable	χ^2	*P*	*d.f.*
Type of building	2.327	0.312	2
Reconstruction during last 10 years	1.888	0.169	1
Relative height of building	2.615	0.106	1
Isolation of building[a]			
Orientation of broad sides of roof	9.111	0.058	4
Construction material used for roof	3.360	0.186	2
Insulation[a]			
Height of the loft	0.001	0.981	1
Habitation by humans	0	1	1

[a] Not tested (all maternity roosts and control buildings were detached and not insulated).

Table 3.—Eigenvalues of the first 4 principal components (PC1–PC4), percentage of explained variance, factor loadings, results of the Mann–Whitney *U*-test (comparing *Myotis myotis* maternity roosts and control buildings in the Moravian Karst Protected Landscape Area and its surroundings, Czech Republic), and *F*-test results (comparing standard deviations of roosts and controls).

	PC 1	PC 2	PC 3	PC 4
Eigenvalue	2.59	1.65	1.56	1.03
% Total variance	28.82	18.28	17.32	11.41
Factor loadings—variable:				
Distance to open water	0.409	−0.261	−0.542	0.242
% Artificial areas	0.246	0.660	−0.609	−0.134
% Arable land	0.698	−0.480	0.458	−0.115
% Other cultivation, pastures and shrubs	−0.624	−0.251	−0.577	−0.195
% Coniferous woodland	−0.725	−0.440	−0.232	−0.025
% Broad-leaved and mixed woodland	−0.505	0.420	0.392	0.420
Distance to woodland	0.833	0.071	−0.299	−0.020
Linear features: tree lines	−0.118	0.108	0.212	−0.846
Linear features: hedges	0.044	−0.679	−0.099	0.069
PC scores median: roosts	−0.441	0.533	0.241	0.071
PC scores median: control buildings	0.890	−0.475	−0.901	0.208
Mann–Whitney *U*-test				
z	1.284	−2.039	−1.662	0.906
P	0.199	0.041	0.097	0.365
F-test				
$F_{16,14}$	1.696	3.235	2.040	3.694
P	0.310	0.027	0.173	0.018

The first 4 PCs accounted for 75.84% of total variance in the habitat data set (Table 3). PC1 was positively associated with percent cover of arable land and distance to woodland, and was negatively associated with percent cover of coniferous woodland, other cultivation, pastures and shrubs, and broad-leaved and mixed woodland. PC2 was positively associated with the percent cover of artificial areas and the percent cover of broad-leaved and mixed woodland, and was negatively associated with percent cover of coniferous woodland, percent cover of arable land, and the percentage of hedges in the linear landscape features. PC3 was positively associated with percent cover of arable land, and was negatively associated with percent cover of artificial areas and percent cover of other cultivation, pastures, and shrubs within a 3.5-km radius, and distance to open water. PC4 was positively associated with percent cover of broad-leaved and mixed woodland and was negatively associated with percentage of trees in the linear landscape features.

The only PC axis that differed between roost and control buildings was PC2 (Table 3). When comparing roost and control buildings for variables that had high PC loadings, we found no difference for the percent cover of artificial areas ($z = -0.680$, $P = 0.497$), percent cover of broad-leaved and mixed woodland ($z = -0.944$, $P = 0.345$), percent cover of coniferous woodland ($z = 1.038$, $P = 0.299$), or percent cover of arable land ($z = -0.566$, $P = 0.571$). The only difference we found was that percentage of hedges in the linear landscape elements connecting the building to the nearest woodland was lower for roost buildings than for control buildings ($z = -2.720$, $P = 0.007$; roosts: median = 0%, range 0–16.4%, controls: median = 10.2%, range 0–37.8%). In addition, the standard deviation differed between roosts and control buildings for PC2 and PC4. Control buildings were more variable than roost buildings on PC2, whereas the opposite was true for PC4 (Table 3). Post hoc *F*-tests on variables that had high PC2 loadings showed that controls were more variable in the percent cover of artificial areas ($F_{14,16} = 4.843$, $P = 0.004$) and the percentage of hedges as linear landscape features ($F_{14,16} = 6.533$, $P = 0.001$). We found no difference between roosts and controls when comparing individual variables that had high PC4 loadings with *F*-tests: percent cover of broad-leaved and mixed woodland ($F_{16,14} = 2.050$, $P = 0.184$) and percentage of trees as linear landscape features ($F_{16,14} = 1.430$, $P = 0.507$).

We found no significant relationship between colony size and habitat variables at any of the 17 roosts (Spearman's rank correlation; Table 4).

Discussion

In general, the features of buildings containing *M. myotis* maternity colonies in our study were consistent with the results of previous studies (Stutz and Haffner 1983–1984; Rudolph and Liegl 1990; Rodrigues et al. 2003; Zahn et al. 2006). It is likely that the preference shown for spacious attics in uninhabited buildings (e.g., castles and churches) and church towers is connected with an absence of human disturbance (Rudolph and Liegl 1990). However, it may also be that inhabited buildings are usually kept in a better state of repair and lack suitable access points (Schofield 1996). Further, the larger space and height of attics probably offer wider temperature gradients and bats may take advantage of a variety of temperatures by moving within the attic (Humphrey and Cope 1976; Zahn and Henatsch 1998; Zahn 1999; Rodrigues et al. 2003). Although prevailing southerly roof exposures may be an artifact of the typical construction method of churches (i.e., east–west alignment), they may also influence temperatures in the roof space during the day, and help to retain heat, which benefits bats during the reproduction period. Indeed, high average roost temperatures are known to favor juvenile development (Zahn 1999) and survival (Audet 1990), with

Table 4.—Spearman rank order correlation between *Myotis myotis* colony size and habitat variables at the 17 occupied roosts in the Moravian Karst Protected Landscape Area and its surroundings, Czech Republic, during the maternity season, 14 May–12 June 2002.

Variable	r_s	*P*
Distance to woodland	−0.098	0.727
Linear features: tree lines	0.264	0.342
Linear features: hedges	−0.270	0.331
Distance to open water	0.050	0.859
% Artificial areas	−0.252	0.365
% Arable land	0.088	0.756
% Other cultivation, pastures, and shrubs	−0.148	0.598
% Coniferous woodland	0.136	0.628
% Broad-leaved and mixed woodland	−0.084	0.766

larger juvenile *M. myotis* at the end of summer having a higher survival rate over the winter.

Analyses of building variables provided no support for our hypothesis on the role of building structure in the selection of roost site. However, the absence of any difference between buildings containing maternity colonies and those unoccupied does not necessarily mean that *M. myotis* were not selective in their choice of roost site with respect to the structural attributes of the building. As we focused on *M. myotis* maternity roosts, our study was biased toward looking for certain types of buildings that were potentially suitable for nursery colonies. Consequently, the control sample probably also contains buildings that are available to bats as potential roosts in terms of the construction features monitored. This preselection makes statistical differences between roosts and controls difficult to detect.

However, not all potential roost sites may be suitable for long-term use by bats. Rates of survival and fecundity of bats using unsuitable sites may be inadequate to sustain a viable population (Brigham and Fenton 1986). Intensive roost investigation in the area under study revealed that 50.5% of 222 building roof spaces contained live bats (individuals or colonies) or fresh bat droppings (indicating that the building is accessible to bats and sometimes used by them) of 9 bat species, though only 7.7% of buildings were occupied by *M. myotis* nursery colonies (Pokorný et al. 2003). The rest were solitary males, individuals, or colonies of other species, or fresh droppings. In Germany, *M. myotis* roosts were found in 83% of 360 buildings investigated (churches and castles) in an area of 4,000 km^2; however, nursery colonies were only found at 22 locations (6.1%), the rest being roosts of solitary males (Zahn et al. 2006). In addition to male roosts, the other buildings might also represent alternative roosts, or suboptimal roosts inaccessible as *M. myotis* nursery colonies. A limiting factor for nursery colonies could potentially be the number or size of exit points (Neubaum et al. 2007; Williams and Brittingham 1997), which is difficult to assess in an unoccupied building, as we cannot observe bats using them.

Analyses of habitat variables provided limited support for our hypothesis on the role of surrounding habitat in the selection of roost site, suggesting that building occupation is unlikely to be random. Relative to controls, roost sites were only associated with a lower percentage of hedges as linear landscape features. In addition, this result is limited because PC2 explains only 18% of the variation in the multidimensional data set. Several studies have shown an importance of linear vegetation features and habitat continuity to bats (Limpens and Kapteyn 1991; Walsh and Harris 1996; Jenkins et al. 1998; Downs and Racey 2006; Moussy 2011). Studies that make a distinction between hedgerows and tree lines find positive associations with tree lines more often than with hedgerows, and suggest that landscape context may influence the use of hedgerows (Walsh and Harris 1996; Downs and Racey 2006). The strength of association between bats and linear features varies among species (Boughey et al. 2011). Tree lines probably provide more benefits to the bats than other linear elements. Apart from facilitating the orientation of bats, they are associated with high insect densities and appear to have a role in feeding in some species (Limpens and Kapteyn 1991; Downs and Racey 2006). Trees provide protection against wind and may provide cover from predators and allow bats to emerge earlier, thus prolonging evening foraging time and efficiency (Entwistle et al. 1996; Jenkins et al. 1998). The linear elements with relatively higher percentage of shrubs connecting unoccupied control buildings to the nearest woodland could probably not provide sufficient cover from aerial predators and/or wind during commuting to foraging areas. Controls were also more variable in the percentage of hedges as linear landscape features. However, when interpreting the biological importance of this variable for *M. myotis* we should take into account that the variable is interconnected with the percentage of trees in the linear element connecting the building to the nearest woodland, although the higher percentage of trees connecting roosts was not statistically significant, and the median values of the percentage of hedges for both roosts and controls were very low (representing 0.0% and 10.2%, respectively, of the length of the linear element connecting the building to the nearest woodland).

Despite differences in foraging strategies, woodland habitat is an important factor influencing roost selection and population density in many bat species (Walsh and Harris 1996; Entwistle et al. 1997; Moussy 2011), including *M. myotis* (Zahn et al. 2006). Zahn et al. (2006) found a correlation between *M. myotis* population density in southeastern Bavaria, Germany, and percent area of mixed forest within a 10-km radius of the roosts. Interestingly, we did not find any relationship between colony size and the percent cover of broad-leaved and mixed woodland, nor a selection of buildings with higher percent cover of broad-leaved and mixed woodland within a 3.5-km radius. Arlettaz (1996), however, characterized *M. myotis* as an opportunistic predator that maximizes its average rate of food intake by switching to habitats offering more abundant or profitable prey. The overall character of the surrounding landscape, as well as the way the land is managed (especially arable land), can have a strong influence on the distribution and abundance of preferred prey up to 3.2–8.7 km from the roost (average distance to foraging sites according to Drescher [2004] and Arlettaz [1995]) and, therefore, may play a significant role in the selection of roost sites. The landscape surrounding maternity roosts in the study area is probably diversified to such an extent that it does not have a significant effect on the choice of roost sites. Furthermore, maternity roosts of *M. myotis* are generally interconnected and members of a given colony may for a short time use other roosts in the vicinity of their own roost (Horáček 1981; Zahn 1998). What is more, they are capable of moving long distances (up to 25 km) from the nursery roost over a short period of time.

Maternity roost sites are critical to species persistence as they contain most of the adult females in a population and, presumably, all the young of the year (Threlfall et al. 2013). The majority of buildings suitable for *M. myotis* nursery colonies in the study area are in need of structural repair and

we are concerned that these roosts may soon disappear. Brigham and Fenton (1986) observed that, after eviction of *Eptesicus fuscus* from their maternity roosts, the bats were able to move short distances to new roosts but they then tended to produce fewer offspring. From the perspective of *M. myotis* conservation, therefore, significant changes to roost sites or exclusion of the colony from well-established roosts should be avoided.

Acknowledgments

We thank E. Jánová for field assistance, J. Gaisler for proofreading, and the reviewers for valuable comments on the manuscript. We are also grateful to all the property owners that allowed us access to their buildings. This study was supported by a grant from the Grant Agency of the Czech Republic (No. 506/12/1064).

Literature Cited

Arlettaz, R. 1995. Ecology of the sibling mouse-eared bats (*Myotis myotis* and *Myotis blythii*): zoogeography, niche, competition and foraging. Ph.D. dissertation, Faculté des Sciences, Université de Lausanne, Lausanne, Switzerland.

Arlettaz, R. 1996. Feeding behaviour and foraging strategy of free-living mouse-eared bats *Myotis myotis* and *Myotis blythii*. Animal Behaviour 51:1–11.

Audet, D. 1990. Foraging behaviour and habitat use by a gleaning bat, *Myotis myotis* (Chiroptera: Vespertilionidae). Journal of Mammalogy 71:420–427.

Benda, P., et al. 2003. Bats (Mammalia: Chiroptera) of the eastern Mediterranean. Part 3. Review of bat distribution in Bulgaria. Acta Societatis Zoologicae Bohemicae 67:245–357.

Bilo, M. 1990. Verhaltensbeobachtungen in einer Wochenstube des Mausohrs, *Myotis myotis* (Borkhausen, 1797). Nyctalus (N.F.) 3:99–118. [In German.]

Bossard, M., J. Feranec, and J. Otahel. 2000. CORINE land cover technical guide—addendum 2000. European Environment Agency, Copenhagen, Denmark.

Boughey, K. L., I. R. Lake, K. A. Haysom, and P. M. Dolman. 2011. Improving the biodiversity benefits of hedgerows: how physical characteristics and the proximity of foraging habitat affect the use of linear features by bats. Biological Conservation 144:1790–1798.

Brigham, R. M., and M. B. Fenton. 1986. The influence of roost closure on the roosting and foraging behaviour of *Eptesicus fuscus* (Chiroptera: Vespertilionidae). Canadian Journal of Zoology 64:1128–1133.

Di Ciaccio, A., M. Coli, and J. M. Angulo Ibanez. 2012. Advanced statistical methods for the analysis of large data-sets. Springer–Verlag, Berlin, Germany.

Downs, N. C., and P. A. Racey. 2006. The use by bats of habitat features in mixed farmland in Scotland. Acta Chiropterologica 8:169–185.

Drescher, C. 2004. Radiotracking of *Myotis myotis* (Chiroptera, Vespertilionidae) in South Tyrol and implications for its conservation. Mammalia 60:387–395.

Entwistle, A. C., P. A. Racey, and J. R. Speakman. 1996. Habitat exploitation by a gleaning bat, *Plecotus auritus*. Philosophical Transactions: Biological Sciences 351:921–931.

Entwistle, A. C., P. A. Racey, and J. R. Speakman. 1997. Roost selection by the brown long-eared bat *Plecotus auritus*. Journal of Applied Ecology 34:399–408.

ESRI. 2010. ArcGIS. Version 10.1. Environmental Systems Research Institute, Redlands, California.

Gaisler, J., Z. Bauerová, M. Vlašín, and J. Chytil. 1988. The bats of S-Moravian lowlands over thirty years: *Rhinolophus* and large *Myotis*. Folia Zoologica 37:1–16.

Gaisler, J., J. Chytil, and M. Vlašín. 1990. The bats of S-Moravian lowlands (Czechoslovakia) over thirty years. Acta Scientiarum Naturalium Brno 24:1–50.

Gebhard, J., and M. Ott. 1985. Etho-ökologishe Beobachtungen an einer Wochenstube von *Myotis myotis* (Borkh., 1797) bei Zwingen (Kanton Bern, Schweiz); Mitteilungen der Naturforschenden Gesellschaft in Bern (N.F.) 42:129–144. [In German.]

Hanák, V., and M. Anděra. 2006. Atlas of the mammals of the Czech Republic—a provisional version. V. Bats (Chiroptera)—part 2. Vespertilionid bats (Vespertilionidae—genus *Myotis*). Národní museum, Praha. [In Czech with a summary in English.]

Horáček, I. 1981. Population ecology of *Myotis myotis* in Central Bohemia (Mammalia: Chiroptera). Acta Universitatis Carolinae—Biologica:161–267.

Horáček, I., and M. Uhrin. 2010. A tribute to bats. Lesnická Práce, s.r.o., Kostelec nad Černými lesy.

Humphrey, S. R., and J. B. Cope. 1976. Population ecology of the little brown bat, *Myotis lucifugus*, in Indiana and north-central Kentucky. Special Publication No. 4. The American Society of Mammalogists, Stillwater, Oklahoma.

Hutson, A. M., S. P. Mickleburgh, and P. A. Racey (comp.). 2001. Microchiropteran bats: global status survey and conservation action plan. IUCN/SSC Chiroptera Specialist Group. IUCN, Gland, Switzerland and Cambridge, United Kingdom.

Jenkins, E. V., T. Laine, S. E. Morgan, K. R. Cole, and J. R. Speakman. 1998. Roost selection in the pipistrelle bat, *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae), in northeast Scotland. Animal Behaviour 56:909–917.

Limpens, H. J. G. A., and K. Kapteyn. 1991. Bats, their behaviour and linear landscape elements. Myotis 29:39–48.

Manly, B. F. J. 1994. Multivariate statistical methods. A primer. 2nd ed. Chapman & Hall, London, United Kingdom.

Moussy, C. 2011. Selection of old stone buildings as summer day roosts by the brown long-eared bat *Plecotus auritus*. Acta Chiropterologica 13:101–111.

Neubaum, D. J., K. R. Wilson, and T. J. O'Shea. 2007. Urban maternity-roost selection by big brown bats in Colorado. Journal of Wildlife Management 71:728–736.

Pandurska, R. 1998. Reproductive behaviour and conservation status of nursery colonies of *Myotis myotis* (Borkhausen, 1797) in Bulgaria. Myotis 36:143–150.

Petrželková, K. J., and J. Zukal. 2003. Does a live barn owl (*Tyto alba*) affect emergence behaviour of serotine bats (*Eptesicus serotinus*)? Acta Chiropterologica 5:177–184.

Pikula, J., et al. 2012. Histopathology confirms white-nose syndrome in bats in Europe. Journal of Wildlife Diseases 48:207–211.

Pokorný, M., H. Berková, J. Gaisler, Z. Řehák, and J. Zukal. 2003. Summer occurrence of bats in buildings in the Moravian Karst and its wider surroundings. Vespertilio 7:161–168. [In Czech with English summary.]

Puechmaille, S. J., et al. 2011. White-nose syndrome: is this emerging disease a threat to European bats? Trends in Ecology and Evolution 26:570–576.

Rodrigues, L., A. Zahn, A. Rainho, and J. M. Palmeirim. 2003. Contrasting the roosting behaviour and phenology of an insectiv-

orous bat (*Myotis myotis*) in its southern and northern distribution ranges. Mammalia 67:321–335.

Rudolph, B.-U., and A. Liegl. 1990. Sommerverbreitung und Siedlungsdichte des Mausohrs (*Myotis myotis*) in Nordbayern. Myotis 28:19–38. [In German.]

Schofield, H. W. 1996. The ecology and conservation biology of *Rhinolophus hipposideros*, the lesser horseshoe bat. Ph.D. dissertation, University of Aberdeen, Aberdeen, United Kingdom.

StatSoft, Inc. 2013. STATISTICA (data analysis software system). Version 12. www.statsoft.com.

Stutz, H.-P., and M. Haffner. 1983–1984. Maternity roosts of the mouse-eared bat *Myotis myotis* (Borkhausen, 1797) in the central and eastern parts of Switzerland. Myotis 21–22:180–184.

Threlfall, C. H., B. Law, and P. B. Banks. 2013. Roost selection in suburban bushland by the urban sensititve bat *Nyctophilus gouldi*. Journal of Mammalogy 94:307–319.

Tuttle, M. D. 1976. Population ecology of the gray bat (*Myotis grisescens*): factors influencing growth and survival of newly volant young. Ecology 57:587–595.

Walsh, A. L., and S. Harris. 1996. Foraging habitat preferences of vespertilionid bats in Britain. Journal of Applied Ecology 32:508–518.

Wibbelt, G., et al. 2013. Skin lesions in European hibernating bats associated with *Geomyces destructans*, the etiologic agent of white-nose syndrome. PLoS ONE 8:e74105.

Williams, L. M., and M. C. Brittingham. 1997. Selection of maternity roosts by big brown bats. Journal of Wildlife Management 61:359–368.

Zahn, A. 1999. Reproductive success, colony size and roost temperature in attic-dwelling bat *Myotis myotis*. Journal of Zoology (London) 247:275–280.

Zahn, A., and B. Henatsch. 1998. Bevorzugt *Myotis emarginatus* kühlere Wochenstubenquartiere als *Myotis myotis*? Zeitschrift für Säugetierkunde 63:26–31. [In German.]

Zahn, A., A. Rottenwallner, and R. Güttinger. 2006. Population density of the greater mouse-eared bat (*Myotis myotis*), local diet composition and availability of foraging habitats. Journal of Zoology (London) 269:486–493.

Zima, J., M. Kovařík, J. Gaisler, Z. Řehák, and J. Zukal. 1994. Dynamics of the number of bats hibernating in the Moravian karst in 1983 to 1992. Folia Zoologica 43:109–119.

Submitted 25 April 2013. Accepted 30 April 2014.

Associate Editor was Matina C. Kalcounis-Rüppell.

Výletová a návratová aktivita u vchodu do úkrytu

Berková, H. & Zukal, J. (2004) Sezónní změny letové aktivity netopýrů u vchodu do jeskyně zjištěné pomocí automatického monitorovacího systému. Vespertilio 8, 45-54.

Vespertilio 8: 45–54, 2004
ISSN 1213-6123

Sezónní změny letové aktivity netopýrů u vchodu do jeskyně zjištěné pomocí automatického monitorovacího systému

Hana Berková[1] & Jan Zukal[1, 2]

[1] *katedra zoologie a ekologie PřF MU, Kotlářská 2, CZ–611 37 Brno, Česko*
[2] *Ústav biologie obratlovců AV ČR, Květná 8, CZ–603 65 Brno, Česko*

Seasonal changes in flight activity of bats at the entrance of the Kateřinská cave revealed by an automatic monitoring system. Double infrared light barrier was used to monitor flight activity of bats at the entrance of a natural karstic cave (Kateřinská cave, Czech Republic). The system allowed discrimination between the bats leaving and entering the cave. Individual species could not be distinguished. How-ever, it is known from a previous study (Řehák et al. 1994) that *Myotis myotis*, *M. emarginatus*, *M. daubentonii*, *M. nattereri* and *M. bechsteinii* are the five most abundant bat species. Five periods were defined on the basis of the amount of bat flight activity: (A) Hibernation period (November – late March), with very low activity. (B1) Departure period 1 (late March – mid April), with intensive departures during the first quarter of the night. (B2) Departure period 2 (mid April – beginning of June), with departure activity in the first quarter, and a small number of bats entering the cave in the fourth quarter of the night. The peak of activity was in the second quarter of the night. (C) Summer period (mid June – mid July), with low activity. (D) Autumn period (late July – late October), with very high activity and increasing number of bats entering the cave. The peak of activity was around midnight. There was a positive correlation between the number of bat passes through the entrance and the outside ambient temperature, and a negative correlation between the number of passes and air pressure. Rain had no significant effect on the level of bat activity.

Bat flight activity, cave, IR light barrier, Moravian Karst

Úvod

Jeskyně Moravského krasu jsou významným a tradičně sledovaným zimovištěm netopýrů. Pozornost byla věnována především výzkumu uvnitř jeskyní (sledování dlouhodobého vývoje početnosti netopýrů, ale i sezónní dynamice a ekologii netopýrů během hibernace) (např. Gaisler 1975, Bauerová & Zima 1988b, Bauerová et al. 1989, Řehák et al. 1994, Zima et al. 1994). Výzkum letové aktivity netopýrů zahájil teprve v roce 1971 Gaisler (1973, 1975), který poprvé použil k odchytu nárazové sítě. Sítě byly exponovány před vchody jeskyní a nad říčkou v jižní části Moravského krasu. V 80. letech sledovali aktivitu netopýrů u vchodů jeskyní Hladomorna a Býčí skála Bauerová & Zima (1988a, b). Jejich publikované výsledky jsou souhrnem údajů z několika sezón. V 90. letech navázali na předchozí výzkumy Řehák a Zukal, kteří sledovali letovou aktivitu nejen u jeskynních vchodů, ale i na jiných biotopech (Řehák et al. 1994, Řehák 1995).

Použití sítí nebo odchytových klecí však může mít rušivý vliv na odchycené netopýry. Jednou odchycení netopýři se po vypuštění sítím vyhýbají (LaVal & LaVal 1980). Stres vyvolaný odchytem a manipulací s netopýry někdy může vést k opuštění lokality. Dochází tak k podhodnocení intenzity letové aktivity ve srovnání s přirozeným stavem (Kunz 1973). Odchytové metody jsou také druhově selektivní. Snáze ulovitelné jsou druhy s menší manévrovací schopností (Řehák

1995). Efektivita odchytu se také snižuje s rostoucí dobou expozice sítí. Intenzita letové aktivity v pozdějších nočních hodinách proto může být podhodnocena.

Cílem práce bylo sledovat letovou aktivitu ve vchodu do jeskyně a její změny během noci a roku bez rušení netopýrů a posoudit vliv klimatických faktorů na přirozenou letovou aktivitu netopýrů.

Popis lokality, materiál a metodika

Kateřinská jeskyně se nachází v severní části Moravského krasu při ústí Suchého žlebu do údolí Punkvy. Její zeměpisné souřadnice jsou 49° 21' s. š. a 16° 48' v. d., čtverec zoologického mapování ČR 6666.

Jediný vchod do jeskyně je orientován na jihozápad a leží v nadmořské výšce 345 m. Je tvořen vysokým „gotickým" portálem, nad nímž je skalní stěna. Vstupní část jeskyně, tzv. Předsíň, je uzavřena železnou branou, v jejíž horní části je vletový otvor 25×18 cm. Následuje asi 55 m dlouhá Chodba, na kterou navazují tři velké Dómy. Celá jeskyně je dlouhá cca 500 m.

Na základě odchytů do nárazových sítí bylo zjištěno, že *Myotis myotis*, *M. nattereri*, *M. bechsteinii*, *M. daubentonii* a *M. emarginatus* jsou eudominantními druhy společenstva netopýrů využívajícího vchod Kateřinské jeskyně (Řehák et al. 1994). Letová aktivita netopýrů u vchodu do jeskyně byla sledována v obdobích od 1. 10. 1997 do 6. 10. 1998 a od 31. 3. 1999 do 26. 10. 1999 v přibližně 14 denních intervalech. Z celkového počtu 45 nocí byla spolehlivá data získána pro 40 nocí. Kvůli technickým závadám monitorovacího zařízení, které byly způsobeny hlavně vysokou vlhkostí vzduchu v jeskyni, bylo vyřazeno 5 pozorování.

Na výletový otvor byla instalována infračervená průletová brána s datovým záznamníkem. Zařízení se skládá ze 2 emitorů infračerveného světla (IČ LED diod) a 2 fototranzistorů umístěných naproti sobě. Proletující netopýr přerušuje paprsky IČ světla. Podle pořadí přerušení paprsků lze rozlišit směr letu netopýra. Počty vletů a výletů byly ukládány do paměti počítadla a každou celou hodinu odečítány. Sledování byla zahájena vždy před západem Slunce a ukončena po východu Slunce.

Za míru letové aktivity je považován počet záznamů na počítadle (tj. počet vletů a výletů, případně jejich součet). Pro hodnocení letové aktivity v průběhu noci byla perioda mezi západem a východem Slunce rozdělena do čtyř stejně dlouhých úseků. Časové údaje jsou uváděny ve středoevropském čase (SEČ).

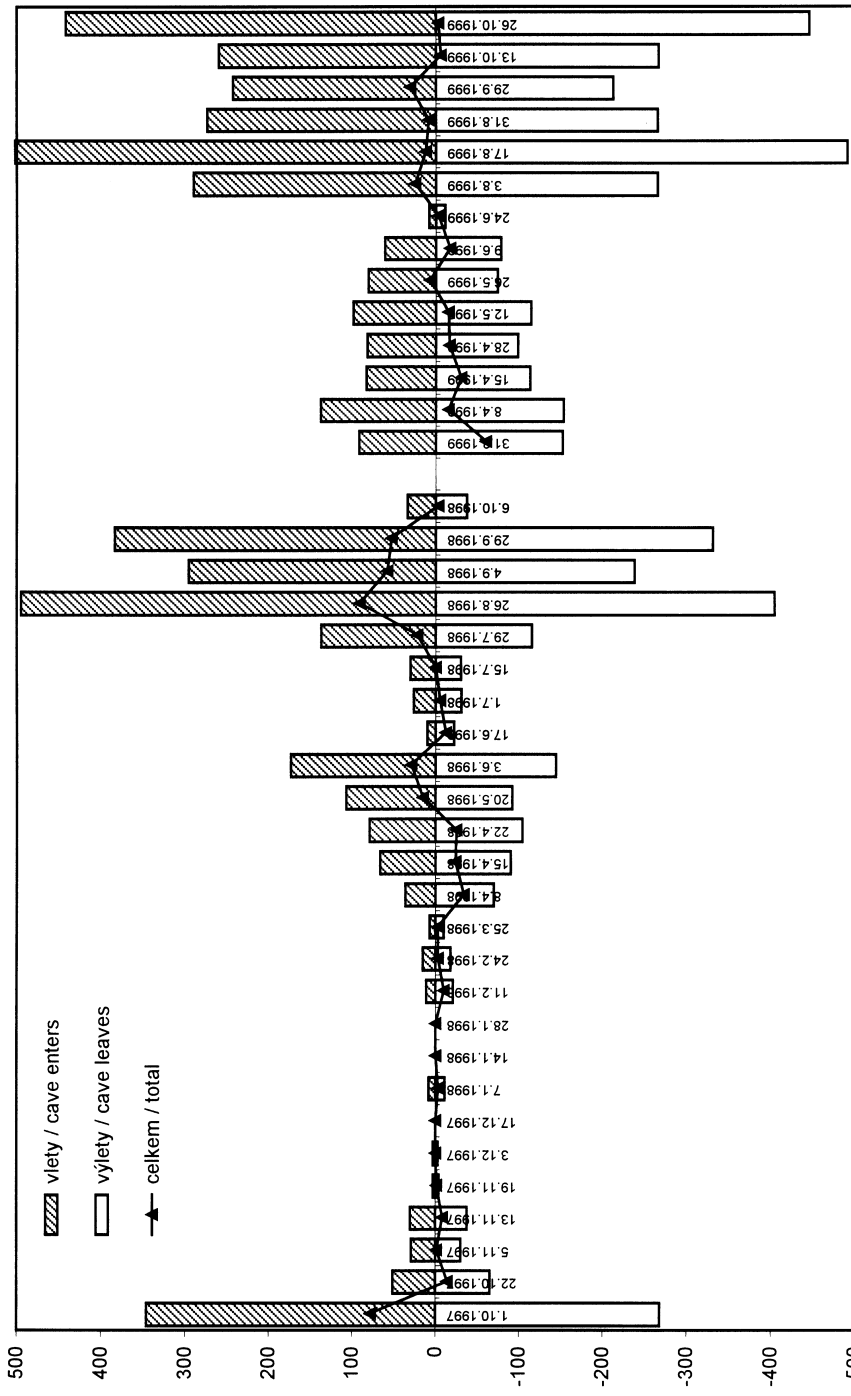
Byl testován vliv následujících klimatických faktorů na letovou aktivitu: T – průměrná teplota dne sledování, TLAK – průměrný atmosférický tlak dne sledování a stav srážek ve 2 stupních (0 – bez srážek, 1 – déšť). Použity byly údaje z meteorologické stanice v Bořitově (11 km severozápadně od Kateřinské jeskyně).

Jednotlivé noci byly na základě hodnot letové aktivity (součet vletů a výletů za noc) rozděleny pomocí shlukové analýzy (UPGMA, euklidovské vzdálenosti). Pomocí k-means shlukové analýzy bylo dále rozděleno jarní období (B) na dvě části na základě rozložení aktivity v průběhu noci (Berková & Zukal, in prep.). Protože hodnoty letové aktivity měly nenormální rozložení, byly při statistickém hodnocení použity neparametrické alternativy testů (Mann-Whitneyův test, Kruskal-Wallisův test). Korelační vztahy byly popsány pomocí Spearmanova koeficientu pořadové korelace. Jako kritická hranice zamítnutí hypotézy byla považována hodnota $p<0,05$. Výpočty byly prováděny s použitím programu Statistica for Windows 6.0.

Výsledky

Sezónní změny letové aktivity

Letová aktivita netopýrů u vchodu do jeskyně podléhá sezónním změnám (obr. 1). Podle výsledků shlukové analýzy (Berková & Zukal in prep.) a s ohledem na roční cyklus aktivity netopýrů byla vyčleněna 4 období: A: 5. 11. – 25. 3. s velmi nízkou až nulovou aktivitou během hibernace; B: 31. 3. – 9. 6. s vyšší aktivitou během jarních přeletů; C: 17. 6. – 15. 7. s nízkou aktivitou; D: 29. 7. – 26. 10. charakterizované vysokou letovou aktivitou. Rozdíly v letové aktivitě v jednotlivých obdobích jsou statisticky významné (Kruskal-Wallisův test: $H_3=31,052$, $p<0,0001$, $n=40$) (obr. 2).



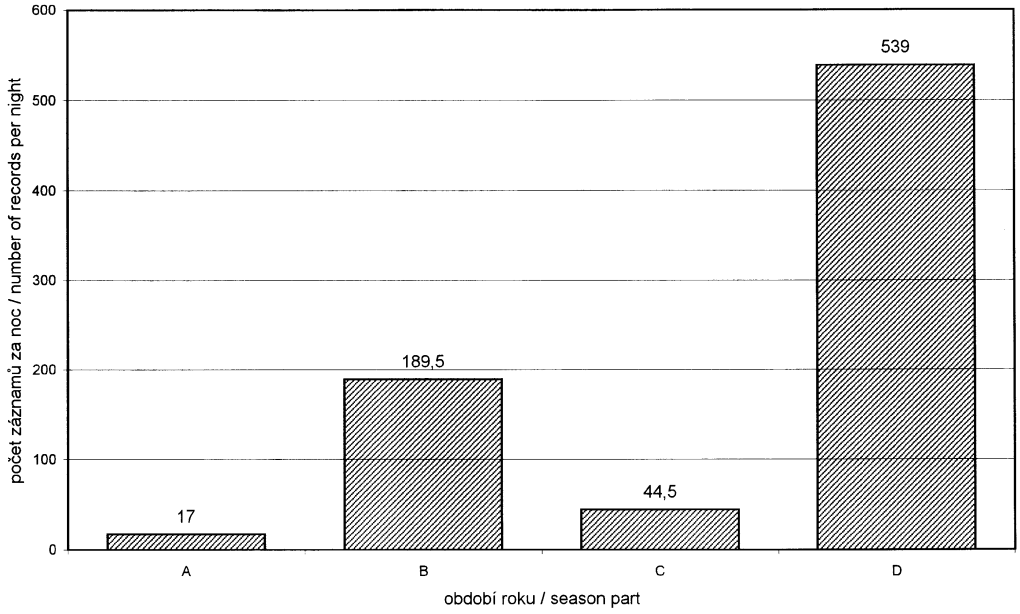
Obr. 1. Sezónní změny letové aktivity netopýrů v období říjen 1997 – říjen 1999.
Fig. 1. Seasonal variation in bat flight activity in the period October 1997 – October 1999.

Tab. 1. Změny letové aktivity v průběhu noci. Výsledky Kruskal-Wallisova testu (H), v závorce je uveden počet stupňů volnosti; p = pravděpodobnost; n = velikost vzorku
Tab. 1. Night variation in flight activity. Results of Kruskal-Wallis test (H), degrees of freedom are given in brackets; p = probability; n = sample size

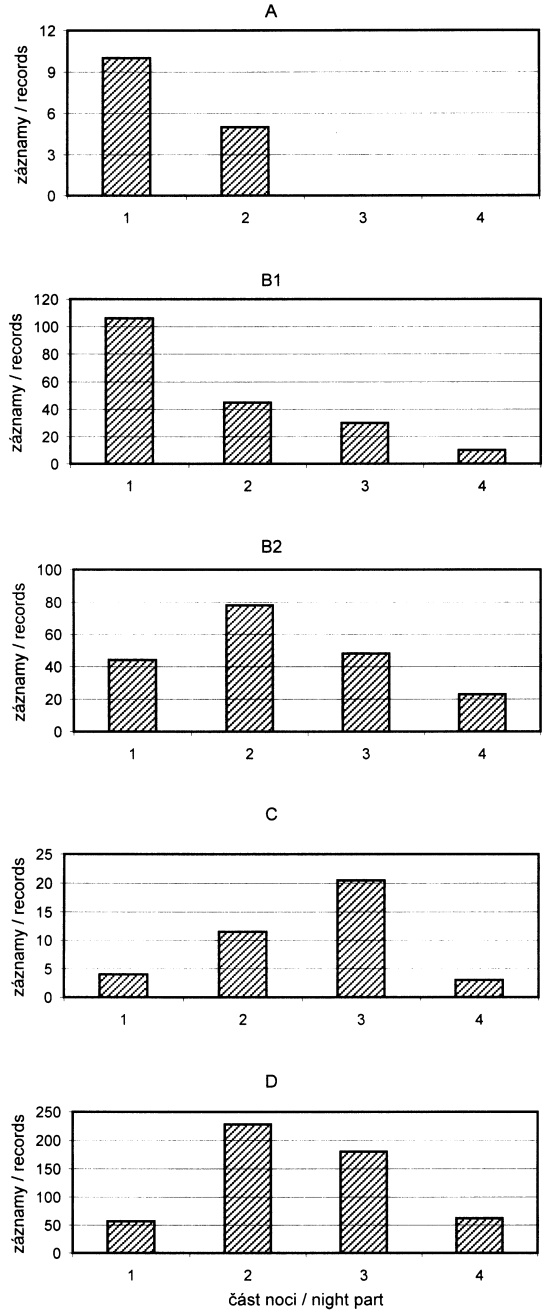
období / period	H (d.f.= 3)	p	n
A	6,287	0,099	44
B1	13,384	**0,004**	20
B2	15,588	**0,001**	28
C	5,891	0,117	16
D	18,978	**<0,001**	52

Letová aktivita v průběhu noci

Průběh noční letové aktivity netopýrů se během sezóny mění. V zimě (období A) a v létě (období C) není statisticky významný rozdíl v rozložení aktivity ve 4 částech noci. V jarních obdobích (B1 a B2) a na podzim (D) je tento rozdíl významný (Kruskal-Wallisův test, tab. 1). V období B1 (31. 3. – 15. 4.) je nejvyšší aktivita v první čtvrtině noci a pak klesá. Přičemž se hodnoty letové aktivity v 1. čtvrtině noci se významně liší od všech ostatních částí noci (Mann-Whitneyův test: 1 & 2: z= –2,611, p=0,009; 1 & 3: z= –2,611, p=0,009; 1 & 4: z= –2,611, p=0,009). Dále se od sebe liší části 2 a 4 (z= –2,193, p=0,028). V období B2 (22. 4. – 3. 6.) je aktivita rozložena více rovnoměrně s maximem ve 2. čtvrtině. Čtvrtá perioda se liší od všech předchozích částí noci



Obr. 2. Mediány letové aktivity netopýrů ve čtyřech obdobích roku (A až D).
Fig. 2. Medians of flight activity in four season parts (A to D).



Obr. 3. Mediány letové aktivity v jednotlivých čtvrtinách noci pro období roku A až D.
Fig. 3. Medians of flight activity in four quaters of the night in parts A to D.

a první čtvrtina se liší od druhé (4 & 1: z= –2,811, p=0,005; 4 & 2: z= –3,131, p=0,002; 4 & 3: z= –2,428, p=0,015; 1 & 2: z= –2,108, p=0,035). V období D je nejvyšší aktivita uprostřed noci. Části 2 a 3 se statisticky významně liší od částí 1 a 4 (2 & 1: z= –3,282, p=0,001; 3 & 1: z= –2,949, p=0,003; 2 & 4: z= –0,103, p=0,002; 3 & 4: z= –2,743, p=0,006) (obr. 3).

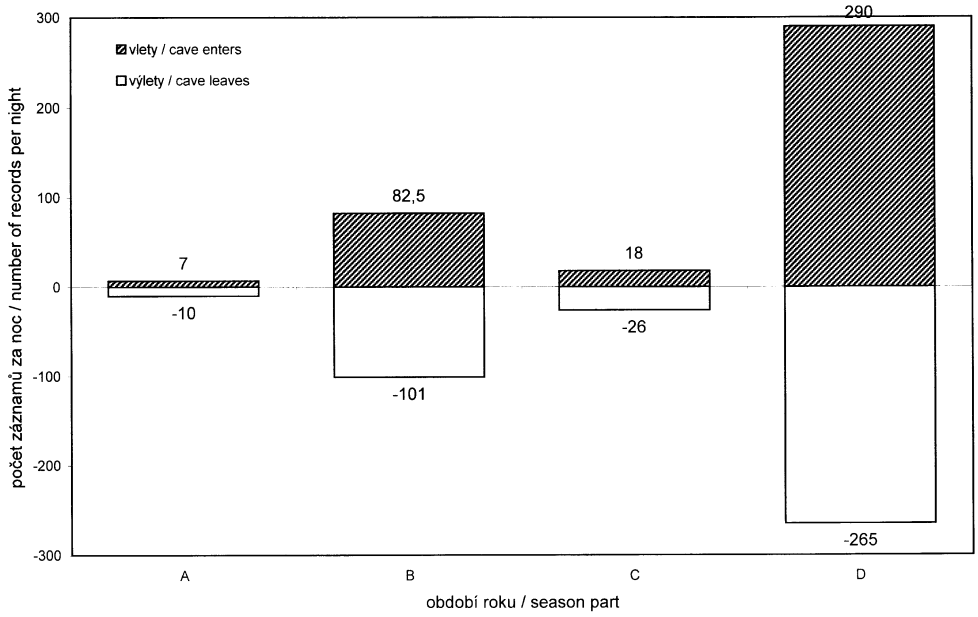
Vletová a výletová aktivita

Směr letové aktivity se mění v průběhu roku v návaznosti na životní cyklus netopýrů. Na jaře (období B) převažují výlety z jeskyně, na podzim (D) naopak vlety do jeskyně. V zimě a v létě (období A a C) je aktivita celkově velmi nízká, při kontrolách převažovaly výlety (obr. 4).

V období B1 dochází v 1. části noci k intenzivním výletům. Ve zbývajících částech je aktivita velmi nízká a převažují vlety. V období B2 je v 1. části noci poměrně vysoká výletová aktivita a v poslední periodě se část netopýrů vrací do jeskyně. V první části noci období C vylétává jen velmi malý počet netopýrů. V 2. části netopýři vlétávají do jeskyně a vylétávají ve 3. části. Na podzim (období D) netopýři přilétají v 2. čtvrtině noci a část z nich jeskyni ve 3. a 4. části opouští.

Vliv klimatických faktorů na letovou aktivitu

Pro testování vlivu klimatických faktorů jsou použity pouze hodnoty celkové aktivity (součet vletů a výletů) v jednotlivých nocích v období od 1. 10. 1997 do 26. 10. 1999. Aktivita netopýrů koreluje s průměrnou teplotou dne sledování (r_s=0,674, p<0,001, n=40). Dále byl zjištěn významný negativní vztah mezi aktivitou netopýrů a průměrnou hodnotou atmosférického tlaku v den pozorování (r_s= –0,359, p=0,023, n=40).



Obr. 4. Mediány vletové a výletové aktivity ve čtyřech obdobích roku (A až D).
Fig. 4. Medians of inflights and outflights in four season parts (A to D).

Po rozdělení dat do jednotlivých období (A až D) však nebyl zjištěn statisticky významný vztah mezi klimatickými faktory a letovou aktivitou.

Vliv srážek na letovou aktivitu byl testován pouze v období B (B1 a B2 dohromady) a D (velikost vzorku z období A a C byla nedostatečná). Bylo zjištěno, že déšť nemá na aktivitu netopýrů ve vchodu do jeskyně vliv (Mann-Whitneyův test: období B: z= –0,568, p=0,570; období D: z = –0,439, p=0,661).

Diskuse

Letová aktivita netopýrů u vchodu do Kateřinské jeskyně byla sledována pomocí infračervené průletové brány. Podobné přístroje byly použity ke sledování aktivity netopýrů na zimovištích např. v Nizozemí a Dánsku (Daan 1970, 1973, Degn et al. 1995). Jejich výhodou je, že neovlivňují přirozenou aktivitu netopýrů. Použitá metoda však neumožňuje rozlišit, které druhy netopýrů jsou aktivní, a proto lze infračervenou průletovou bránu kombinovat s detektorem ultrazvuku, fotoaparátem nebo videorekorderem. Použití blesku však ovlivňuje přirozené chování netopýrů (Daan 1970, Ransome 1990). Daan (1970) navíc připouští, že identifikace druhů na fotografiích není příliš spolehlivá. Počet záznamů na počítadle průletové brány můžeme považovat za míru letové aktivity, která však nemusí odpovídat počtu aktivních netopýrů. Někteří jedinci totiž mohou proletět výletovým otvorem několikrát tam a zpět.

Intenzita letové aktivity netopýrů u vchodu do Kateřinské jeskyně se mění v závislosti na období roku. Tyto změny souvisí s ročním cyklem netopýrů a jejich ekologickými nároky. Vzhledem k celkovým klimatickým poměrům oblasti nejsou jeskyně Moravského krasu vhodné pro letní kolonie samic a mláďat netopýrů a žádná taková kolonie také není z jeskyní Moravského krasu známa. Pro mnoho druhů netopýrů jsou však významnými zimovišti (Zima et al. 1994). Proto je maximum letové aktivity zaznamenáno v období jarních a zejména podzimních přeletů. Podobné sezónní změny v aktivitě netopýrů u vchodů do jeskyní a štol v ČR byly zaznamenány i při odchytech do sítí (Horáček & Zima 1978, Bauerová & Zima 1988a, Anděra et al. 1992, Řehák et al. 1994, Řehák 1995, Hanzal & Průcha 1996).

Na jaře (období B) dochází k intenzivním výletům ze zimoviště. V období B1 (brzké jaro) je nejvyšší aktivita v 1. čtvrtině noci, kdy z jeskyně vylétává velký počet netopýrů, pak úroveň aktivity klesá. Podobný průběh aktivity pozorovali např. Degn et al. (1995) a Řehák (1995). Aktivita je relativně vysoká až do poloviny června (období B2). Nejintenzivnější výlety probíhají opět v 1. čtvrtině noci, maximální aktivita je ale až ve 2. čtvrtině. V poslední části noci převažují vlety do jeskyně. Toto chování naznačuje, že jeskyně je využívána i jako přechodný úkryt při jarních migracích a pravděpodobně také samci v době, kdy samice již vytvářejí letní kolonie. Na zimovišti v Dánsku Degn et al. (1995) zaznamenali aktivitu také ještě v období od poloviny května do poloviny června. Na stejné lokalitě Degn (1989) zjistil, že tito netopýři byli samci *Myotis daubentonii*. Většina z nich navštívila důl pouze jedenkrát v daném období, takže se zřejmě jednalo o jakýsi druh přechodného úkrytu. Skiba (1987) pozoroval masový výlet *Myotis daubentonii* ze zimoviště ve štolách v západním Harcu v březnu a v dubnu. V této době, v rámci jarních migrací, přilétávali do štol netopýři z jiných zimovišť a později jimi byly štoly využívány jako přechodný úkryt.

Od poloviny června do konce července (období C) byla aktivita u vchodu Kateřinské jeskyně velmi nízká. V 2. části noci několik netopýrů vletuje do jeskyně a ve 3. části ji zase opouští. Tento typ aktivity nasvědčuje tomu, že malý počet jedinců pravděpodobně využívá jeskyni jako přechodný noční úkryt mezi vrcholy lovecké aktivity (Schofield 1996). Pro vchody jeskyní je v letním období charakteristické téměř výhradní zastoupení samců (Bauerová & Zima 1988a, Whitaker

& Rissler 1992a, Degn 1989, Řehák 1995). Dospělé samice žijí v této době v letních koloniích, kde se rodí a jsou odchovávána mláďata. Během období laktace se samice mezi vrcholy lovecké aktivity vracejí do mateřské kolonie a noční úkryty využívají jen sporadicky a krátce (Anthony et al. 1981).

Na konci července se aktivita začíná zvyšovat v souvislosti s rozpadem letních kolonií. U vchodů jeskyní se začínají objevovat adultní samice a juvenilní jedinci (Horáček & Zima 1978, Řehák 1995). Koncem léta a začátkem podzimu (období D) dosahuje aktivita maximálních hodnot. Vrchol letové aktivity je posunut až do doby kolem půlnoci a v druhé části noci netopýři vlétávají do jeskyně. Ve třetí a ve srovnání s ostatními obdobími i ve čtvrté části je aktivita ještě značně vysoká, nevýrazně převažují výlety. Hall & Brenner (1965, 1968) také zaznamenali během srpna nejvyšší aktivitu u jeskyně uprostřed noci (mezi 22:00 a 24:00 hodinou). Netopýři často přilétávali v malých skupinkách (2 až 12 jedinců). Každou noc vlétávala do jeskyně jiná skupina netopýrů a vylétávala před rozedněním, takže přes den se v jeskyni netopýři většinou nevyskytovali. Podobné chování netopýrů jsme zjistili v Kateřinské jeskyni (nepubl. údaje). Podzimní aktivita u vchodů jeskyní pravděpodobně umožňuje seznámení juvenilních jedinců s potenciálními zimovišti a setkání jedinců opačného pohlaví, kteří v letním období žijí odděleně (Fenton 1969, Cope & Humphrey 1977). Davis & Hitchcock (1965) vidí jeho význam v konečném výběru zimoviště.

Během listopadu aktivita klesá a do začátku února je téměř nulová. Velmi nízká zůstává až do konce března, jen zřídka dochází k výletům z jeskyně (období A). Přerušení letargie může být vyvoláno změnou podmínek vnějšího prostředí, fyziologickým stavem hibernujícího netopýra, případně přímým vyrušením (Speakman & Racey 1989, Thomas 1995). Aktivita netopýrů mimo úkryt během hibernačního období je částečně určená druhem netopýra a zejména klimatickými podmínkami, kterým jsou jedinci vystaveni (Daan 1973, Park et al. 1999). Netopýři mohou v průběhu zimy i přelétávat na jiná zimoviště (Ransome 1968, Bogdanowicz & Urbańczyk 1983, Masing 1987). V oblastech s mírnější zimou, kde se občas vyskytují periody s maximální denní teplotou přesahující 6 °C, mohou alespoň někteří jedinci některých druhů lovit (Ransome 1990). Speakman & Racey (1989) považují za primární funkci výletů z úkrytu doplnění vody.

Byla prokázána pozitivní korelace mezi letovou aktivitou netopýrů u vchodu do Kateřinské jeskyně a průměrnou teplotou dne sledování. Nízké průměrné denní teploty vysvětlují zařazení dvou podzimních pozorování (6. 10. 1998 a 22. 10. 1997) při shlukové analýze mezi zimní a jarní kontroly, kdy je aktivita nízká (Berková & Zukal in prep.). Dále byl zjištěn statisticky významný negativní vztah mezi aktivitou netopýrů a průměrnou hodnotou atmosférického tlaku v den pozorování (cf. Nagel & Nagel 1993). Řehák (1995) zjistil při odchytech do sítí před vchody jeskyní pozitivní korelaci mezi teplotou a počtem aktivních netopýrů jen u některých druhů (*M. myotis*, *M. emarginatus*, *M. bechsteinii* a *Barbastella barbastellus*). Druhy *M. nattereri* a *R. hipposideros* se běžně vyskytovali i při relativně nízkých teplotách. Prokázal také negativní vztah mezi aktivitou *M. myotis* a atmosférickým tlakem a pozitivní mezi aktivitou *R. hipposideros* a atmosférickým tlakem. Aktivita netopýrů u vchodu Kateřinské jeskyně byla zaznamenána i při teplotách nižších než 0 °C (cf. Ransome 1968, Skiba 1987, Whitaker & Rissler 1992b, Baroň 2000).

Vliv srážek na letovou aktivitu nebyl potvrzen. Fenton (1969) naopak zjistil znatelný vliv srážek na podzimní aktivitu netopýrů u jeskyně. V případě Kateřinské jeskyně má nepochybně vliv umístění vletového otvoru až za prostornou Předsíní, kde jsou netopýři před deštěm chráněni. Ani při odchytech do sítí (Řehák 1995) se totiž vliv srážek na aktivitě netopýrů v portálu neprojevil.

Poděkování

Autoři děkují Martinu Pokornému a Kláře J. Petrželkové, kteří se podíleli na práci v terénu a Davidu Motlovi za opravy průletové brány a záznamníku. Výzkum a zpracování dat bylo podpořeno granty MSM 143100010 a GA ČR 206/01/1555.

Literatura

ANDĚRA M., ZBYTOVSKÝ P. & BÜRGER P., 1992: Bat community of Chýnovská jeskyně cave (Southern Bohemia, Czechoslovakia) in 1981–1986. Pp.: 1–11. In: HORÁČEK I. & VOHRALÍK V. (eds.): *Prague Studies in Mammalogy*. Charles Univ. Press, Praha, 246 pp.

ANTHONY E. L. P., STACK M. H. & KUNZ T. H., 1981: Night roosting and the nocturnal time budget of the little brown bat, *Myotis lucifugus*: effects of reproductive status, prey density, and environmental conditions. *Oecologia*, **51**: 151–156.

BAROŇ I., 2000: *Sezónní změny v letové aktivitě vrápence malého* (*Rhinolophus hipposideros*) *ve vchodu jeskyně*. Diplomová práce. PřF MU Brno, 53 pp.

BAUEROVÁ Z., GAISLER J., KOVAŘÍK M. & ZIMA J., 1989: Variation in numbers of hibernating bats in the Moravian Karst: results of visual censuses in 1983–1987. Pp.: 499–505. In: HANÁK V., HORÁČEK I. & GAISLER J. (eds.): *European Bat Research 1987*. Charles Univ. Press, Praha, 720 pp.

BAUEROVÁ Z. & ZIMA J., 1988a: Seasonal changes in visits to a cave by bats. *Folia Zool*., **37**: 97–111.

BAUEROVÁ Z. & ZIMA J., 1988b: Výzkum netopýrů v jeskyni Býčí skála v letech 1977–1986. *Čs. Kras*, **39**: 51–59.

BERKOVÁ H. & ZUKAL J., in prep.: Flight activity of bats at the entrance of a natural cave. *Folia Zool.*

BOGDANOWICZ W. & URBAŃCZYK Z., 1983: Some ecological aspects of bats hibernating in city of Poznań. *Acta Theriol*., **28**: 371–385.

COPE J. B. & HUMPHREY S. R., 1977: Spring and autumn swarming behavior in the Indiana bat, *Myotis sodalis*. *J. Mammal*., **58**: 93–95.

DAAN S., 1970: Photographic recording of natural activity in hibernating bats. *Bijdr. Dierk*., **40**: 13–16.

DAAN S., 1973: Activity during natural hibernation in three species of vespertilionid bats. *Netherlands J. Zool*., **23**: 1–71.

DAVIS W. H. & HITCHCOCK A., 1965: Biology and migration of the bat, *Myotis lucifugus*, in New England. *J. Mammal*., **46**: 296–313.

DEGN H. J., 1989: Summer activity of bats at a large hibernaculum. Pp.: 523–526. In: HANÁK V., HORÁČEK I. & GAISLER J. (eds.): *European Bat Research 1987*. Charles Univ. Press, Praha, 720 pp.

DEGN H. J., ANDERSEN B. B. & BAAGØE H., 1995: Automatic registration of bat activity through the year at Monsted Limestone Mine, Denmark. *Ztschr. Säugetierk*., **60**: 129–135.

FENTON M. B., 1969: Summer activity of *Myotis lucifugus* (Chiroptera: Vespertilionidae) at hibernacula in Ontario and Quebec. *Can. J. Zool*., **47**: 597–602.

GAISLER J., 1973: Netting as a possible approach to study bat activity. *Period. Biol*., **75**: 129–134.

GAISLER J., 1975: A quantitative study of some populations of bats in Czechoslovakia (Mammalia: Chiroptera). *Acta Sci. Natur. Brno*, **9**(5): 1–44.

HALL J. S. & BRENNER F. J., 1965: A behavior of bats, not related to roosting, in the use of caves in summer. *Am. Zool*., **5**: 225.

HALL J. S. & BRENNER F. J., 1968: Summer netting of bats at a cave in Pennsylvania. *J. Mammal*., **49**: 779–781.

HANZAL V. & PRŮCHA M., 1996: Annual course of cave visitation by bats (Mammalia: Chiroptera) in the Bohemian karst (Czech Republic). *Acta Soc. Zool. Bohem*., **60**: 25–30.

HORÁČEK, I. & ZIMA, J., 1978: Net-revealed cave visitation and cave-dwelling on European bats. *Folia Zool*., **27**: 135–148.

KUNZ T. H., 1973: Resource utilization: temporal and spatial components of bat activity in central Iowa. *J.Mammal*., **54**: 14–32.

LaVal R. K. & LaVal M. L., 1980: *Ecological studies and management of Missouri bats*, *with emphasis on cave-dwelling species*. *Terrestrial Series No*. *8*. Missouri Department of Conservation, Jefferson City, 93 pp.

Masing M., 1987: Movements of bats between winter quarters. *Acta Comm*. *Univ*. *Tartu*., **15**: 56–60.

Nagel A. & Nagel R., 1993: Activity of hibernating bats in their natural habitat recorded by automatic data logging. Pp.: 37–38. In: Palmeirim J. M., Rodriguez L. & Rabaça J. (eds.): *VI European Bat Research Symposium*. *Évora*, *Portugal*, *22–27 August 1993*. *Programme*. *Abstracts*. *List of Participants*. European Bat Research Organization, 65 pp.

Park J. K., Jones G. & Ransome R. D., 1999: Winter activity of a population of greater horseshoe bats (*Rhinolophus ferrumequinum*). *J*. *Zool*., *Lond*., **248**: 419–427.

Ransome R. D., 1968: The distribution of the greater horseshoe bat, *Rhinolophus ferrumequinum*, during hibernation, in relation to environmental factors. *J*. *Zool*., *Lond*., **154**: 77–112.

Ransome R. D., 1990: *The Natural History of Hibernating Bats*. Christopher Helm, London, 226 pp.

Řehák Z., 1995: *Letová aktivita netopýrů v Moravském krasu*. Dizertační práce. PřF MU Brno, 184 pp.

Řehák Z., Zukal J. & Kovařík M., 1994: Long- and short-term changes in the bat community of the Kateřinská cave (Moravian karst) – a fundamental assessment. *Folia Zool*., **43**: 425–436.

Schofield H. W., 1996: *The ecology and conservation biology of Rhinolophus hipposideros*, *the lesser horseshoe bat*. PhD Thesis, University of Aberdeen, 197 pp.

Skiba R., 1987: Bestandsentwicklung und Verhalten von Fledermäusen in einem Stollen des Westharzes. *Myotis*, **25**: 95–103.

Speakman J. R. & Racey P. A., 1989: Hibernal ecology of the pipistrelle bat: energy expenditure, water requirements and mass loss, implications for survival and the function of emergence flights. *J*. *Animal Ecol*., **58**: 797–813.

Thomas D. W., 1995: Hibernating bats are sensitive to nontactile human disturbance. *J*. *Mammal*., **76**: 940–946.

Whitaker J. O. Jr. & Rissler L. J., 1992a: Seasonal activity of bats at Copperhead cave. *Proc*. *Indiana Acad*. *Sci*., **101**: 127–134.

Whitaker J. O. Jr. & Rissler L. J., 1992b: Winter activity of bats at a mine entrance in Vermillion county, Indiana. *Am*. *Midl*. *Natur*., **127**: 52–59.

Zima J., Kovařík M., Gaisler J., Řehák Z. & Zukal J., 1994: Dynamics of the number of bats hibernating in the Moravian Karst in 1983 to1992. *Folia Zool*., **43**: 109–119.

došlo 16. 10. 2004

Výletová a návratová aktivita u vchodu do úkrytu

Berková, H. & Zukal, J. (2006) Flight activity of bats at the entrance of a natural cave. Acta Chiropterologica 8, 187-195.

Acta Chiropterologica, 8(1): 187–195, 2006
PL ISSN 1508-1109 © Museum and Institute of Zoology PAS

Flight activity of bats at the entrance of a natural cave

HANA BERKOVÁ[1, 2] and JAN ZUKAL[1, 2]

[1]*Department of Zoology and Ecology, Faculty of Science, Masaryk University, Kotlářská 2, CZ-611 37 Brno, Czech Republic; E-mail: berkova@brno.cas.cz*
[2]*Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, CZ-603 65 Brno, Czech Republic*

Activity patterns of bats were investigated at the entrance of a natural karstic cave (Kateřinská cave, Czech Republic). The activity was recorded automatically with a double infrared light barrier allowing discrimination between those bats leaving and those entering the cave. Five periods were defined on the basis of bat flight activity: A) Hibernation period (November–late March), with very low activity; B1) Departure period 1 (late March–mid April), with intensive departure during the first quarter of the night; B2) Departure period 2 (mid April–beginning of June), with emergence activity in the first quarter, and a small number of bats entering the cave in the fourth part of the night. The peak of activity was in the second part of the night. C) Summer period (mid June–mid July), with low activity. D) Autumn period (late July–late October), with very high activity and increasing number of bats entering the cave. The peak of activity was around midnight. All periods showed a non-random temporal distribution and a concentration of flight activity around specific time. There was a positive correlation between the number of bat passes through the entrance and outside ambient temperature and a negative correlation between the number of passes and barometric pressure. Rain had no significant effect on the level of bat activity.

Key words: flight activity, caves, seasonal changes, IR light barrier

INTRODUCTION

The caves in the area of Moravian Karst belong to the most important hibernacula of bats in Europe. Although some of them have been studied for the longest time in the Czech Republic, the research has mainly been focused on the long-term monitoring of the numbers of bats (Zima *et al.,* 1994). A few studies on the seasonal dynamics and ecology of bats during the hibernation period have also been produced (Gaisler, 1970, Bauerová and Zima, 1988*b*, Řehák *et al.,* 1994). In 1971, J. Gaisler initiated the research of flight activity in the non-hibernation period, using mist nets to capture bats (Gaisler, 1973, 1975). In the 1980s, Bauerová and Zima (1988*a,* 1988*b*) carried out a mist netting study of bats at two cave entrances. However, their published results are a summary of data from several seasons. In the 1990s, Z. Řehák and J. Zukal continued the research of flight activity in different habitats, using mist nets and ultrasound detectors (Řehák *et al.,* 1994; Řehák, 1995).

However, using mist nets or harp-traps involves disturbance of bats. Stress caused by capturing the bats and manipulating with them may result in leaving the locality and

thus underestimation of the intensity of flight activity in comparison with the natural state (Kunz, 1973). Once trapped, the bats try to avoid the nets (LaVal and LaVal, 1980) and effectivity of netting decreases with increasing time of nets exposition (Řehák, 1995). The trapping methods are also species selective, as the species with less flight manoeuvrability are easier to catch (Francis, 1989; see also Berry *et al*., 2004). The use of automatic recording devices can remove these problems. The main drawback is their inability to distinguish individual bats, or even species (Ransome, 1990). However, this is a minor problem if we focus on the activity of the bat community as a whole.

The aim of this study was to monitor the natural pattern of flight activity of bats at a cave entrance without any disturbance and to evaluate its changes in the course of a year. We predicted that the overnight distribution of activity depends on the time of a year and tested the hypothesis that the activity is concentrated around specific time (i.e., is non-random) during the whole year. The influence of climatic factors on the level of bat flight activity was also tested.

Materials and Methods

Activity of bats was investigated at the entrance to Kateřinská cave, which is a natural limestone cave situated in the northern part of the Moravian Karst Protected Landscape Area, Czech Republic (49°21'N, 16°48'E). It is an important hibernaculum in the area. The overall length of the cave is about 500 m and it consists of 55 m long corridor and three large domes. An iron gate with a hole (25 × 20 cm) in its upper part closes the only entrance to the cave. Typical species of the community of bats hibernating in the Kateřinská cave are *Myotis myotis* and *Rhinolophus hipposideros* (over 80%). Although *Myotis emarginatus, M. daubentonii, M. nattereri* and *M. bechsteinii* are rarely found hibernating inside the cave, they are eudominant in both autumn and spring netting samples from the cave entrance (Řehák *et al.,* 1994).

The study was carried out between 1 October 1997 and 26 October 1999. Bat movements through the hole in the iron gate were monitored automatically with a double infrared light barrier and datalogger, once every fortnight. Each recording started before sunset and ended after sunrise. Reliable data were collected for 40 nights. The recording system consisted of two infrared light emitters (diodes) and two receivers (phototransistors). This technique allows discrimination between those bats leaving and those entering the cave. However, it does not allow species identification of active bats. The numbers of in-flights and out-flights were stored in the datalogger and read at hourly intervals. As the bats can fly in and out through the entrance hole several times, the number of bat passes does not need to be equal to the number of active bats. However, it was used as a measure of activity. The main problem was high cave air humidity that was causing repeated equipment failures due to corrosion of connectors and other metal components of the datalogger or water condensation on the infrared light emitters. Because of these technical problems five nights out of 45 had to be omitted from activity analyses.

The individual nights were grouped by using the cluster analysis (UPGMA, Euclidean distances), according to the level of flight activity (sum of inflights and outflights per night — Fig. 1). Three nights of sampling were included in different periods than expected. For the subsequent analyses we put 29.07.1998 into the period C, and 06.10.1998 and 22.10.1997 into the period D (see also Discussion). As the overnight activity pattern changes during the period B, although the amount of activity remains the same, K-means clustering was used to minimise the variability and two clusters (B1 and B2) of greatest possible distinction were produced, on the basis of activity distribution during the night. To assess the temporal distribution of activity during the night, nights (i.e., the time between sunset and sunrise) were divided into four periods of equal length. Nonparametric tests (Mann-Whitney *U*-test, Kruskal-Wallis ANOVA, Wilcoxon matched pairs test) were used, as data could not be normalised.

Nonparametric Rayleigh's test and Moore's *R*' test for randomness of the distribution around a circle were used to test whether there was a bias in the temporal distribution of activity (Zar, 1984). The time of each reading (absolute time was used) was converted to degrees. Since the sunset times within the period D varied considerably (up to 175 min), for this analysis it was divided in two subperiods, so as the variance in sunset times was minimal (D1: 29 July–04 September, and D2: 29 September–26 October). For each period (A–D2), the direction ($\bar{a}$) and length (r) of the mean vector were calculated. The mean time of activity corresponding to the mean ($\bar{a}$) angle was then

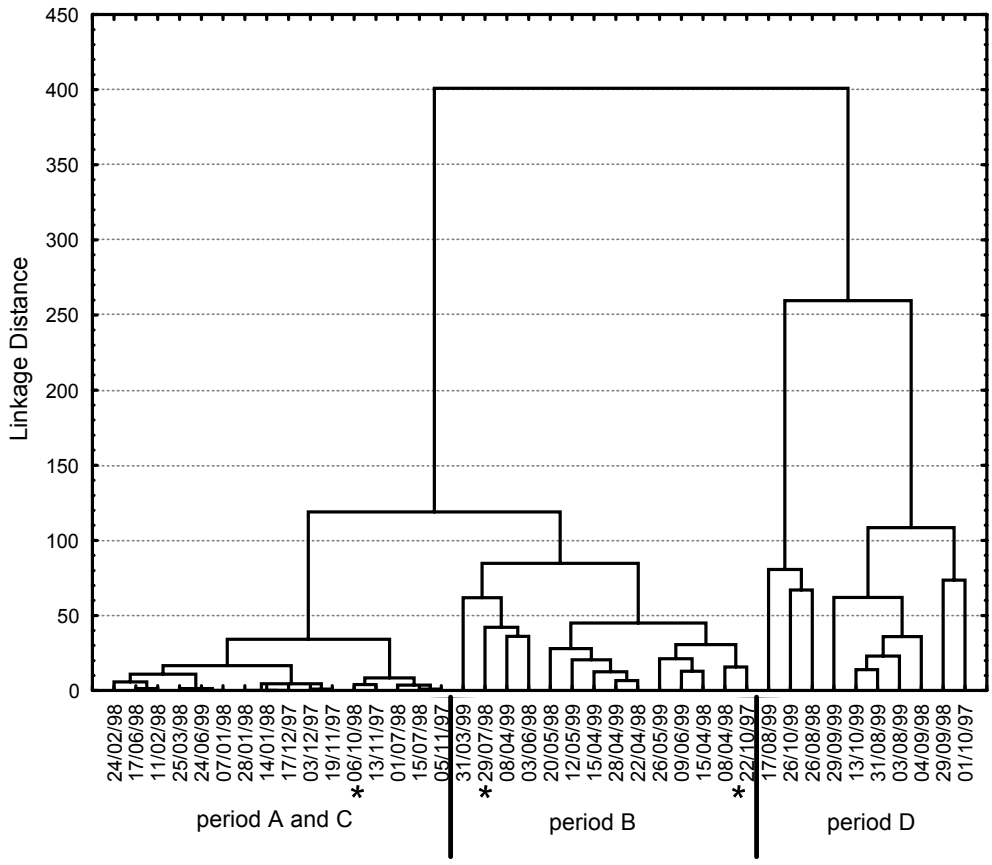


FIG. 1. Results of cluster analysis (UPGMA, Euclidean distances). The nights marked with an asterisk were shifted to different periods for the analyses

recalculated. The value of r is a measure of concentration and varies from 0 (dispersion) to 1 (all the data are concentrated at the same direction).

The influence of mean daily ambient temperature, mean barometric pressure (Spearman's rank correlation coefficient) and rain (Mann-Whitney *U*-test; 0 = no rain, 1 = rain) on bat activity levels was tested. The measurements were obtained from The Meteorological Office in Bořitov (ca. 11 km north-west of the Kateřinská cave). Statistical analyses were performed by using Statistica 6.0 (StatSoft, Inc. 1984–2001).

RESULTS

Seasonal Changes in Flight Activity

The amount of flight activity at the entrance to the cave changes seasonally. However, bats use the cave year round. With respect to annual cycle of bat activity, four periods were defined according to the results of cluster analysis (Fig. 1): A) Hibernation period (November–late March), with very low activity; B) Departure period (late March–beginning of June), with higher activity during spring migrations; C) Summer period (mid June–mid July), with low activity; D) Autumn period (late July–late October), with very high flight activity. The differences in flight activity in individual periods are statistically significant (Kruskal-Wallis test: $H_3 = 31.05$, $P < 0.001$, $n = 40$ — see Fig. 2).

Furthermore, the departure period 'B' was split into two parts (K-means clustering), on the basis of the distribution of bat

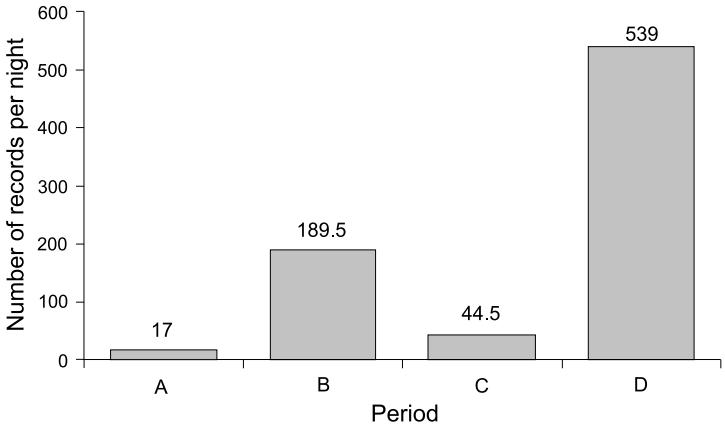


FIG. 2. Temporal distribution of flight activity. Columns represent medians for each period

activity during individual nights. They differed significantly (ANOVA, $P < 0.05$) in the number of inflights in the 1st, 2nd and 4th part of the night and the number of outflights in the 1st and 2nd part. The 1st subgroup (B1) (late March–mid April) is characterised by intensive departure activity during the first part of the night. For the 2nd subgroup (B2) (mid April–beginning of June), departure activity in the first quarter, and a small number of bats entering the cave in the fourth part of the night is typical.

Temporal Distribution of Flight Activity

The distribution of the flight activity in the particular quarters of the night of the total sample is not uniform (Kruskal-Wallis test: $H_3 = 8.454$, $P < 0.05$, $n = 160$). Nevertheless, during the winter (period A) and summer (period C), the difference in the distribution of activity in the four parts of the night was not significant. In periods B1, B2 and D, the levels of activity in these parts of the night differed significantly (Kruskal-Wallis test: B1: $H_3 = 13.38$, $P < 0.01$, $n = 20$; B2: $H_3 = 15.59$, $P < 0.001$, $n = 28$; D: $H_3 = 18.98$, $P < 0.001$, $n = 52$). Most of activity in the period B1 occurs in the 1st part of the night and then the activity of bats decreases. The level of flight activity in the 1st part of the night is significantly different from the other parts of the night (Mann-Whitney *U*-test: 1st and 2nd: $z = -2.61$, $P < 0.01$; 1st and 3rd: $z = -2.61$, $P < 0.01$; 1st and 4th: $z = -2.61$, $P < 0.01$). The 2nd part is different from the 4th part ($z = -2.19$, $P < 0.05$).

The activity in period B2 is distributed more evenly, with the peak in the 2nd part. The 4th night period is significantly different from the previous night periods (*U*-test: 4th and 1st: $z = -2.81$, $P < 0.01$; 4th and 2nd: $z = -3.13$, $P < 0.01$; 4th and 3rd: $z = -2.43$, $P < 0.05$). Furthermore, the 1st part differs from the 2nd part ($z = -2.11$, $P < 0.05$).

The peak of activity in period D (autumn) is around midnight. Parts 2 and 3 are significantly different from parts 1 and 4 (*U*-test: 2nd and 1st: $z = -3.28$, $P < 0.001$; 3rd and 1st: $z = -2.95$, $P < 0.01$; 2nd and 4th: $z = -0.10$, $P < 0.01$; 3rd and 4th: $z = -2.74$, $P < 0.01$).

Further, the data from hourly readings were tested for randomness of the time distribution of activity. All periods (A–D2) showed a non-random temporal distribution, which means that the flight activity was concentrated around specific time (Rayleigh's test and Moore's *R*' test — Table 1). Detailed analysis of individual

TABLE. 1. Results of Rayleigh's and Moore's tests for randomness on flight activity times for each period. Abbreviations: ā — mean vector direction converted to hours, *r* — mean vector length, *z* — Rayleigh's test statistic, *P* — probability, *n* — number of intervals, *R*' — Moore's test statistic

Period	ā (hrs)	Mean sunset time	*r*	*z*	*P*	*n*	*R*'	*P*	*d.f.*
A	20:06	16:36	0.642	6.183	<0.01	15	2.192	<0.01	8
B1	21:07	18:36	0.723	6.801	<0.01	13	2.226	<0.01	5
B2	23:13	19:30	0.820	8.737	<0.001	13	3.939	<0.01	7
C	23:54	20:00	0.907	6.585	<0.001	8	2.474	<0.01	4
D1	23:54	19:02	0.852	8.704	<0.001	12	3.475	<0.01	6
D2	23:57	17:15	0.729	7.963	<0.001	15	3.333	<0.01	7

nights revealed that only on the 7th January 1998, the activity was randomly distributed around the night (Rayleigh's test: $z = 0.68$, $P >> 0.05$, $n = 15$). The difference between the mean time of activity and mean sunset time varied during the season. It was lowest in period B1 (151 min) and highest in D2 (402 min).

In-flights and Out-flights

The ratio of emergencies and arrivals varies during the season (Fig. 3). Out-flights significantly dominated during period B (Wilcoxon matched pairs test: $z = 3.06$, $P < 0.01$, $n = 12$), whereas a reverse trend was apparent during period D ($z = 3.18$, $P < 0.001$, $n = 13$). Significant difference was found also for period A ($z = 2.52$, $P < 0.05$, $n = 11$).

The activity is generally very low during the winter (period A). However, a significant difference was found between the number of in-flights and out-flights in the 1st part of the night (Wilcoxon matched pairs test: $z = 2.37$, $P < 0.05$, $n = 11$).

Intensive departures occur during the 1st part of the night in period B1 ($z = 2.02$, $P < 0.05$, $n = 5$). Then the activity is very low and the number of arrivals slightly (but not significantly) exceeds the number of departures. During the 1st part of the night in period B2, the departure is quite intensive ($z = 2.37$, $P < 0.05$, $n = 7$), and significant prevalence of returning bats is apparent in the last part of the night ($z = 2.20$, $P < 0.05$, $n = 7$). In period C, only small number of

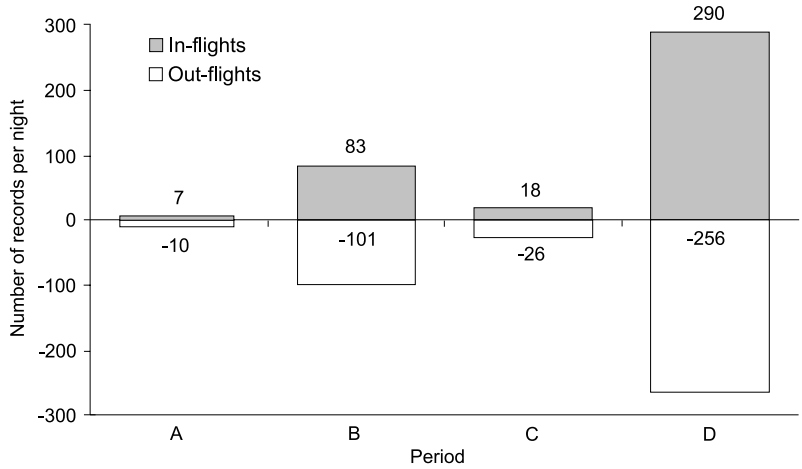


FIG. 3. In-flight and out-flight medians for each period

bats leaves the cave in the 1st part of the night. In the 2nd part bats fly to the cave and leave it again in the 3rd part. However, these differences are not statistically significant. The period D is typical of increasing number of bats entering the cave. Bats arrive during the 2nd part of the night (z = 3.18, P < 0.001, n = 13) and some of them leave the cave again during the 3rd (z = 2.00, P < 0.05, n = 13) and 4th part (z = 2.93, P < 0.01, n = 13 — see Fig. 4).

Effect of Climatic Factors

To assess the influence of climatic factors on bat activity, the sum of in-flights and out-flights for individual nights was used. There was a significant positive relationship between the number of bat passes through the entrance and the mean outside ambient temperature (Spearman's rank correlation: r_s = 0.67, P < 0.001, n = 40) and a negative correlation between the number of passes and the mean barometric pressure (r_s = -0.36, P < 0.05, n = 40). No relationship was found between the activity in the separate periods and climatic factors.

Rain had no significant effect on the level of bat activity (U-test: period B: z = -0.57, P >> 0.05; period D: z = -0.44, P >> 0.05). Test was performed for periods B (data from B1 and B2 pooled) and D only, because the sample size from two other periods was insufficient.

Discussion

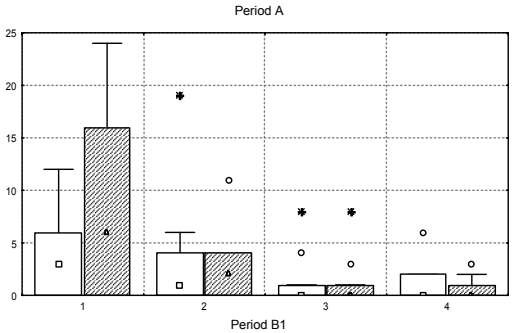
Seasonal Changes in Flight Activity

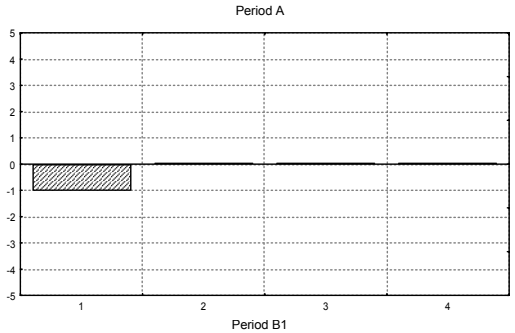
The amount of bat activity at the entrance to Kateřinská cave varies seasonally. These changes are related to the annual cycle of bat activity and their ecological demands. The Moravian Karst's caves are not suitable for summer breeding colonies. Nevertheless, the caves are important hibernacula for many bat species (Zima *et al.,* 1994). Therefore the peaks of activity occur during the spring and mainly autumn movements.

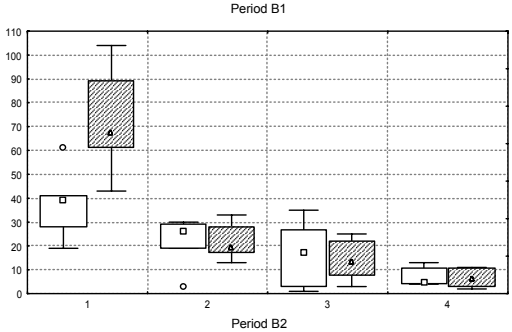
In the spring (period B), the activity level was relatively high over a period of more than two months (from the end of March until half of June). However, the activity distribution over the night was changing. This implies that the cave may serve as a transitional roost during spring migrations and probably as a temporary roost by males when females already form summer colonies (Skiba, 1987). The activity pattern during mid June–mid July period suggests that the cave may be used as a night roost between the peaks of foraging activity (Schofield, 1996; Szkudlarek and Paszkiewicz, 1997) or a transitional day roost (Degn, 1989; Degn *et al.,* 1995) mainly by males (Gaisler, 1975; Horáček and Zima, 1978; Bauerová and Zima, 1988*a*; Degn, 1989). Adult females occupy maternity roosts during lactation and return to them between foraging bouts, and night roosts use only briefly and sporadically (Anthony *et al.,* 1981). The increase in activity in the end of July corresponds with the break-up of summer breeding colonies and adult females and juveniles arrive into caves (Horáček and Zima, 1978). During November, the level of activity decreases and until the beginning of February is almost zero. It remains very low until the end of March and departures from the cave are still rare during this month. This was in accordance with

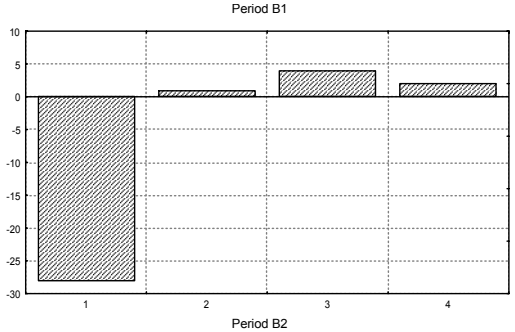
➪

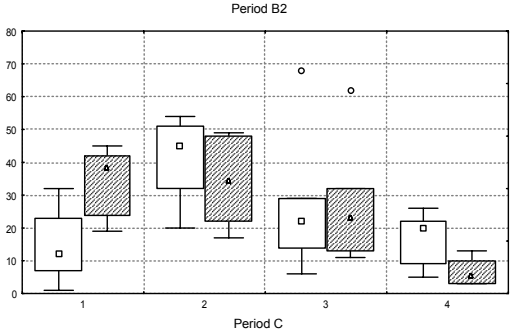
Fig. 4. In-flight and out-flight activity in each of the four parts of the night for periods A–D. Box and whisker plots showing in-flights and out-flights. Box 25–75%, whisker — non-outlier minimum and maximum, middle point — median, ○ — outliers, * — extremes. Bar charts showing medians of the difference between in-flights and out-flights (positive values — prevalence of arrivals, negative values — prevalence of departures)

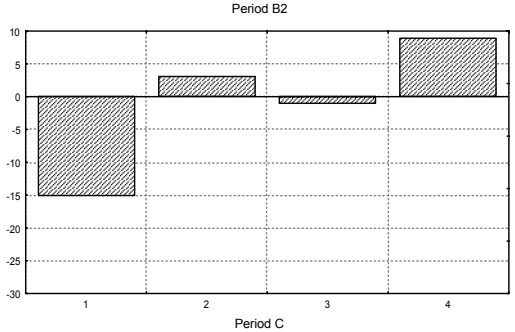

Period A
25
20
15
10
5
0
1
2
3
4

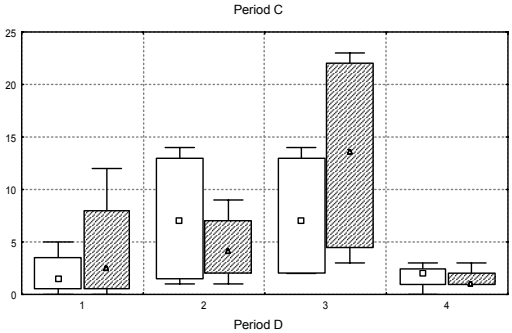

Period A
5
4
3
2
1
0
-1
-2
-3
-4
-5
1
2
3
4

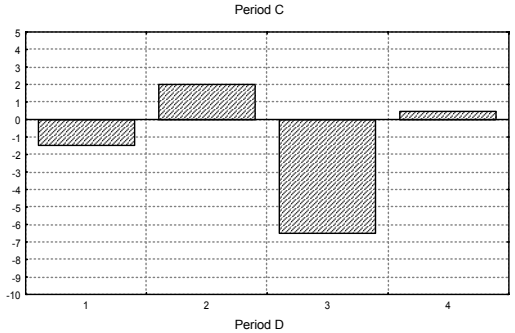

Period B1
110
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4

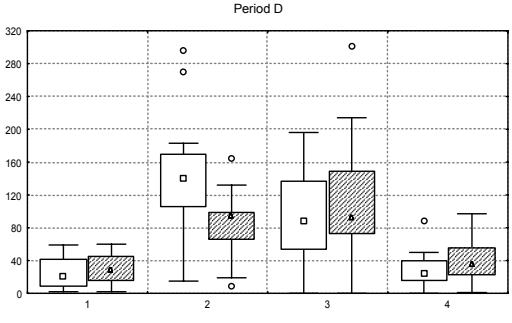

Period B1
10
5
0
-5
-10
-15
-20
-25
-30
1
2
3
4

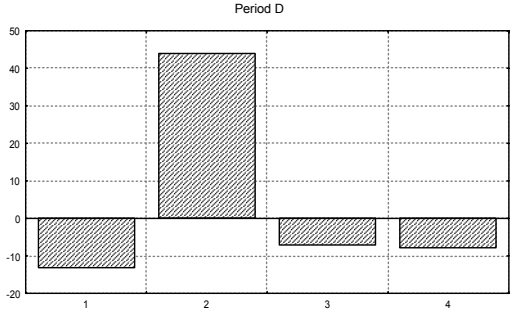

Period B2
80
70
60
50
40
30
20
10
0
1
2
3
4


Period B2
10
5
0
-5
-10
-15
-20
-25
-30
1
2
3
4


Period C
25
20
15
10
5
0
1
2
3
4


Period C
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
1
2
3
4


Period D
320
280
240
200
160
120
80
40
0
1
2
3
4


Period D
50
40
30
20
10
0
-10
-20
1
2
3
4

the results of netting at the same locality (Řehák *et al.,* 1994).

Temporal Distribution of Activity

Our data clearly indicated that in all periods the flight activity showed a non-random temporal distribution, i.e., it was concentrated around specific time. Important finding is that even in winter (period A), a concentration of activity (3–3.5 hours after sunset) was apparent, if some activity occurred of course. The only exception when the activity was randomly distributed around the night was one winter night (7th January 1998). Nevertheless, the activity started after the sunset and ended before the sunrise, i.e., remained nocturnal. This finding was in accordance with Park *et al.* (1999) who proved that in *Rhinolophus ferrumequinum* the activity remains nocturnal throughout winter. Similarly, Nagel and Nagel (1993) recorded most of activity between sunset and sunrise. On the contrary Thomas (1993) suggested that arousal times of *Myotis lucifugus* and *M. septentrionalis* become random in winter. However, since these authors monitored activity inside the hibernacula, our data cannot be directly compared with their results.

The variation in the difference between the mean time of activity and mean sunset time during the season probably does not reflect any changes in the timing of emergence activity. The difference was lowest in period B1 (151 min) as bats emerged from the hibernaculum soon after the sunset and highest in autumn (period D2 — 402 min) because the majority of bats was roosting elsewhere and came probably after the foraging bout.

Effect of Climatic Factors

In general, there was a positive correlation between daily activity level and the mean ambient temperature and an increase in activity with a reduction in barometric pressure. However, some of the highest temperatures were recorded during the summer period (C), when the flight activity is quite low. Our failure to detect relationship between the activity in the separate periods (A–D) and climatic factors may have been a consequence of the small number of nights of observations.

It is obvious, that the activity level considerably decreases if the temperature is below 10°C. This compares with the temperature-related feeding activity found by Rydell (1989*a,* 1989*b*): in 6–10°C interval, feeding activity of bats was reduced and at very low air temperatures (≤ 6°C) the bats did not forage at all. The low temperatures are the reason why the two autumn controls (6 October 1998 and 22 October 1997 with maximum daily temperatures 6.8°C and 5.1°C, respectively) were grouped with the low activity periods in the cluster analysis. Nevertheless, even below 0°C activity at the entrance to the cave still occurred, although it was minimal.

Rainfall was not found to be important factor influencing bat activity at the cave entrance. In contrast, Fenton (1969) found a noticeable effect of rain on the numbers of bats that visited a mine during the autumn swarming. However, bats are protected from rain at the entrance part of Kateřinská cave, which is formed by a large portal.

Acknowledgements

We thank to Martin Pokorný and Klára J. Petrželková for field assistance and to David Motl for technical service of the monitoring device. We are also grateful to the reviewers for valuable comments on the manuscript. This study was supported by the grant GA ČR 206/01/1555, GA ČR 524/05/4536, and grant No. LC06073 of the Czech Ministry of Education.

Literature Cited

Antony, E. L. P., M. H. Stack, and T. H. Kunz. 1981. Night roosting and the nocturnal time budget of

the little brown bat, *Myotis lucifugus*: effects of reproductive status, prey density, and environmental conditions. Oecologia, 51: 151–156.

BAUEROVÁ, Z., and J. ZIMA. 1988*a*. Seasonal changes in visits to a cave by bats. Folia Zoologica, 37: 97–111.

BAUEROVÁ, Z., and J. ZIMA. 1988*b*. Výzkum netopýrů v jeskyni Býčí skála v letech 1977–1986. Československý Kras, 39: 51–59.

BERRY, N., W. O'CONNOR, M. W. HOLDERIED, and G. JONES. 2004. Detection and avoidance of harp traps by echolocating bats. Acta Chiropterologica, 6: 335–346.

DEGN, H. J. 1989. Summer activity of bats at a large hibernaculum. Pp. 523–526, *in* European bat research (V. HANÁK, I. HORÁČEK, and J. GAISLER, eds.). Charles University Press, Praha, xxii + 718 pp.

DEGN, H. J., B. B. ANDERSEN, and H. J. BAAGØE. 1995. Automatic registration of bat activity through the year at Monsted Limestone Mine, Denmark. Zeitschrift für Säugetierkunde, 60: 129–135.

FENTON, M. B. 1969. Summer activity of *Myotis lucifugus* (Chiroptera: Vespertilionidae) at hibernacula in Ontario and Quebec. Canadian Journal of Zoology, 47: 597–602.

FRANCIS, C. M. 1989. A comparison of mist nets and 2 designs of harp traps for capturing bats. Journal of Mammalogy, 70: 865–870.

GAISLER, J. 1970. Remarks on the thermopreferendum of palearctic bats in their natural habitats. Bijdragen tot de Dierkunde, 40: 33–35.

GAISLER, J. 1973. Netting as a possible approach to study bat activity. Periodicum Biologorum, 75: 129–134.

GAISLER, J. 1975. A quantitative study of some populations of bats in Czechoslovakia (Mammalia: Chiroptera). Acta Scientiarium Naturalium Academiae Scientiarum Bohemoslovacae, 9: 1–44.

HORÁČEK, I., and J. ZIMA. 1978. Net-revealed cave visitation and cave-dwelling on European bats. Folia Zoologica, 27: 135–148.

KUNZ, T. H. 1973. Resource utilization: temporal and spatial components of bat activity in central Iowa. Journal of Mammalogy, 54: 14–32.

LAVAL, R. K., and M. L. LAVAL. 1980. Ecological studies and management of Missouri bats, with emphasis on cave-dwelling species. Terrestrial Series No. 8, Missouri Department of Conservation, Jefferson City, 93 pp.

NAGEL, A., and R. NAGEL. 1993. Activity of hibernating bats in their natural habitat recorded by automatic data logging. Pp. 37–38, *in* Abstracts of the 6th European Bat Research Symposium, Évora, Portugal, 22–27 August 1993, 65 pp.

PARK, J. K., G. JONES, and R. D. RANDÍME. 1999. Winter activity of a population of greater horseshoe bats (*Rhinolophus ferrumequinum*). Journal of Zoology, 248: 419–427.

RANSOME, R. D. 1990. The natural history of hibernating bats. Christopher Helm, London, 226 pp.

ŘEHÁK, Z. 1995. Letová aktivita netopýrů v Moravském krasu. Ph.D. Thesis, PřF MU Brno, 184 pp.

ŘEHÁK, Z., J. ZUKAL, and M. KOVAŘÍK. 1994. Long- and short-term changes in the bat community of the Kateřinská cave (Moravian karst) — a fundamental assessment. Folia Zoologica, 43: 425–436.

RYDELL, J. 1989*a*. Feeding activity of the northern bat *Eptesicus nilssoni* during pregnancy and lactation. Oecologia, 80: 562–565.

RYDELL, J. 1989*b*. Occurence of bats in the northernmost Sweden (65°N) and their feeding ecology in summer. Journal of Zoology (London), 227: 517–529.

SCHOFIELD, H. W. 1996. The ecology and conservation biology of *Rhinolophus hipposideros*, the lesser horseshoe bat. Ph.D. Thesis, University of Aberdeen, Aberdeen, 197 pp.

SKIBA, R. 1987. Bestandsentwicklung und Verhalten von Fledermäusen in einem Stollen des Westharzes. Myotis, 25: 95–103.

SZKUDLAREK, R., and R. PASZKIEWICZ. 1997. Summer activity of the lesser horseshoe bat in caves — preliminary observations. Pp. 155–156, *in* Tagungsband: 'Zur Situation der Hufeisennasen in Europa', Nebra, 26–28 Mai 1995, 182 pp.

THOMAS, D. W. 1993. Lack of evidence for a biological alarm clock in bats (*Myotis* spp.) hibernating under natural conditions. Canadian Journal of Zoology, 71: 1–3.

ZAR, J. H. 1984. Biostatistical analysis. 2nd edition. Prentice Hall, New Jersey, 718 pp.

ZIMA, J., M. KOVAŘÍK, J. GAISLER, Z. ŘEHÁK, and J. ZUKAL. 1994. Dynamics of the number of bats hibernating in the Moravian Karst in 1983 to 1992. Folia Zoologica, 43: 109–119.

Received 17 August 2005, accepted 30 December 2005

Výletová a návratová aktivita u vchodu do úkrytu

Berková, H. & Zukal, J. (2010) Cave visitation by temperate zone bats: effects of climatic factors. Journal of Zoology (London) 280, 387-395.

Journal of Zoology

Journal of Zoology. Print ISSN 0952-8369

Cave visitation by temperate zone bats: effects of climatic factors

H. Berková[1,2] & J. Zukal[1,2]

1 Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic
2 Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic

Keywords
automatic registration; bats; flight activity; hibernaculum; seasonal changes.

Correspondence
Hana Berková, Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, CZ-60365 Brno, Czech Republic.
Email: berkova@brno.cas.cz

Editor: Günther Zupanc

Received 27 October 2009; accepted 4 November 2009

doi:10.1111/j.1469-7998.2009.00673.x

Abstract

We investigated the effects of climatic variables on the flight activity of bats at the entrance of a hibernaculum (Kateřinská cave, Moravian Karst, CZ). Activity was recorded automatically using a double infrared-light logging system. Climatic factors influenced not only seasonal but also night-to-night and overnight patterns in cave visitation. The effect of individual variables and their contribution to variability in activity levels changed during the year. (1) Flight activity during late hibernation (5 March–14 April) was positively affected by the mean ambient temperature (T_{avg}) and negatively affected by previous day minimal temperature. (2) During the departure period (15 April–4 June), nightly activity correlated with T_{avg} and P_{avg} (mean barometric pressure). Previous day rainfall caused a decline in the activity levels. (3) Summer activity (5 June–26 July) increased as the range of daily temperature ($T_{dif\ max-min}$) increased and was suppressed by previous day rainfall. In contrast, a higher amount of rainfall (>10 mm) in the study day caused an increase in activity, likely due to bats sheltering. (4) During swarming (5 September–14 November), activity was positively related to T_{avg}, P_{avg} and the amount of rainfall. (5) During hibernation (15 November–4 March), temperature (T_{avg} and $T_{dif\ max-min}$) was the best predictor of the activity level. The percentage of nights on which activity occurred increased with increasing temperature during hibernation and late hibernation. Activity occurred even at temperatures $<0\,^{\circ}C$ ($T_{min} = -13.2\,^{\circ}C$). The recordings were all positive at $T_{max} \geq 6.2\,^{\circ}C$. The activity within corresponding temperature groups was significantly lower during hibernation than during late hibernation. We review possible explanations for the patterns observed.

Introduction

The ecology and behaviour of temperate zone microchiropteran bats are fundamentally affected by seasonal changes in day length and associated climatic variables (Erkert, 1982). These changes require flexible behavioural adjustments of their circadian as well as circannual activity patterns.

A characteristic feature of the annual cycle of insectivorous temperate zone bats is hibernation. Selection of a suitable hibernation site is crucial for overwinter survival, and caves and mines are the most common type of hibernacula. Hibernation is usually interrupted by periodical arousals. Such arousals may concern switching hibernation site, drinking, feeding or even mating (e.g. Ransome, 1971; Daan, 1973; Speakman & Racey, 1989; Schofield, 1996; Zukal, Berková & Řehák, 2005). In Central Europe, departures from hibernacula occur between March and May (Skiba, 1987; Degn, Andersen & Baagoe, 1995; Berková & Zukal, 2006). During this time, caves may serve as transitional roosts during spring migrations or as temporary roosts (Skiba, 1987). Females then usually move to maternity colonies to give birth. All known maternity roosts in the study area (Moravian Karst, CZ) and its environs are in buildings or tree hollows, as caves are not suitable for nurseries (Pokorný *et al.*, 2003). Males tend to roost solitarily or in small groups but the location of their roosting sites remains largely unknown. Bat activity at cave entrances is generally low during summer. However, some bats, almost exclusively males, often use underground sites as resting places between the peaks of foraging activity (Schofield, 1996) or as day roosts (Gaisler, 1963*b*; Degn *et al.*, 1995), while adult females return to maternity roosts between foraging bouts, using only sporadically particular night roosts during lactation (Anthony, Stack & Kunz, 1981). Breeding colonies disperse in late summer (end of July onwards) and adults and juveniles of both sexes move to 'swarming' sites (often caves and mines), where many eventually hibernate (Horáček & Zima, 1978; Řehák, Zukal & Kovařík, 1994).

Caves may thus serve a plurality of needs and many of them are probably used the year round. Recently, automatic

loggers allow the collection of a large quantity of data over a long period and are frequently used to monitor activity. In monitoring cave entrances, this method was verified to provide a reliable index of activity levels (Parsons, Jones & Greenaway, 2003; Rivers, Butlin & Altringham, 2006). Their main advantage is the lack of disturbance to bats compared with netting. The drawback is their inability to distinguish individual bats or even species (Ransome, 1990; Parsons *et al*., 2003). However, this is a minor problem if we focus on the activity of the bat assemblage as a whole.

So far, most studies have been focused on activity during hibernation or, because the peak of activity occurs during the autumn, on the phenomenon called 'swarming'. Activity patterns at hibernation sites have only rarely been documented over an entire annual cycle. The exceptions are studies by Harrje (1994), Degn *et al*. (1995), Sendor, Kugelschafter & Simon (2000) and Berková & Zukal (2006). In this paper, we present the first comprehensive data on flexibility of the activity patterns under variable weather conditions for bat assemblage of a natural karstic cave.

When trying to identify environmental factors that may affect bat activity, one should take into account that we are dealing with a combination of seasonally varying environmental cues, such as light, temperature, air humidity, atmospheric pressure, lunar cycle, rainfall and wind, as well as insect density.

The aims of this study were (1) to identify those climatic variables that best account for variation in activity levels in the different parts of the annual cycle; (2) to describe their influence on seasonal and nightly activity patterns.

Materials and methods

Study site

The Kateřinská cave is a natural limestone cave situated in the northern part of the Moravian Karst Protected Landscape Area, Czech Republic (49°2′N, 16°48′E). It is an important hibernaculum in the area, monitored for hibernating bats since 1970. Typical bat species hibernating in the Kateřinská cave are *Myotis myotis* and *Rhinolophus hipposideros* (over 80%). *Myotis emarginatus*, *Myotis daubentonii*, *Myotis nattereri* and *Myotis bechsteinii* are eudominant in both autumn and spring netting samples from the cave entrance, but rarely found hibernating inside the cave (probably due to their use of crevices). During visual counts, 160–218 bats were seen in winter (years 1999–2001). Bats were rarely found roosting within the cave from late May until late October (Řehák *et al*., 1994; Zukal *et al*., 2001). The only entrance to the cave is formed by a large portal, which is, after *c*. 15 m, closed by an iron gate with a hole (25 × 20 cm) in its upper part, followed by 55-m-long corridor and three large domes. The overall length of the cave is about 500 m. Microclimatic conditions are dynamic in the corridor (influenced by the outside ambient temperature) and relatively stable in domes (temperature ranging between 6.3 and 8.8 °C during the course of the year) (Zukal *et al*., 2005).

Data collection

We monitored bat movements through the hole in the iron gate using an automatic custom-made double-infrared light barrier connected to a data logger. The recording system consisted of two infrared light emitters (diodes) and two receivers (phototransistors), allowing discrimination between those bats leaving and those entering the cave. However, it did not allow species identification of active bats. After each bat passes the gate, time (hour and minute) and direction (in or out) were stored in the data logger. Reliable data were collected for 469 days between 21 March 2000 and 28 November 2002. Some data were lost owing to equipment malfunction (e.g. corrosion or water condensation).

Climatic variables

Climatic variables are listed in Table 1 together with abbreviations used in the text. Data were obtained from weather stations in Protivanov (15.8 km from the Kateřinská cave): T_{avg}, T_{max}, T_{min}; Brno (23.5 km): P_{avg}; and Blansko (5.7 km): R (Fig. 1). All data were provided by the Czech Hydrometeorological Institute Brno.

Statistical analysis

Data from all years were pooled and individual days arranged into 10-day periods. Each night (i.e. the time between sunset and sunrise) was divided into four periods of equal length, and medians of in-flights and out-flights in the four night periods were calculated for each 10-day period. The 10-day periods were grouped by using the cluster analysis (complete linkage, Euclidean distances), according to the activity in the four night periods. With respect to the annual cycle of bat activity, five periods were defined according to the results of cluster analysis: (1) hibernation (15 November–4 March); (2) late hibernation (5 March–14 April); (3) departure (and transition) (15 April–4 June); (4) summer (5 June–26 July); (5) swarming (5 September–14 November). As we did not obtain any data for the period between 6 August and 4 September, we omitted from analyses also the decade 27 July–5 August (data for 5 days), which was actually the beginning of the swarming period (H. Berková & J. Zukal, unpubl. data). We used 464 days for the subsequent analyses.

Table 1 Abbreviations of climatic variables used in the text

Variable	Description
T_{avg}	Mean daily ambient temperature
T_{max}	Maximum daily ambient temperature
T_{min}	Minimum daily ambient temperature
T_{min-1d}	Minimal temperature of the day preceding the study day
$T_{dif\ max-min}$	Range of daily temperature ($T_{max} - T_{min}$)
P_{avg}	Mean barometric pressure
R	Rainfall on the study day (mm 24 h^{-1})
R_{-1d}	Rainfall on the day preceding the study day

We define nightly activity as the number of bat passes per night. Total activity = number of bat passes and net departures = difference between in-flights and out-flights in the four night periods. We used the Mann–Whitney (MW) *U*-test and Kruskal–Wallis (KW) ANOVA with multiple comparisons for the univariate comparisons, as the data were not normally distributed. We used stepwise multiple regression, using forward selection (F to enter 0.99, F to remove 0.98), to determine the environmental factors that best accounted for variation in activity levels. The square roots of nightly activity and $T_{\text{dif max–min}}$ were used to achieve normality. Data on rainfall (R and R_{-1d}) were log transformed. Two cases (7 and 8 April 2001) were excluded because of extreme values (24 mm) of R and R_{-1d}, respectively. All equations were assessed to ensure that the variables incorporated were both statistically and computationally independent. We computed Pearson's correlation coefficients for all pairs of climatic variables and removed all but one variable from each highly correlated set ($r > 0.6$). We then used T_{avg}, previous day T_{min}, $T_{\text{dif max–min}}$, P_{avg}, R and R_{-1d} for the analysis. A significance criterion of $P < 0.05$ was used in all tests. Statistical analyses were performed using STATISTICA 8.0 (StatSoft Inc. 1984–2007, Tulsa, OK, USA).

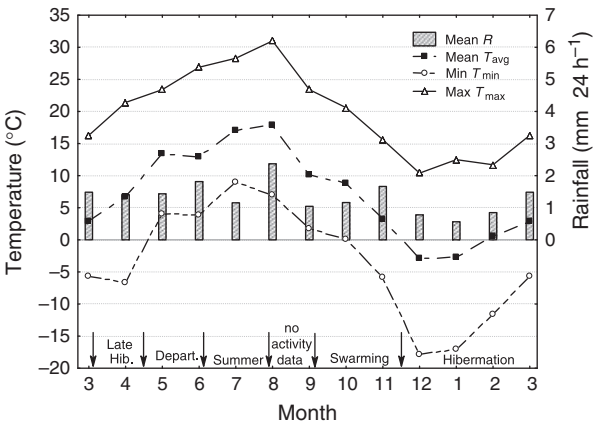


Figure 1 Mean amount of rainfall (mm $24\,h^{-1}$; weather station in Blansko) and mean, maximum and minimum monthly ambient temperatures (°C; weather station in Protivanov) during the study period (21 March 2000–28 November 2002).

Results

Late hibernation

Flight activity was positively correlated with T_{avg} and negatively with $T_{\text{min–1d}}$. The amount of variability (adjusted R^2) explained by the climatic variables was 53.2% (Table 2).

In $T_{\text{avg}} < 0$ °C ([1]), the activity was very low (median 9.5 bat passes), although it occurred even at −6.7 °C. In $T_{\text{avg}} \geq 0$ °C, the nightly activity was higher after a cool day ($T_{\text{min–1d}} < 0$ °C; [2]) than after a day with $T_{\text{min}} \geq 0$ °C ([3]) (median 127.5 and 107, respectively), although the difference was not statistically significant (KW test: $H_2 = 32.232$, $P < 0.001$, $n = 81$; multiple comparisons following the KW test: [1] and [2] $P < 0.001$, [1] and [3] $P < 0.001$, [2] and [3] NS). The increase in activity in $T_{\text{avg}} \geq 0$ °C and $T_{\text{min–1d}} < 0$ °C compared with $T_{\text{min–1d}} \geq 0$ °C was obvious when the between-nights difference in activity was assessed (MW test: $z = 3.688$, $P < 0.001$, $n_1 = 18$, $n_2 = 46$; median +64.5 and −4.5 bat passes, respectively; Fig. 2).

The range of temperature ($T_{\text{dif max–min}}$) was not the main factor influencing activity; however, a significant increase in activity was found with increasing $T_{\text{dif max–min}}$ (KW test: $H_3 = 12.031$, $P = 0.007$, $n = 83$; multiple comparisons: [< 7.0 °C] and [> 10.5 °C] $P = 0.019$; Fig. 3).

Between-nights differences in activity were also influenced by day-to-day changes in T_{max} and T_{min}. A decline in T_{max} or T_{min} by 2–6 °C reduced activity levels and activity significantly increased with an increase in T_{max} or T_{min} by 2–6 °C (Table 3; Fig. 4).

Activity was not different on comparing days with rainfall with dry days (MW test: $z = -0.763$, $P = 0.445$, $n_1 = 45$, $n_2 = 38$). However, precipitations > 5 mm per 24 h negatively affected the proportion of out-flies in the first quarter of the night (median values: dry = 55.0%, < 5 mm = 58.3%, > 5 mm = 46.7%; KW test: $H_2 = 7.318$, $P = 0.026$, $n = 81$; multiple comparisons: [dry] and [< 5 mm] NS, [dry] and [> 5 mm] NS, [< 5 mm] and [> 5 mm] $P = 0.022$).

Table 2 Influence of climatic factors on seasonal flight activity of bats at the entrance of Kateřinská cave

	LH (n=83)		DE (n=72)		SU (n=49)		SW (n=93)		HI (n=167)	
	β	P	β	P	β	P	β	P	β	P
Intercept		**<0.001**		**0.038**		0.646		**<0.001**		**0.001**
T_{avg}	**0.835**	**<0.001**	**0.319**	**0.005**	−0.045	0.803	**0.586**	**<0.001**	**0.748**	**<0.001**
$T_{\text{dif max–min}}$	0.040	0.672	0.009	0.948	**0.423**	**0.002**	−0.167	0.106	**0.120**	**0.023**
$T_{\text{min–1d}}$	**−0.211**	**0.030**	0.075	0.564	−0.213	0.098	−0.078	0.582	0.074	0.384
P_{avg}	−0.068	0.474	**0.252**	**0.024**	−0.046	0.734	**0.396**	**<0.001**	0.037	0.536
R	−0.057	0.484	−0.068	0.548	**0.332**	**0.010**	**0.185**	**0.046**	−0.011	0.839
R_{-1d}	−0.127	0.105	−0.082	0.469	−0.091	0.537	−0.071	0.474	0.055	0.292

Results of forward stepwise multiple regression analysis. Significant correlates ($P < 0.05$) are set in bold.
LH, late hibernation; HI, hibernation; DE, departure; SU, summer; SW, swarming.

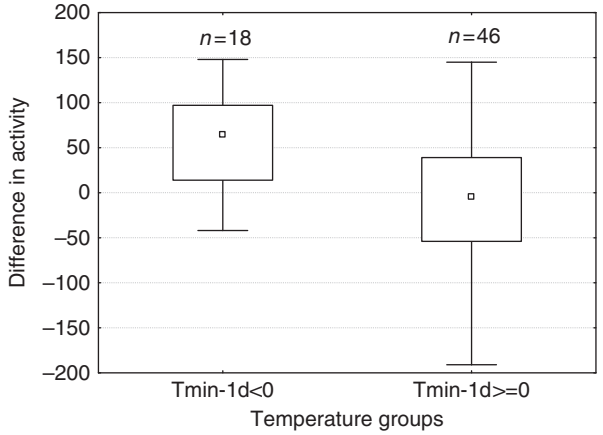


Figure 2 Between-nights difference in nightly activity during late hibernation (5 March–14 April) in $T_{avg} > 0\,^{\circ}C$ after a cool night ($T_{min-1d} < 0\,^{\circ}C$) compared with night with $T_{min-1d} \geq 0\,^{\circ}C$. Middle point, median; box, interquartile range; whisker, non-outlier range.

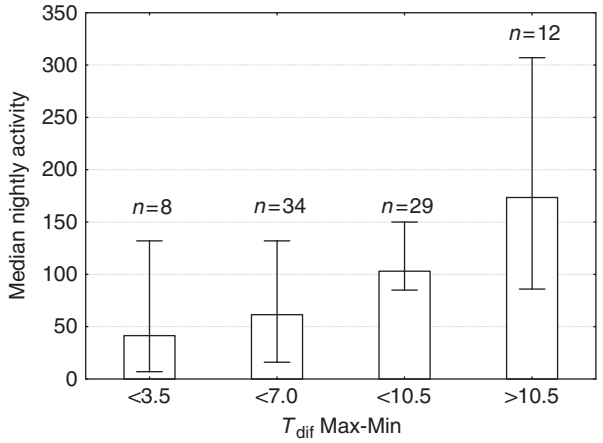


Figure 3 Median nightly number ± interquartile range of bat passes in nights with different ranges of daily temperature ($T_{dif\ max-min}$) during late hibernation (5 March–14 April).

Departure period

A significant positive relationship was found between nightly activity and T_{avg} and P_{avg} (Table 2). However, these variables explained only 15.3% of the variability.

Between-nights differences in activity were also related to the day-to-day changes in T_{max}. The activity decreased with a decline in T_{max} by 2–6 °C (median = −17) and increased with an increase in T_{max} by 2–6 °C (median = 32.5). The multiple comparisons following the KW test, however, fell short of statistical significance (Table 3). Day-to-day changes in T_{min} did not have any effect on changes in bat activity (KW test: $H_2 = 0.020$, $P = 0.990$, $n = 67$). R did not have any influence on activity (MW test: $z = 0.320$, $P = 0.749$, $n_1 = 45$, $n_2 = 27$). R_{-1d} caused a decline in activity levels ($z = 2.102$, $P = 0.036$, $n_1 = 44$, $n_2 = 28$; median: dry = 223.5, rain = 158.5).

Summer

$T_{dif\ max-min}$ and R were among the most important climatic factors influencing bat activity during summer (Table 2). However, the variability explained by the regression equation was only 28.8%.

Activity increased as the $T_{dif\ max-min}$ increased (KW test, group [< 3.5 °C] excluded ($n = 2$): $H_2 = 7.518$, $P = 0.023$, $n = 47$). Multiple comparisons indicated that only group [7.0; < 10.5 °C] was significantly different from group [≥ 10.5 °C] ($P = 0.035$). For further analyses, we divided the data into two groups: $T_{dif\ max-min} < 10\,^{\circ}C$ and $T_{dif\ max-min} \geq 10\,^{\circ}C$. Both differences in nightly activity (MW test: $z = -3.959$, $P < 0.001$, $n_1 = 32$, $n_2 = 17$; median 41.5 and 105) and total activity and net departures in individual quarters of the night were statistically significant (Table 4). Activity was lower in $T_{dif\ max-min} < 10\,^{\circ}C$. In $T_{dif\ max-min} \geq 10\,^{\circ}C$, significantly more bats flew inside the cave in the second part and outside the cave in the third and fourth part of the night (Fig. 5).

The increase in nightly activity in days with rainfall (median = 68) compared with dry days (median = 59) was not statistically significant (MW test). A higher amount of rainfall (> 10 mm) caused a sharp increase in activity, but the statistical outcomes would be meaningless, given the small number of observations ($n = 2$) in group $R > 10$ mm.

R_{-1d} negatively affected nightly activity (MW test: $z = 2.382$, $P = 0.017$, $n_1 = 27$, $n_2 = 22$; median: dry = 90, rain = 47). The activity was also significantly lower in the second and third part of the night if there was rain in the previous day compared with dry in the previous day (Table 4).

The differences in activity due to rainfall are more pronounced if combinations of rain and dry in the study day and the preceding day are assessed (KW test: $H_3 = 9.994$, $P = 0.019$, $n = 49$; multiple comparisons: [R_{-1d} rain, R dry] and [R_{-1d} dry, R rain] $P = 0.012$). Activity was highest when there was rain on the study day but not on the preceding day, followed by dry on both days. Activity decreased when there was rain on both days, and the lowest activity levels were on dry days with preceding day rainfall. Differences were also apparent in the course of the night (Fig. 6).

The combined effect of $T_{dif\ max-min}$ and R was tested. Data were divided into four groups with either $T_{dif\ max-min} < 10\,^{\circ}C$ or $\geq 10\,^{\circ}C$ and dry or rain. The KW test indicated significant differences between groups ($H_3 = 18.654$, $P < 0.001$, $n = 49$). Multiple comparisons showed that activity during nights with $T_{dif\ max-min} < 10\,^{\circ}C$ and dry was significantly different from activity during nights with $T_{dif\ max-min} \geq 10\,^{\circ}C$ and both dry ($P = 0.008$) and rain ($P = 0.001$), confirming the major influence of temperature fluctuation, while the effect of rainfall is just additive. Differences were also apparent among the four night parts (Fig. 7).

Table 3 Influence of day-to-day changes in T_{max} and T_{min} on between-nights difference in nightly activity during late hibernation (5 March–14 April) and the departure period (15 April–4 June)

	LH T_{max} $H_2 = 15\,240$, $n = 74$	LH T_{min} $H_2 = 8322$, $n = 77$	DE T_{max} $H_2 = 6483$, $n = 67$
Kruskal–Wallis test	***P*<0.001**	***P*=0.016**	***P*=0.039**
Temperature groups	Multiple comparisons:		
(−2; −6 °C) & (−2; 2 °C)	$P = 0.821$	$P = 0.741$	$P = 1.000$
(−2; −6 °C) & (2; 6 °C)	***P*=0.001**	***P*=0.027**	$P = 0.069$
(−2;2 °C) & (2; 6 °C)	***P*=0.006**	$P = 0.056$	$P = 0.089$

Significant results ($P < 0.05$) are set in bold. LH, late hibernation; DE, departure.

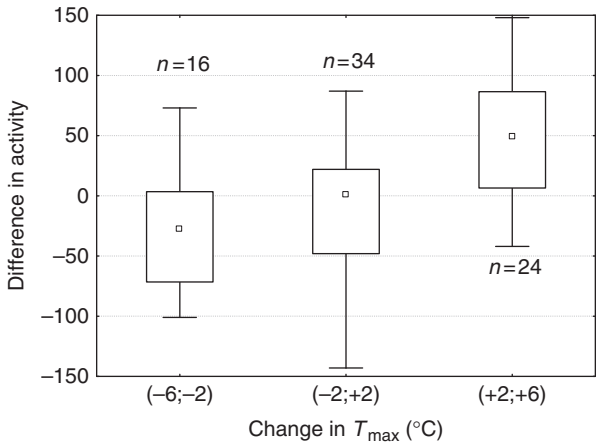


Figure 4 Effect of day-to-day changes in T_{max} on between-nights difference in nightly activity during late hibernation (5 March–14 April). Middle point, median; box, interquartile range; whisker, non-outlier range.

Swarming period

A multiple linear regression analysis indicated that T_{avg}, P_{avg} and R were good climatic predictors of the general activity level (Table 2). The amount of variability explained by the climatic variables was 34.2%.

The day-to-day positive changes in T_{max} correlated with day-to-day changes in activity (KW test: $H_2 = 8.270$, $P = 0.016$, $n = 77$). However, multiple comparisons revealed that activity significantly increased if T_{max} increased by 2–6 °C ($P = 0.014$), but a decline by 2–6 °C in T_{max} did not have a significant effect on bat activity. Activity was particularly reduced when T_{max} declined by >6 °C, but these observations were excluded from the analysis ($n = 2$).

Hibernation

Temperature was the best predictor of the activity level (Table 2). The relationship between T_{avg} and activity also depended on $T_{dif\ max-min}$. The predictor variables explained 56.0% of the variation in activity.

Of the 167 days monitored, activity occurred on 124 nights, even at temperatures <0 °C ($T_{min} = -13.2$ °C). The percentage of nights on which activity occurred increased with increasing temperature. The recordings were all positive at $T_{max} \geq 6.2$ °C. The effect of temperature fluctuations during the day was apparent only at $T_{avg} > 0$ °C. Precipitations did not have any influence on bat activity.

In addition to significant differences in activity levels between the temperature groups within hibernation and late hibernation, the activity within corresponding temperature groups was significantly higher during late hibernation than during hibernation (Fig. 8, Table 5).

Discussion

Temperature

Apparently, the ambient temperature is a key climatic factor influencing not only seasonal changes in activity but also night-to-night flight activity patterns of temperate zone bats. The strongest correlation between activity and climatic variables concerned hibernation and late hibernation. The activity is highly temperature dependent during these cold periods with food scarcity, when large numbers of bats occupy the cave. In contrast, during the warm season periods, low percentage of explained variance suggests a strong influence of non-meteorological factors on activity patterns. In addition to reproductive status, sex and age, the spatiotemporal dynamics of prey availability and abundance, together with diverse foraging strategies of bat species causing different sensitivity to dynamics of environmental conditions, probably play an important role in flight and foraging activity patterns and time budgeting (Anthony *et al.*, 1981; Erkert, 1982; Ciechanowski *et al.*, 2007).

During hibernation, the clearest relationship between activity and climatic variable was for temperature (T_{avg}), which is thought to be a crucial exogenous factor controlling seasonal timing and the course of hibernation (e.g. torpor bout duration) (Erkert, 1982; Park, Jones & Ransome, 2000). A positive relationship between activity and $T_{dif\ max-min}$ suggests that a higher temperature fluctuation during the day together with $T_{avg} > 0$ °C induce arousals and more bats become active. This finding is consistent with Ransome's (1971) assumption that temperature fluctuation near hibernating bats influences arousal frequency. The monitoring device we used, however, registered only those bats that left or entered the cave. Thus, the activity monitored represents only a fraction of arousals

Table 4 Effect of $T_{\text{dif max−min}}$ on the total amount of activity and net departures, and effect of R_{-1d} on the total activity in individual quarters of the night during summer (5 June–26 July)

		Part 1	Part 2		Part 3		Part 4	
		P	*z*	*P*	*z*	*P*	*z*	*P*
$T_{\text{dif max−min}}$ TA	$n_1=32$, $n_2=17$	NS	−3.487	**<0.001**	−4.012	**<0.001**	−2.783	**0.005**
$T_{\text{dif max−min}}$ ND	$n_1=32$, $n_2=17$	NS	−4.001	**<0.001**	2.993	**0.003**	3.203	**0.001**
R_{-1d} TA	$n_1=27$, $n_2=22$	NS	2.251	**0.024**	2.824	**0.005**		NS

Results of Mann–Whitney *U*-tests. Significant results ($P<0.05$) are set in bold.
TA, total amount of activity; ND, net departures; NS, not significant.

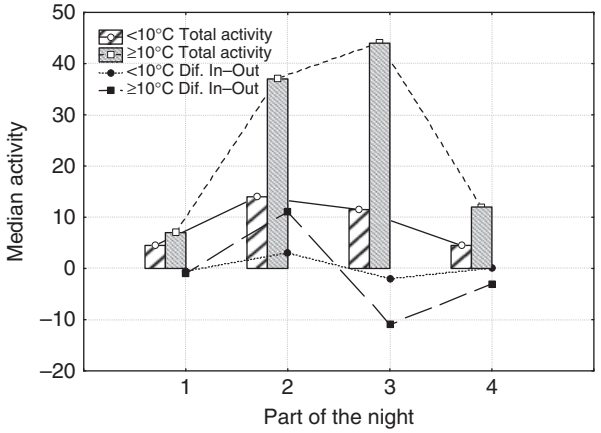


Figure 5 Influence of the range of daily temperature ($T_{\text{dif max−min}}$) on the total activity and net departures (=difference between in-flights and out-flights) during the course of the night during summer (5 June–26 July).

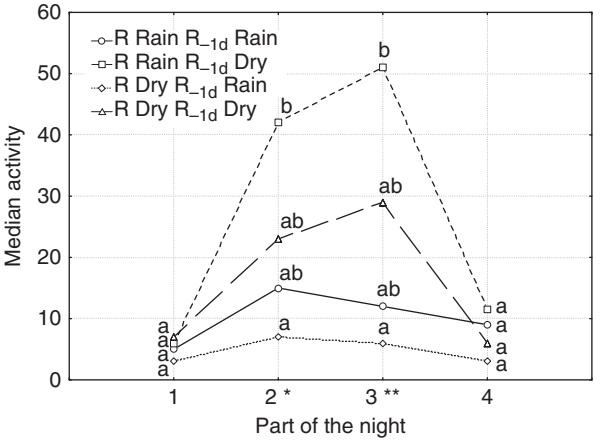


Figure 6 Combined effect of *R* and R_{-1d} (dry or rain) on the nightly pattern of activity during summer (5 June–26 July). Significant differences between groups in individual parts of the night, indicated by the Kruskal–Wallis (KW) test, are marked with *($P<0.05$) and **($P<0.01$). Letters (a, b, c) indicate significant differences between groups calculated using multiple comparisons following the KW test.

as bats often do not change their hibernation site or move only within the hibernaculum after arousal (Daan, 1973; Ransome, 1971; own unpubl. data). The causes of winter activity outside the roost at low temperatures still remain unknown. The most favourable explanation is that bats fly out to replenish water (Speakman & Racey, 1989). However, water is available to bats in the Kateřinská cave.

During late hibernation, the activity was also highly temperature dependent. Apparently, bats immediately reacted on positive changes in temperature, as there was a significant increase in activity after a cool night at temperatures $>0\,^{\circ}$C. Analysis of between-nights differences in activity in relation to $T_{\max}$ and $T_{\min}$ confirmed this. During hibernation (from December onwards) and late hibernation, increasing numbers of bats move to the dynamic part of the Kateřinská cave, where the temperature reflects changes in the outside ambient temperature (Zukal *et al.*, 2005). This evidence implies that synchronization with the outside ambient temperature might be important. Ransome (1971) assumes that the use of dynamic parts of hibernacula probably enables bats to arouse when insect availability is high. Such behaviour may be of advantage to bats if foraging outside is more profitable than remaining inside the cave. The increase in activity with increasing temperature fluctuations ($T_{\text{dif max−min}}$) suggests that higher $T_{\max}$ (usually in the afternoon) is more important than the minimum temperature (usually in the morning) as higher $T_{\max}$ might induce insect activity. However, this will probably not explain why activity occurred at low temperatures, although Ciechanowski *et al.* (2007) observed *M. daubentonii* intensively foraging at temperatures as low as $-3.3\,^{\circ}$C.

In addition to a significant positive correlation between activity and ambient temperature during hibernation and late hibernation, we found significant differences in activity levels between hibernation and late hibernation within corresponding temperature groups. We may assume that the documented temperature effects on activity levels within individual periods largely represent behavioural flexibility in the patterning of activity to reduce energy expenditure, whereas the between-periods differences would reflect seasonal behaviour associated with seasonal (e.g. the weakened state of bats by late hibernation and their need to improve body condition) and sexual (this could be particularly pressing for females before late gestation) differences in energetic demands imposed on the animals, and species differences.

Interestingly, variation in activity during the departure period explained by the climatic variables was the lowest of all periods, suggesting (1) that temperatures were high enough not to be a limiting factor; (2) a strong influence of endogenous rhythms on departure from hibernacula.

It seems that higher temperature fluctuations, that is high T_{max} and low T_{min}, suggesting a rapid decline in temperature towards morning, emphasize the bimodality in bat activity during summer. The activity patterns of insectivorous species are usually interpreted as a response to the times of food availability (Erkert, 1982). Activity can be limited to the beginning of the night, when the abundance of diurnal prey is higher (Jones & Rydell, 1994), and night roosts are occupied for relatively longer periods on cooler nights or when insect density is lower (Anthony *et al*., 1981). Thus, maximal foraging activity will probably occur in the beginning of the night and bats will make a greater use of the night roost departing during the third or fourth quarter of the night, which suggests before-dawn foraging and return to the day roost or just return. However, as meteorological variables explained only 28.8% of the variance in flight activity, other factors, such as spatiotemporal variability in insect resources, may influence the use of the cave.

Consistent with the results of Parsons *et al*. (2003), swarming activity was positively affected by an increase in T_{max} from day to day. This is probably related to higher insect availability during warmer nights, which enables rapid satiation before energetically costly flight and activity at the cave entrance.

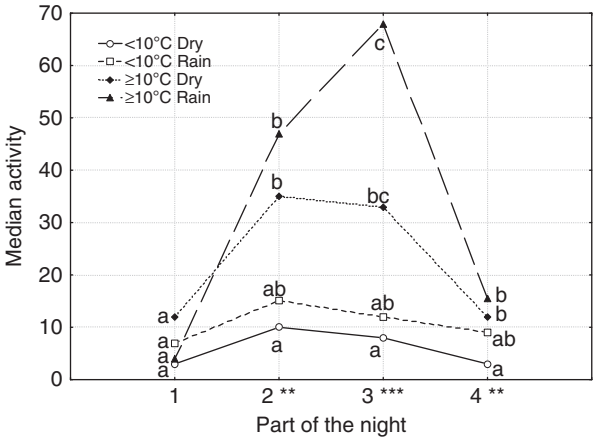


Figure 7 Combined effect of the range of daily temperature ($T_{dif\ max-min}$: $<10\,^{\circ}C$ or $\geq 10\,^{\circ}C$) and *R* (dry or rain) on the nightly pattern of activity during summer (5 June–26 July). Significant differences between groups in individual parts of the night, indicated by the Kruskal–Wallis (KW) test, are marked with **($P<0.01$) and ***($P<0.001$). Letters (a, b, c) indicate significant differences between groups calculated using multiple comparisons following the KW test.

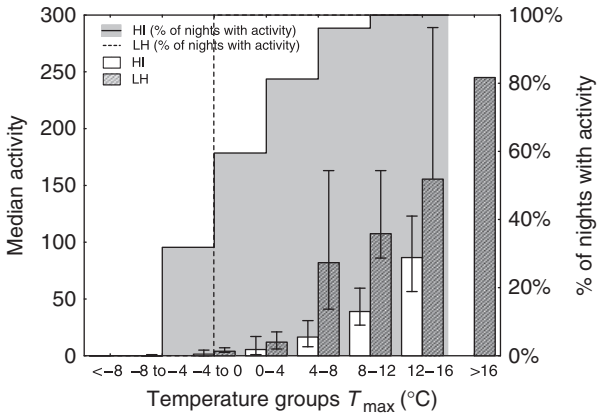


Figure 8 Activity levels (median values ± interquartile range) in individual temperature groups during hibernation (15 November–4 March) and late hibernation (5 March–14 April). The percentage of nights on which activity occurred in individual temperature groups is indicated by the grey area for the hibernation period and the dashed line denotes late hibernation.

Table 5 Activity levels (median values), number of nights and the percentage of nights on which activity occurred in individual temperature groups (T_{max} °C) during hibernation 15 November–4 March) and late hibernation (5 March–14 April)

Temperature groups	<−8	−8 to −4	−4 to 0	0–4	4–8	8–12	12–16	>16
HI								
Median activity	0	0	1.5	5.5	16.5	39	86.5	–
Number of nights	1	22	42	48	26	24	4	–
% with activity	0	31.82	59.52	81.25	96.15	100	100	–
LH								
Median activity	–	–	4	12	82	107.5	155.5	245
Number of nights	–	–	5	11	26	26	14	1
% with activity	–	–	100	100	100	100	100	100
MW								
z	–	–	–	1.674	4.804	5.253	–	–
P	–	–	–	0.094	**<0.001**	**<0.001**	–	–

Results of the Mann–Whitney (MW) *U*-test are given where appropriate. Significant results ($P<0.05$) are set in bold.
LH, late hibernation; HI, hibernation.

Rainfall

The most heterogeneous results were published on the effect of rainfall. The extent to which rain affects bat activity probably depends not only on its intensity but also on species-specific hunting tactics. Drizzle and light rain do not seem to have a significant effect on flight or foraging activity (Rydell, 1989; Řehák, 1995). Gaisler (1963a), Erickson & West (2002) and Ciechanowski *et al*. (2007) found a negative association of flight or foraging activity or time spent outside the roost with rain. In contrast, Swift (1980) did not. While Fenton (1969) and Parsons *et al*. (2003) found a negative relationship between autumn swarming activity and rainfall, Navo, Henry & Ingersoll (2002) did not. In the previous research in the Kateřinská cave, Berková & Zukal (2006) found no significant effect of rain on bat activity at the cave entrance during departure and autumn periods. Similarly, netting captures at the same cave were not influenced by rain (Řehák, 1995). The current study confirms these results only partially.

The increase in activity in summer and autumn with rainfall as well as our failure to detect any effect of rainfall in other periods could be due to bats sheltering, thus protected from rain at the entrance part of the Kateřinská cave, and the activity may remain high despite the rain. As our rainfall data are 24-h readings, it might also well be that it was raining during the daytime and the rain did not directly affect bat activity. However, bat activity could be affected indirectly through insect activity.

In general, previous day rainfall caused a decline in activity at the cave entrance. During summer, the highest activity levels were observed in nights with rainfall but without rainfall on the preceding day, consistent with the assumption that the cave serves as a shelter and night roost. In dry days, the cave is probably used as a night roost too. We assume that in these two cases, the bats successfully foraged the previous night and may thus afford to fly to the night roost. Moreover, in dry days, when foraging is more successful, satiation may motivate return to a night roost (Anthony *et al*., 1981). Activity was reduced when there was rain both on the preceding and on the study day. The bats probably (1) did not emerge from their day roosts; (2) emerged but returned to day roosts again or used some alternative roost closer to their foraging grounds. The lowest activity levels were observed on dry days with preceding rainfall. Previous night rainfall might negatively affect the food intake. Bats probably flew directly to their foraging ground and spent more time foraging.

During the departure period, the cave probably serves as a transitional roost as well and fewer bats probably switch the roost after bad weather.

Atmospheric pressure

Atmospheric pressure is the only environmental cue that bats roosting deep within caves could use to predict insect activity and abundance outside the roost (Paige, 1995). In this study, the activity was positively affected by P_{avg} during the departure and swarming periods, which contrasts with the results of other studies. Nagel & Nagel (1993) found a negative association of bat activity with atmospheric pressure during hibernation and assumed that low P often means warm weather during winter. Řehák (1995) found a negative correlation between activity at cave entrances and P only for *M. myotis*. Berková & Zukal (2006) reported a negative correlation between the number of bat passes through the entrance of Kateřinská cave and P_{avg}. These results were based on univariate analyses of correlation and should be interpreted with caution. However, Paige (1995) showed, using stepwise regression, that P alone explained 87% of the variation in bat activity at a roost during spring, late summer and early autumn, and was negatively correlated with activity.

Acknowledgements

We thank Miroslav Kovařík (CHKO Moravský kras) for field assistance and David Motl for construction and technical service of the monitoring device. Special thanks are due to Prof. Jiri Gaisler for his correction of the English text. This study was supported by grants GAČR 206/01/1555 and MŠM LC06073.

References

Anthony, E.L.P., Stack, M.H. & Kunz, T.H. (1981). Night roosting and the nocturnal time budget of the little brown bat, *Myotis lucifugus*: effects of reproductive status, prey density, and environmental conditions. *Oecologia* **51**, 151–156.

Berková, H. & Zukal, J. (2006). Flight activity of bats at the entrance of a natural cave. *Acta Chiropt*. **8**, 187–195.

Ciechanowski, M., Zajac, T., Bilas, A. & Dunajski, R. (2007). Spatiotemporal variation in activity of bat species differing in hunting tactics: effects of weather, moonlight, food abundance, and structural clutter. *Can. J. Zool*. **85**, 1249–1263.

Daan, S. (1973). Activity during natural hibernation in three species of vespertilionid bats. *Neth. J. Zool*. **23**, 1–71.

Degn, H.J., Andersen, B.B. & Baagoe, H. (1995). Automatic registration of bat activity through the year at Monsted Limestone Mine, Denmark. *Z. Säugetierkd*. **60**, 129–135.

Erickson, J.L. & West, S.D. (2002). The influence of regional climate and nightly weather conditions on activity patterns of insectivorous bats. *Acta Chiropt*. **4**, 17–24.

Erkert, H.G. (1982). Ecological aspects of bat activity rhythms. In *Ecology of bats*: 201–242. Kunz, T.H. (Ed.). New York: Plenum Press.

Fenton, M.B. (1969). Summer activity of *Myotis lucifugus* (Chiroptera: Vespertilionidae) at hibernacula in Ontario and Quebec. *Can. J. Zool*. **47**, 597–602.

Gaisler, J. (1963*a*). Nocturnal activity in the lesser horseshoe bat, *Rhinolophus hipposideros* (Bechstein, 1800). *Zool. listy* **12**, 223–230.

Gaisler, J. (1963*b*). The ecology of lesser horseshoe bat (*Rhinolophus hipposideros* Bechstein, 1800) in Czechoslovakia, Part I. *Věst. Čs. Spol. Zool.* **27**, 211–233.

Harrje, C. (1994). Etho-ökologische Untersuchung der ganzjahrigen Aktivität von Wasserfledermausen (*Myotis daubentonii* Kuhl, 1819) am Winterquartier. *Mitt. natforsch. Ges. Schaffhausen* **39**, 15–52.

Horáček, I. & Zima, J. (1978). Net-revealed cave visitation and cave-dwelling in European bats. *Folia Zool.* **27**, 135–148.

Jones, G. & Rydell, J. (1994). Foraging strategy and predation risk as factors influences emergence time in echolocating bats. *Philos. Trans. Roy. Soc. Lond. Ser. B* **346**, 445–455.

Nagel, A. & Nagel, R. (1993). Activity of hibernating bats in their natural habitat recorded by automatic data logging. *VI European Bat Research Symposium*, Évora, Portugal, 22–27 August 1993, pp. 37–38.

Navo, K.W., Henry, S.G. & Ingersoll, T.E. (2002). Observations of swarming by bats and band recoveries in Colorado. *West. N. Am. Nat.* **62**, 124–126.

Paige, K.N. (1995). Bats and barometric pressure: conserving limited energy and tracking insects from the roost. *Funct. Ecol.* **9**, 463–467.

Park, J.K., Jones, G. & Ransome, R.D. (2000). Torpor, arousal and activity of hibernating greater horseshoe bats (*Rhinolophus ferrumequinum*). *Funct. Ecol.* **14**, 580–588.

Parsons, K.N., Jones, G. & Greenaway, F. (2003). Swarming activity of temperate zone microchiropteran bats: effects of season, time of night and weather conditions. *J. Zool. (Lond.)* **261**, 257–264.

Pokorný, M., Berková, H., Gaisler, J., Řehák, Z. & Zukal, J. (2003). Summer occurrence of bats in buildings in the Moravian Karst and its wider surroundings. *Vespertilio* **7**, 161–168.

Ransome, R.D. (1971). The effect of ambient temperature on the arousal frequency of the hibernating greater horseshoe bat, *Rhinolophus ferrumequinum*, in relation to site selection and the hibernation state. *J. Zool. (Lond.)* **164**, 353–371.

Ransome, R.D. (1990). *The natural history of hibernating bats*. London: Christopher Helm.

Řehák, Z. (1995). *Flight activity of bats in the Moravian Karst*. Dissertation thesis, PřF MU Brno, 184pp.

Řehák, Z., Zukal, J. & Kovařík, M. (1994). Long- and short-term changes in the bat community of the Kateřinská cave (Moravian karst) – a fundamental assessment. *Folia Zool.* **43**, 425–436.

Rivers, N.M., Butlin, R.K. & Altringham, J.D. (2006). Autumn swarming behaviour of Natterer's bats in the UK: population size, catchment area and dispersal. *Biol. Conserv.* **27**, 215–226.

Rydell, J. (1989). Feeding activity of the northern bat *Eptesicus nilssoni* during pregnancy and lactation. *Oecologia* **80**, 562–565.

Schofield, H.W. (1996). *The ecology and conservation biology of Rhinolophus hipposideros, the lesser horseshoe bat*. Dissertation thesis, University of Aberdeen, 197pp.

Sendor, T., Kugelschafter, K. & Simon, M. (2000). Seasonal variation of activity patterns at a pipistrelle (*Pipistrellus pipistrellus*) hibernaculum. *Myotis* **38**, 91–109.

Skiba, R. (1987). Bestandsentwicklung und Verhalten von Fledermäusen in einem Stollen des Westharzes. *Myotis* **25**, 95–103.

Speakman, J.R. & Racey, P.A. (1989). Hibernal ecology of the pipistrelle bat: energy expenditure, water requirements and mass loss, implications for survival and the function of emergence flights. *J. Anim. Ecol.* **58**, 797–813.

Swift, S.M. (1980). Activity pattern of Pipistrelle bats (*Pipistrellus pipistrellus*) in north-east Scotland. *J. Zool. (Lond.)* **190**, 285–295.

Zukal, J., Berková, H. & Řehák, Z. (2005). Activity and shelter selection by *Myotis myotis* and *Rhinolophus hipposideros* hibernating in the Kateřinská cave (Czech Republic). *Mamm. Biol.* **70**, 271–281.

Zukal, J., Kovařík, M., Řehák, Z. & Berková, H. (2001). Numbers of bats hibernating in two caves in the northern part of Moravian Karst and their long-term changes. *Vespertilio* **5**, 321–328.

Výletová a návratová aktivita u vchodu do úkrytu

Zukal, J., Berková, H. & Madaraszová, J. (submitted) Flying or sleeping: Flight activity of bats in natural cave with confirmed WNS. Folia Zoologica.

Flying or sleeping: Flight activity of bats in natural cave with confirmed WNS

Jan ZUKAL*, Hana BERKOVÁ and Jana MADARASZOVÁ

Institute of Vertebrate Biology AS CR v.v.i., Květná 8, 603 65 Brno, Czech Republic; e-mail: zukal@ivb.cz, berkova.hana@gmail.com, madaraszova@sci.muni.cz

Abstract

White-nose Syndrome (WNS) decimates bat populations in North America but similar impact was not registered in Europe. WNS-affected bats exhibit abnormal hibernation behavior that prematurely deplete fat reserves and ultimately causes death by starvation. In the deep hibernation period (December – March) of 2006/07 (pre-WNS) and 2010/11 (post-WNS), we monitored bat hibernation behavior and flight activity to test potential impact of WNS on European bats. We registered no abnormal changes in bat hibernation behavior (movement to visible sites, utilization of dynamic cave sections), flight activity level, its direction or seasonal pattern remained unchanged following WNS infection. Flight activity inside the cave and at its entrance was generally low during deep hibernation period and temperature remained the best predictor of activity level. In general, stable hibernation behavior and activity patterns suggests that they are apparently optimized for European winter conditions and support the hypothesis that the fungus has been present in Europe for a long time and has only recently invaded North America.

Keywords

WNS, bat activity, hibernation behavior, *Myotis myotis*, hibernacula

Introduction

Hibernation is a characteristic annual feature of the annual cycle of temperate zone bats and represents is hibernation, as an optimal energetic adaptation to a prolonged decline in ambient temperature and reduction in prey availability. Overwinter survival is influenced by many critical factors, including amount of stored energy, level and length of torpor and hibernacula, and microhabitat selection. While deep hibernation may be is interrupted by periods of arousal, which requires costly thermogenesis (internal heat production) (Thomas et al. 1990), flight activity of bats is minimal (Berková & Zukal 2006). Arousal may occur for a number of behavioral and physiological reasons, including switching of hibernation site, drinking, feeding (in mild periods or regions), excretion, or even mating (Zukal et al. 2005, Hope & Jones 2012). Regular arousal may also boost the immune system as hibernation is known to negatively affect the innate and adaptive immune systems (Bouma et al. 2010). These factors are not mutually exclusive, and the reason for arousal may depend largely on the ecology of a given species, its distribution and the local environment (Hope & Jones 2012).

A previous study on bat flight activity at the cave entrance showed a non-random temporal distribution of activity shortly after the sunset. In general, flight activity remained nocturnal (between sunset and sunrise) and was associated with regular periods of arousal (Berková & Zukal 2006). Temperature appears to be the best exogenous predictor of the activity level and course of hibernation, the percentage of nights on which activity occurs during hibernation increasing with increasing temperature (Berková & Zukal 2010).

Several bat species in North America and Europe are presently threatened by White-nose Syndrome (WNS). This new infectious disease was discovered in February 2006 in a cave in the north-east of the USA (Blehert et al. 2009, Puechmaille et al. 2011a) and is associated with a newly identified psychrophylic fungus *Pseudogymnoascus destructans* (Gargas et al. 2009, Kubátová et al. 2011). Since 2006 it has spread to caves throughout the eastern part of the USA. Hibernating bat populations at impacted localities have experienced dramatic population declines ranging from 30-

100% (Blehert et al. 2009, Frick et al. 2010). Presence of WNS was not confirmed in Europe prior to 2008, but has subsequently been identified at many localities from France to Turkey (Puechmaille et al. 2011b, Pikula et al. 2012). In contrast with sites in North America, *P. destructans* in Europe does not appear to be associated with dramatic bat mortalities (Puechmaille et al. 2011a). In the Czech Republic, only slight population fluctuations have been observed in the most affected species, *Myotis myotis*, lying within the population trend predictions (Martínková et al. 2010).

WNS-affected bats typically have a visible white cover on the muzzles, nose, wings and ears (Courtin et al. 2010, Wibbelt et al. 2010), and exhibit abnormal hibernation behavior that results in premature fat reserve depletion (Blehert et al. 2009, Boyles & Willis 2010, Puechmaille et al. 2011a). Such as more frequent and unusual arousals during hibernation, winter day-flight activity, premature emergence from hibernacula, and roosting near entrances of hibernacula entrances (Hallam & Federico 2012, Reeder et al. 2012). The first *P. destructans* suspected bats are noted in January/February, but their number is the highest in March (Puechmaille et al. 2011b, Sachanowicz et al. 2014), when maximum number of hibernating *M. myotis* is observed.

Our objective was to test the influence of WNS on European bat hibernation behavior and flight activity under natural conditions by repeating previous research at the same site carried out prior to WNS detection (Zukal et al. 2005, Berková & Zukal 2006, 2010), thus allowing a comparison between "unknown" (or unaffected) and WNS-affected stages of the bat community. We predict that if behavior is affected by WNS, hibernating bats will exhibit abnormal flight activity (higher level and sooner onset) both inside the cave and at the cave entrance, respectively, and different hibernation pattern.

Material and Methods

Hibernation behavior was studied at a regularly monitored natural limestone hibernaculum (Kateřinská cave, Czech Republic). Cave total length is around 500 m with one entrance that is closed by an iron gate with a vertical hole in its upper part (Berková & Zukal 2006). The cave consists of two

main habitats, stable temperature sections and an outer (dynamic) section. Two species, *M. myotis* and *Rhinolophus hipposideros*, dominate the hibernating bat community, representing over 80% of individuals. WNS-affected specimens (*M. myotis*) were first registered in the winter season 2008/09 even there was realized intensive winter bat research from 1992 (Zukal et al. 2005). WNS prevalence now approaches 2-3 % of hibernating bats (Horáček et al. 2014 and unpublished data). The presence of WNS was repeatedly confirmed by UV trans-illumination and histopathology (Turner et al. 2014, Zukal et al. 2014) in various bat species. Although *Myotis emarginatus, M. daubentonii, M. nattereri* and *M. bechsteinii* are rarely found hibernating inside the cave, they are dominant in netting samples from the cave entrance (Řehák et al. 1994).

The study was undertaken over two winter seasons before (2006/07) and after (2010/11) WNS outbreak. Only data from the „deep hibernation" period were analyzed, i.e. from mid-December to mid-March (Zukal et al. 2005). Seven biweekly controls were realized which included visual monitoring (no handling of hibernating bats minimizing any disturbance (Zukal et al. 2005)) and observation bat flight activity in the cave. Bat position was registered along with species present and clustering behavior. Small species of *Myotis* genus were pooled as *Myotis* sp. group as we were unable to determine all of them exactly. At the same days, flight activity inside the cave was observed using a Pathfinder 2000s night-vision scope one hour before sunset and two hour after sunset. The observer, sitting at narrow stairs separating the exit corridor from inner cave, recorded time and direction (IN vs. OUT) of each bat.

Bat movements through the gate hole were monitored continuously by a custom-made double infrared-light barrier connected to a data-logger. This system allows discrimination between bats leaving and those entering the cave (Berková & Zukal 2010), though species identification is not possible. After each bat passes the gate, time (hour and minute) and direction were stored by data-logger. As bats can fly in and out through the entrance hole several times, the number of bat passes will not always equal the number of active bats. However, this was used as a measure of activity

entitled “emergence activity”. Reliable data were collected for 65 days pre-WNS and 79 post-WNS; these data being pooled into two-week periods for subsequent analyses.

Ambient outside temperature data were obtained from weather station at Macocha abyss, 1,5 km from the study locality. An average of 24 hourly measurements was used for statistical analysis. The data logger malfunctioned between 21.2.2011 11:00 PM and 23.2.2011 9:00 PM due to extremely low temperatures.

Daily average temperatures for both sample seasons were compared using the t-test, while level of bat fluctuation during hibernation was evaluated using the Pearson Chi-square test. Nonparametric tests (Mann-Whitney U-test, Kruskal-Wallis ANOVA, Spearman correlation coefficient) were used for all other analyses as data could not be normalized. Two sided binomial test was calculated to compare the proportion of nights without bat activity. All statistical analyses were performed by Statistica for Windows 10.0 (Zar 1998).

Results

Highest numbers of hibernating *M. myotis* were registered near the end of deep hibernation in both seasons, though there was no statistically significant differences (Pearson Chi-square tests) in the model of bat number fluctuation (Table 1). *M. myotis* tended to move to dynamic parts of cave during winter, with their dominance in the hibernating bat guild increasing to nearly 50%.

Flight activity inside the cave was low and onset was desynchronized i.e. starting before sunset (Fig. 1). Mean ambient temperature differed between winter seasons, with winter 2006/07 (before WNS) being significantly warmer (t-test, $t = 6.95$, $p < 0.001$, d.f. = 179). During winter 2006/07, the activity started earlier and reached higher level; with highest activity noted during the first hour after sunset (Table 2). However, median flight activity and flight direction (IN *vs.* OUT) did not differ significantly between winters (Mann-Whitney test). General activity patterns, therefore, remained the same after the appearance of WNS.

Overall, there was no significant difference in total level of emergence activity at the cave entrance (Mann-Whitney test, $z = 1.65$, $p = 0.10$, $n_1 = 65$, $n_2 = 79$) or the ratio of IN *vs.* OUT flights (Mann-Whitney test, $z = -0.01$, $p = 0.99$, $n_1 = 65$, $n_2 = 79$) between the two study seasons, i.e. bats did not leave the hibernaculum more often following outbreak of WNS (Fig. 2). Similarly, there was no difference in the proportion of nights with no emergence activity (Binomial test, $p = 0.43$, $n_1 = 65$, $n_2 = 79$). Total flight activity was correlated with mean ambient temperature (Spearman correlation, $r_S = 0.43$, $n = 65$, $p < 0.05$ in 2006 and $r_S = 0.90$, $n = 79$, $p < 0.05$ in 2010), which also influenced the overall course of emergence activity. Nevertheless, activity fluctuation was higher during 2010/11 (Kruskal-Wallis ANOVA, Table 3), probably due to the colder winter.

Discussion

Since its discovery, WNS has killed millions of bats in northeastern USA and Canada. Such large-scale mortalities have not been documented in Europe, however, despite *P. destructans* being presently widespread (Martínková et al. 2010, Puechmaille et al. 2011b). This suggests that fungus may have been introduced to North America and European bats having developed immunity or evolved genetic or behavioral resistance to pathogen following historical exposure to *P. destructans* (Martínková et al. 2010, Puechmaille et al. 2011a, Leopardi et al. 2015). Moreover, previously published experimental study demonstrated that isolates of *P. destructans* from North America and Europe were both lethal to a North American bat species (Warnecke et al. 2012).

In our study, we registered no abnormal changes in bat hibernation behavior, activity level or its seasonal pattern after WNS occurrence. Flight activity inside the cave and at its entrance was generally low during deep hibernation period and temperature remained the best predictor of activity level (Berková & Zukal 2006, 2010). Higher ambient temperatures resulted in an increase in flight activity of bats and increasingly desynchronized activity onset, bats leaving the cave even during January in some cases. Such activity patterns largely reflected the findings from *Rhinolophus ferrumequinum* studied by Park et al. (1999) at caves in south-west England. We believe, however,

that winter cave emergence was caused by foraging activity of bats and not disturbance due to *P. destructans* infection, despite laboratory grown *P. destructans* showing optimal growth at temperatures typical in hibernacula (i.e. 3–15°C (Gargas et al. 2009, Verant et al. 2012)) and declines in *Myotis lucifugus* were higher in hibernacula with higher temperatures (Langwig et al. 2012). Increased flight activity under such conditions may be protective adaptation as it is always coupled with grooming behavior that damages the fungus cover. The lack of a continual increase of prevalence of *P. destructans* suspected bat in late March was registered in abandoned Polish ore mine and seems to be related to increasing arousals and activity of bats too (Sachanowicz et al. 2014). Nevertheless, the absence of visible white fungal growth on bats does not mean the lack of *P. destructans* infection.

Flight direction, movement to visible sites, utilization of dynamic cave sections, and emergence activity also remained unchanged following WNS infection, with activity patterns apparently optimized for European winter conditions (Fuszara et al. 1996, Zukal et al. 2005, Berková & Zukal, 2006). Moreover, movement of bats to the colder and drier parts of cave may result in a decline *P. destructans* growth and such roosts will provide a refuge from disease (Langwig et al. 2012, Verant et al. 2012). This will be most important at the end of hibernation when European bats are maximally immunosuppressed and show high prevalence of infection (Martínková et al. 2010, Puechmaille et al. 2011a). Bats hibernating in colder outer spaces will save more fat stores for the rest of hibernation (Thomas et al. 1990, Boyles and McKechnie, 2010).

Observed behavioral adaptations should help hibernating bats to survive pathogen attacks and they should be a result of previous evolutionary development when WNS acted as a strong selection force (Martínková et al. 2010). Therefore, low prevalence of WNS (2 – 3 % of bats) registered at locality under study combined with stable hibernation behavior support the hypothesis that the fungus has been present in Europe for a long time and has only recently invaded North America. Alternatively, low prevalence should bias our results and the behavioral changes cannot be detected under such conditions.

Acknowledgement

The authors thank Miroslav Kovařík and many other colleagues for their help during field work. We also thank Zdeněk Řehák and Jiří Gaisler for critically reading an earlier version of this manuscript. We are grateful to Kevin Roche for his correction and improvement of the English text. This study was supported by the grant of GA CR 506/12/1064, and through institutional support grant RVO:68081766.

Literature

Berková H. & Zukal J. 2006: Flight activity of bats at the entrance of a natural cave. *Acta Chiropt. 8: 187–195.*

Berková H. & Zukal J. 2010: Cave visitation by temperate zone bats: effects of climatic factors. *J. Zool. (Lond.) 280: 387–395.*

Blehert D.S., Hicks A.C., Behr M., Meteyer C.U., Berlowski-Zier B.M., Buckles E.L., Coleman J.T.H., Darling S.R., Gargas A., Niver R., Okoniewski J.C., Rudd R.J. & Stone W.B. 2009: Bat white-nose syndrome: an emerging fungal pathogen? *Science 323: 227.*

Bouma H.R., Carey H.V. & Kroese F.G. 2010: Hibernation: the immune system at rest? *J. Leukoc. Biol. 88: 619–624.*

Boyles J.G. & McKechnie A.E. 2010: Energy Conservation in Hibernating Endotherms: Why "Suboptimal" Temperatures Are Optimal. *Ecol. Model. 221: 1644–1647*.

Boyles J.G. & Willis C.K.R. 2010: Could Localized Warm Areas Inside Cold Caves Reduce Mortality of Hibernating Bats Affected by White-Nose Syndrome? *Front. Ecol. Environ. 8: 92–98*.

Courtin F., Stone W.B., Risatti G., Gilbert K. & Van Kruiningen H.J. 2010: Pathologic Findings and Liver Elements in Hibernating Bats With White-Nose Syndrome. *Vet. Pathol. 47: 214–219.*

Frick W.F., Pollock J.F., Hicks A.C., Langwig K.E., Reynolds D.S., Turner G.G., Butchkoski C.M. & Kunz T.H. 2010: An emerging disease causes regional population collapse of a North American bat species. *Science 329: 679–682*.

Fuszara E., Kowalski M., Lesinski G. & Cygan J.P. 1996: Hibernation of bats in underground shelters of central and northeastern Poland. *Bonn. Zool. Beitr. 46: 349–358*.

Gargas A., Trest M.T., Christensen M., Volk T.J. & Blehert D.S. 2009: *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. *Mycotaxon 108: 147–154.*

Hallam T.G. & Federico P. 2012: The Panzootic White-nose Syndrome: An Environmentally Constrained Disease? *Transboundary and Emerging Diseases 59: 269–278.*

Hope P.R. & Jones G. 2012: Warming up for dinner: torpor and arousal in hibernating Natterer's bats (*Myotis nattereri*) studied by radio telemetry. *J. Comp. Physiol. B 182: 569–578*.

Horáček I., Bartonička T., Lučan R.K. & the Czech Bat Conservation Trust 2014: Macroecological characteristics of bat geomycosis in the Czech Republic: results of five years of monitoring. *Vespertilio 17: 65–77.*

Kubátová A., Koukol O. & Nováková A. 2011: *Geomyces destructans*, phenotypic features of some Czech isolates. *Czech Mycology, 63: 65–75*.

Langwig K.E., Frick W.F., Bried J.T., Hicks A.C., Kunz T.H. & Kilpatrick A.M. 2012: Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecol. Lett. 15: 1050–1057.*

Leopardi S., Blake D. & Puechmaille S.J. 2015: White-nose syndrome fungus introduced from Europe to North America. *Curr. Biol. 25: 217-219.*

Martínková N., Bačkor P., Bartonička T., Blažková P., Červený J., Falteisek L., Gaisler J., Hanzal V., Horáček D., Hubálek Z., Jahelková H., Kolařík M., Korytár L., Kubátová A., Lehotská B., Lehotský R., Lučan R.K., Májek O., Matějů J., Řehák Z., Šafář J., Tájek P., Tkadlec E., Uhrin M., Wagner J., Weinfurtová D., Zima J., Zukal J. & Horáček I. 2010: Increasing incidence of *Geomyces destructans* fungus in bats from the Czech Republic and Slovakia. *PLoS ONE 5: e13853.*

Park K.J., Jones G. & Ransome R.D. 1999: Winter activity of a population of greater horseshoe bats (*Rhinolophus ferrumequinum*). *J. Zool. (Lond.) 248: 419–427.*

Pikula J., Banďouchová H., Novotný L., Meteyer C.U., Zukal J., Irwin N.R., Zima J. & Martínková N. 2012: Histopathology Confirms White-Nose Syndrome in Bats in Europe. *Journal of Wildlife Diseases 48: 207-211.*

Puechmaille S.J., Frick W.F., Kunz T.H., Racey P.A., Voigt C.C., Wibbelt G. & Teeling E.C., 2011a: White-nose syndrome: is this emerging disease a threat to European bats? *Trends Ecol. Evol. 26: 570–576.*

Puechmaille S.J., Wibbelt G., Korn V., Fuller H., Forget F., Mühldorfer K., Kurth A., Bogdanowicz W., Borel C., Bosch T., Cherezy T., Drebet M., Görföl T., Haarsma A.J., Herhaus F., Hallart G., Hammer M., Jungmann C., Le Bris Y., Lutsar L., Masing M., Mulkens B., Passior K., Starrach M., Wojtaszewski A., Zöphel U. & Teeling E.C. 2011b: Pan-European Distribution of White-Nose Syndrome Fungus (*Geomyces destructans*) Not Associated with Mass Mortality. *PLoS ONE 6: e19167.*

Reeder D.M., Frank C.L., Turner G.G., Meteyer C.U., Kurta A., Britzke E.R., Vodzak M.E., Darling S.R., Stihler C.W., Hicks A.C., Jacob R., Grieneisen L.E., Brownlee S.A., Muller L.K. & Blehert D.S. 2012: Frequent Arousal from Hibernation Linked to Severity of Infection and Mortality in Bats with White-Nose Syndrome. *PLoS ONE 7: e38920.*

Řehák Z., Zukal J. & Kovařík, M. 1994: Long- and short-term changes in the bat community of the Kateřinská cave (Moravian karst) – fundamental assessment. *Folia Zool. 43: 425–436.*

Sachanowicz K., Stepien A. & Ciechanowski M. 2014: Prevalence and phenology of white-nose syndrome fungus *Pseudogymnoascus destructans* in bats from Poland. *Cent. Eur. J. Biol. 9: 437-443.*

Thomas D.W., Dorais M. & Bergeron J.M. 1990: Winter energy budgets and cost of arousals for hibernating little brown bats, *Myotis lucifugus*. *J. Mammal. 71: 475–479.*

Turner G.G., Meteyer C.U., Barton H., Gumbs J.F., Reeder D.A., Overton B., Banďouchova H., Bartonička T., Martínková N., Pikula J., Zukal J. & Blehert D.S. 2014: Nonlethal screening of

bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. *Journal of Wildlife Diseases 50: 566-573.*

Verant M.L., Boyles J.G., Waldrep W. Jr., Wibbelt G. & Blehert D.S. 2012: Temperature-Dependent Growth of *Geomyces destructans*, the Fungus That Causes Bat White-Nose Syndrome. *PLoS ONE 7: e46280.*

Warnecke L., Turner J.M., Bollinger T.K., Lorch J.M., Misra V., Cryan P.M., Wibbelt G., Blehert D.S. & Willis C.K.R. 2012: Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proc. Natl. Acad. Sci. USA 109: 6999–7003.*

Wibbelt G., Kurth A., Hellmann D., Weishaar M., Barlow A., Veith M., Prüger J., Görföl T., Grosche L., Bontadina F., Zöphel U., Seidl H.P., Cryan P.M. & Blehert D.S. 2010: White-Nose Syndrome fungus (*Geomyces destructans*) in bats, Europe. *Emerg. Infect. Dis. 16: 1237–1242.*

Zar J. H. 1998: Biostatistical Analysis, 3rd edn. New Jersey, Upper Saddle River: Prentice Hall, Inc.

Zukal J., Banďouchová H., Bartonička T., Berková H., Brack V., Brichta J., Dolinay M., Jaroň K.S., Kováčová V., Kovařík M., Martínková N., Ondráček K., Řehák Z., Turner G.G. & Pikula J. 2014: White-Nose Syndrome Fungus: A Generalist Pathogen of Hibernating Bats. *PLoS One 9: e97224.*

Zukal J., Berková H. & Řehák Z. 2005: Activity and shelter selection by *Myotis myotis* and *Rhinolophus hipposideros* hibernating in the Kateřinská cave (Czech Republic). *Mamm. Biol. 70: 271–281.*

Table 1. Total number of bats, number of hibernating *M. myotis* registered during deep hibernation and their dominance in dynamic parts of cave during winter seasons. Significant results ($p < 0.05$) are set in bold.

Table 2. Parameters of bat flight activity registered by night vision scope inside the cave. Explanations: IN-OUT difference – the difference between the numbers of bats flying in deeper parts of cave and from this part to the Corridor. Significant results ($p < 0.05$) are set in bold.

Table 3. Results of Kruskal-Wallis ANOVA on the total level of emergence activity at the cave entrance and the ratio of IN *vs.* OUT flights during two seasons under study. Significant results ($p < 0.05$) are set in bold.

Figure 1. The percentage of flight activity inside the cave during ten-minute periods. The periods before sunset are indicated by the grey area.

Figure 2. Total level of emergence activity of bats at the cave entrance. Bat activity has not been affected by WNS and the bats did not leave the hibernaculum more often. Data pooled into two-week periods. Middle point, median; box, interquartile range; whisker, non-outlier range.

Table 1

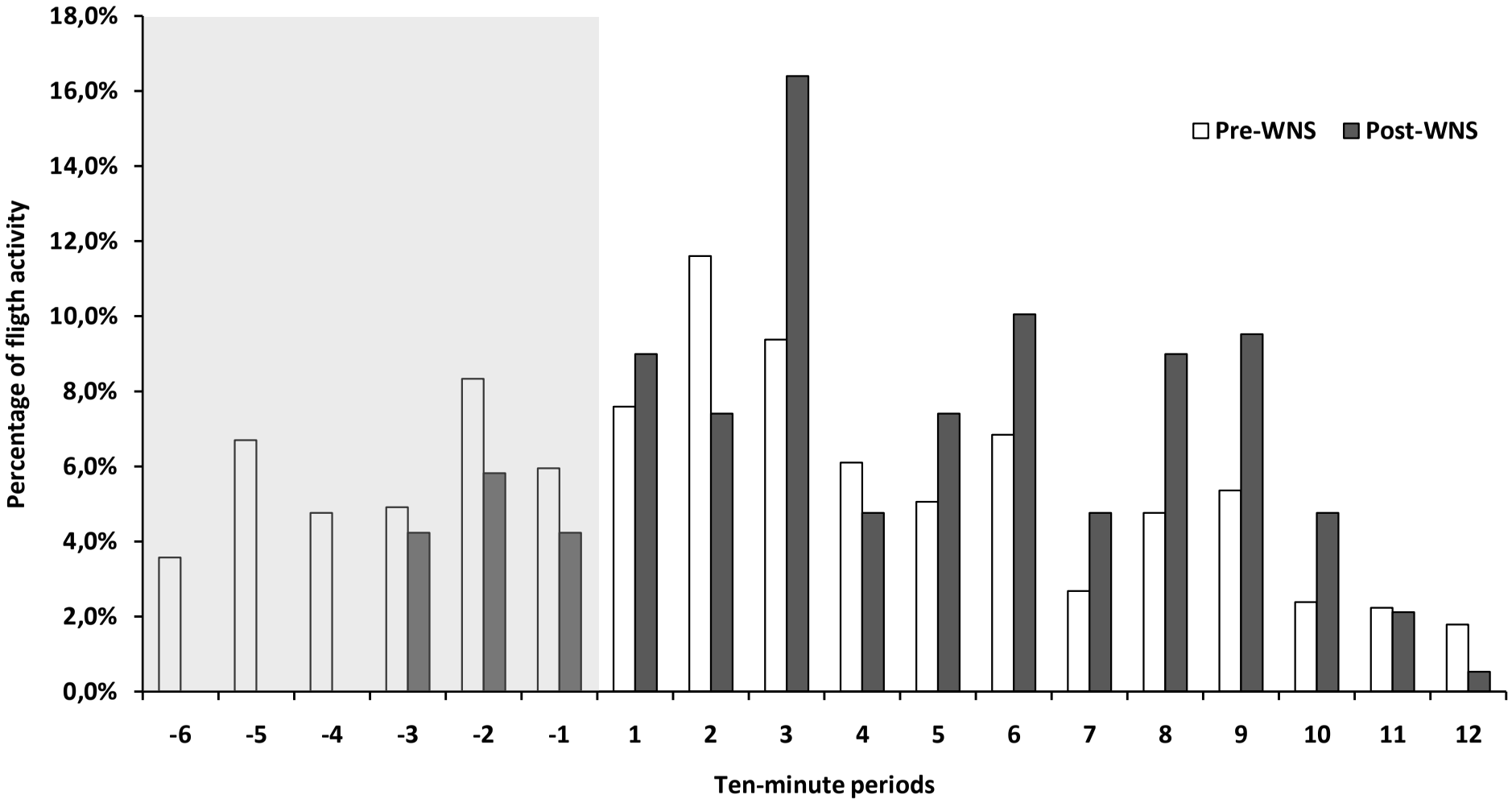
	No. of bats		No. of *Myotis myotis*		*Myotis myotis* dynamic part	
Month	Pre-WNS	Post-WNS	Pre-WNS	Post-WNS	Pre-WNS	Post-WNS
XII	138	104	31	23	38.7%	47.8%
XII/I	192	137	67	41	61.2%	65.9%
I	227	190	89	62	73.0%	75.8%
I	228	199	93	72	77.4%	79.2%
II	256	229	114	97	84.2%	82.5%
II	282	233	125	108	83.2%	85.2%
III	268	251	128	120	85.2%	89.2%
Perason Chi-square test	5.019		4.724		0.008	
p	0.541		0.580		1.000	

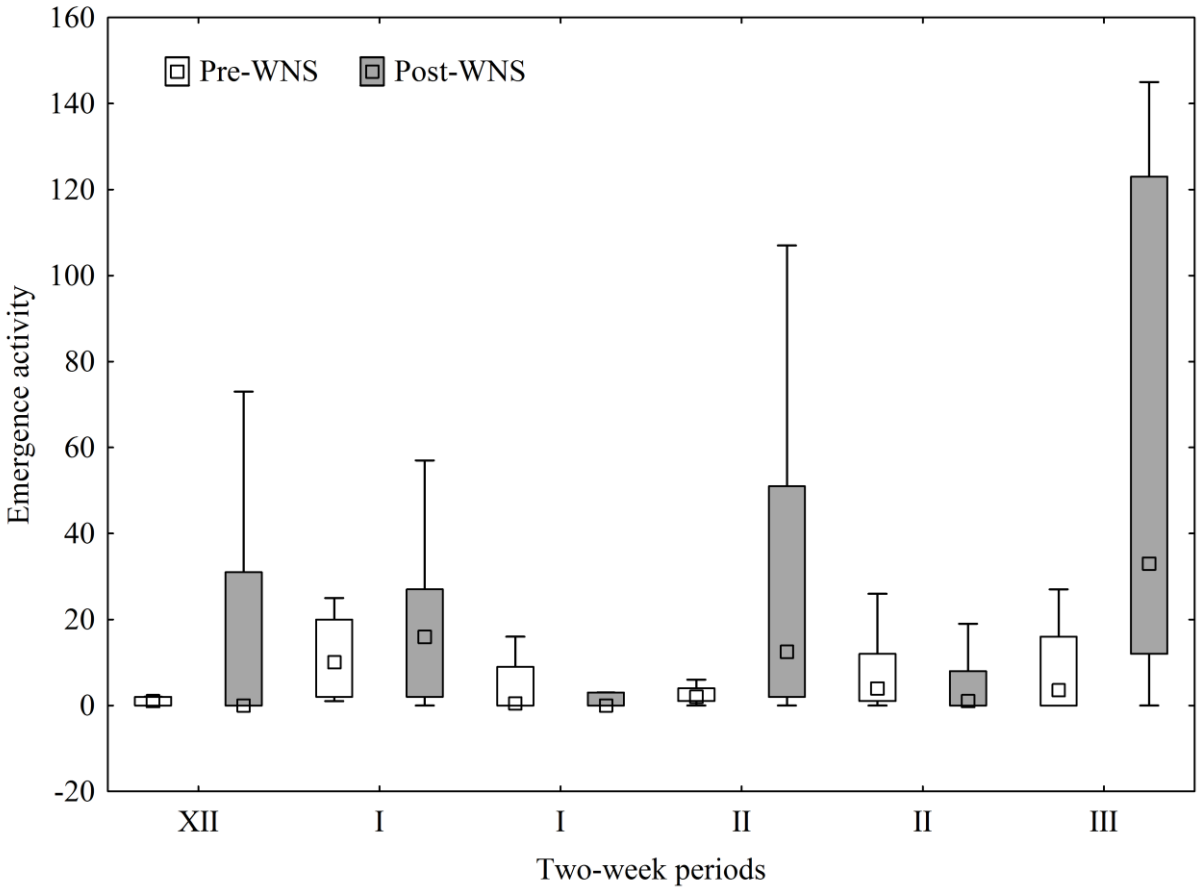
Table 2

	Total level of activity		IN-OUT difference		Onset of activity after sunset		Median of activity after sunset	
Month	Pre-WNS	Post-WNS	Pre-WNS	Post-WNS	Pre-WNS	Post-WNS	Pre-WNS	Post-WNS
XII	19	14	1	0	-39	29	-4	32
XII/I	79	5	-3	-1	-53	-17	24	5
I	59	56	3	-2	-48	-18	36	34
I	63	58	1	0	-57	-29	8	26
II	99	3	1	-1	-43	26	43	26
II	157	0	7	0	-58		7	
III	196	53	-24	1	-60	-30	4	57
Mann-Whitney U test	3.0		15.5		0.0		13.0	
p	**0.007**		0.277		**0.003**		0.284	

Table 3

Month	Pre-WNS			Post-WNS		
	n	H	p	n	H	p
Total level of bat activity	65	29007	0.24	79	23.26	**< 0.001**
IN-OUT difference	65	19419	0.62	79	16.13	**0.007**


18,0%
16,0%
14,0%
12,0%
10,0%
8,0%
6,0%
4,0%
2,0%
0,0%
Percentage of fligth activity
Pre-WNS
Post-WNS
-6
-5
-4
-3
-2
-1
1
2
3
4
5
6
7
8
9
10
11
12
Ten-minute periods


Pre-WNS
Post-WNS
Emergence activity
160
140
120
100
80
60
40
20
0
-20
XII
I
I
II
II
III
Two-week periods

Antipredační chování během výletové a návratové aktivity

Petrželková, K. & Zukal, J. (2001) Emergence behaviour of the serotine bat (*Eptesicus serotinus*) under predation risk. Netherlands Journal of Zoology 51, 395-414.

Netherlands Journal of Zoology
51 (4): 395-414 (2001)

EMERGENCE BEHAVIOUR OF THE SEROTINE BAT (*EPTESICUS SEROTINUS*) UNDER PREDATION RISK

by

KLÁRA PETRŽELKOVÁ [1,2] and JAN ZUKAL [2]

([1] *Department of Zoology and Ecology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic. E-mail: petrzel@sci.muni.cz*;
[2] *Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic. E-mail: zukal@brno.cas.cz*)

ABSTRACT

Emergence activity of a maternity colony of *Eptesicus serotinus* was monitored from May to August 1997 and 1998 at Střelice, Czech Republic. We focused mainly on the impact of predation risk on emergence parameters, but the effects of reproductive and climatic factors were assessed too. Observations were made with 10-days intervals during two consecutive nights from which the first was a control and the second a treatment night. On treatment nights a stuffed specimen of *Tyto alba* (in 1997) or *Falco tinnunculus* (in 1998) was placed close to the roost exits and recorded calls of the particular species were played back towards the roost. In 1997 the bats emerged earlier during lactation than during gravidity, while in 1998 the trend was opposite. This could be explained by a different course of food availability in each year. During poor weather the number of emerged bats decreased and bats probably used an alternative roost. The predation risk did not affect the values of any emergence parameter but induced changes in relationships among emergence parameters. When bats emerged earlier and thus were exposed to increased potential predation pressure, they increased their degree of clustering to decrease the probability of being attacked. The perception of predation risk was not affected by weather conditions or reproductive period.

KEY WORDS: *Eptesicus serotinus*, emergence, predation risk, climatic factors, reproductive period.

INTRODUCTION

During the reproductive period of many bat species, females aggregate in roosts to form maternity colonies, where the young are born and raised to independence. These colonies vary in size from less than ten to several thousand individuals, depending on the species involved (KUNZ, 1982). One potential cost of living in groups is the risk of predation and the attractiveness of bat colonies to predators is enhanced by predictable emergences through small openings (ERKERT, 1982). However, the records of predators such as nocturnal and diurnal raptors, snakes or other carnivores taking bats emerging from roosts are sporadic

(*e.g.*, ALLEN, 1939; GILLETTE & KIMBROUGH, 1970; HILL & SMITH, 1984; BARCLAY *et al.*, 1982; FENTON *et al.*, 1994).

Very few predator species make bats a major component of their diet, except tropical bat hawks *Macheiramphus alcinus* and *Falco rufigularis* (CADE, 1987; BROWN, URBAN & NEWMAN, 1983) and probably *Strix nigrolineata* (IBÁÑEZ *et al.*, 1992; GERHARDT, 1994). But individuals of some predatory bird species, *e.g.*, *Tyto alba* (BAUER, 1956), *Strix aluco* (OBUCH, 1992) or *Falco tinnunculus* (NEGRO *et al.*, 1992) have specialized on catching bats. SPEAKMAN (1991) assessed that predation by avian predators would account for about 11% of the annual mortality of British bats despite the apparent low representation of bats in the diets of predatory birds. So the effects of predation on bat behaviour and population probably cannot be ignored.

Many aspects of bat behaviour, like coloniality and roost selection (KUNZ, 1982; BARCLAY *et al.*, 1982; FENTON *et al.*, 1994; JENKINS *et al.*, 1998), using linear landscape elements (VERBOOM, 1998), emergence behaviour (FENTON *et al.*, 1994; JONES & RYDELL, 1994; SPEAKMAN *et al.*, 1995; DUVERGÉ *et al.*, 2000) and especially phenomena of bat nocturnality (RYDELL & SPEAKMAN, 1994; SPEAKMAN, 1995), are believed to be at least partly affected by predation risk. But there is no clear evidence for these assumptions.

The evening emergence of bat colonies is one of most frequently studied aspects of bat behaviour. In addition to predation — environmental, climatic and reproductive factors, social relationships and foraging strategy are likely to affect the timing and pattern of emergence (rewieved by ERKERT, 1982). SPEAKMAN *et al.* (1992) and KALCOUNIS & BRIGHAM (1994) studied the impact of predation risk on emergence behaviour using a predator model. FENTON *et al.* (1994) quantified bat responses to a real predator pressure at the colonies in South Africa, which were attacked by raptors. Some authors who conducted studies at attacked colonies also mentioned the possibility of antipredator mechanisms (*e.g.*, MCWILLIAM, 1989; RODRÍGUEZ-DÚRAN & LEWIS, 1985; NEGRO *et al.*, 1992).

The aims of this study were to assess the effects of a predator model (stuffed specimen of a bird and its recorded voice) on the emergence activity of a maternity colony of the serotine bat (*Eptesicus serotinus*), to test the effects of reproductive and climatic factors and to find out interactions between these factors and the effects of predation risk. We expected that the bats would change their emergence behaviour under the predation risk, *i.e.* emergence would be faster and delayed with a higher degree of clustering.

MATERIAL AND METHODS

Study colony

Field work was carried out from the beginning of May to the end of August in 1997 and 1998 at the maternity colony of serotine bats at Střelice, a small village near Brno, South Moravia, Czech Republic. Female bats were moving into the roost in late April or early May and we started our experiments after the establishment of the colony. Bats roosted in the attic of a family house at the periphery of a village and emerged from a few exit holes (crevices between the tiles or between body belt molding and tiles). One hole was used much more frequently (>80%) and it is described as the main exit hole. The front part of the house faced to a street (south-west) and the back part had exit holes to a complex of gardens (north-east). The garden was covered by branches of walnut tree (*Juglans regia*). The village was surrounded mainly by agrocoenoses with patches of woods.

Data collecting

Emergence activity was monitored with approximately 10-day intervals, always during two consecutive nights. The first night was a control and the emergence of bats was recorded without the presence of a predator model. The second night a treatment mimicking predation risk was applied. Second by second observations of emergence were made directly by one observer positioned outside the roost. Each observation started approximately 15 to 30 minutes before the expected onset of the emergence period and lasted until no bat had emerged for 15 minutes. On treatment nights in 1997, a stuffed specimen of the barn owl (*Tyto alba*) was placed on the corner of the roof about 5 m from the roost exits. A stuffed specimen of the common kestrel (*Falco tinnunculus*) was used in 1998. In addition to the models, recorded calls of the respective bird species were played back towards the roost exits using a tape-recorder fixed beside the predator model. The 'barn owl' tape lasted 30 minutes and consisted of five 2-min sequences of territorial calls with four 5-min intervals. The 'common kestrel' tape also lasted 30 minutes and consisted of three 5-minute sequences of the most common vocalisations with two 7.5-min intervals. Playbacks began approximately 15 minutes before the expected onset of emergence (according to the time of the first emergence the day before). We chose these predator species because they were recorded in the area (J. VAČKAŘ, pers. com.) and have been reported to prey on bats (*e.g.*, SPEAKMAN, 1991). The presence of the observer

and his voice in both control and treatment night were considered neutral stimuli.

All data concerning emergence behaviour were continually spoken into a tape-recorder. The tapes were later transcribed with second accuracy of emergence times, using a stop watch and/or program TIMER (J.R. Speakman, Dept. of Zoology, Univ. of Aberdeen, Aberdeen, Scotland). The emergence model was characterized by eight parameters: 1) onset of emergence — the time of first emerged bat, 2) median of emergence — the time of middle emerged bat, 3) end of emergence — the time of last emerged bat, all time parameters were given with respect to sunset which was calculated for the studied locality on the basis of the Astronomical Yearbooks (Prague 1997, 1998), 4) the duration of emergence — period between the first and the last emerged bat, 5) rate of emergence — number of bats per minute, 6) exit preference — percent of bats which used the main exit hole, 7) direction preference — percent of bats which departed in the direction north, 8) degree of clustering — expressed as G-value. The level of light intensity was measured at the onset of emergence using a luxmeter (GOSEN) placed close to the roost of the colony. Because of inaccessibility of the roost and because we did not want to disturb the colony, the reproductive stage of the females could not be evaluated precisely. June 15th was defined as the turning-point between gravidity and lactation, based on literature data (SCHOBER & GRIMMBERGER, 1993; CATTO *et al*., 1995) and according to the results of research which was carried out simultaneously at another maternity colony of *Eptesicus serotinus* nearby (POKORNÝ, 1998). Lactation and postlactation periods were lumped into a single class called 'lactation' and therefore the breeding season was divided only into two periods — 'gravidity' and 'lactation'. Climatic information was supplied by the Hydrometeorological Institute in Brno, Czech Republic. Temperature, wind speed and relative humidity were recorded at Troubsko, a village nearby (2 km), at 9 p.m. Atmospheric pressure was recorded in Brno-Tuřany (cca 15 km) at 9 p.m. Minimal day temperature and mean day temperature from Troubsko were also used in the analyses.

Statistics

The nonparametric Mann-Whitney U test was used to test the single effect of year and also the effect of reproductive and climatic factors. Wilcoxon matched pairs test was used to determine if the predator model affected the parameters of emergence activity. Each treatment night (with predator model) was paired with the previous control night to minimalize the influence of reproductive and climatic factors.

The analysis of clustering behaviour was performed by the behavioural temporal clustering analysis program Clustan (J.R. Speakman, Dept. of Zoology, Univ. of Aberdeen, Aberdeen, Scotland). The emergences, where the number of bats was lower than 30, had to be excluded from this analysis. The software calculates an explicit comparison of the observed distribution of intervals between two consecutive individual emergence events with the distribution expected for random emergence and tests the significance of this difference using the χ^2 and G test. The G-value was chosen to reflect the extent of clustering. In addition, the program avoids spurious detection of clustering by trimming the tails from the distributions (SPEAKMAN *et al*., 1992). The statistical artefact of the size of emerged bats was removed by the procedures outlined in SPEAKMAN *et al*. (1999). We analysed the climatic, reproductive and predation effect on the corrected estimate of clustering, using one-way and two-way ANCOVA with the number of emerged bats as a covariate.

To examine the relationship between the emergence activity and climatic factors we performed a principal component analysis of all weather variables (see above) which had been standardized before the analysis. To divide all observation days into 2 groups according to climatic conditions, the K-means clustering was run on the factor scores of the first three principal components. The distances were first sorted and then objects at constant intervals were chosen as initial cluster centres. We performed Spearman's correlation among emergence parameters and between emergence parameters and the factors scores.

Two sets of non-parametric two-way ANOVA (Scheirer-Ray-Hare test, DYTHAM, 1999) by reproduction and year, and by weather and predation, were run on parameters of emergence activity.

RESULTS

General description of emergence

Approximately 15 minutes prior to first emergence, bats congregated at the exit holes and audible vocalisation was heard during this time. Bats mostly departed from one preferred exit hole, at the height of the hole or dropped a little before picking up height again to fly away, usually in a straight northern direction. In each year the data were collected on 14 nights, of which 7 were controls and 7 treatment nights (table 1). The number of emerged bats was stable after the establishment of the colony (about 50 bats) and the bats left the roost when the young began to fly. In August 1997, bats returned to the roost (fig. 1).

TABLE 1

Summary of emergence parameters. Time parameters (s, or seconds after sunset), level of light intensity (lx), exit preference (percent of bats which used the main exit hole), direction preference (percent of bats which departed in the direction north), degree of clustering (expressed as G-value).

Parameter	Mean	Std. Dev.	Median	Min	Max	Mean	Std. Dev.	Median	Min	Max
	control 1997, $N = 7$					control 1998, $N = 7$				
number of emerged bats	46.29	15.89	50.00	16.00	65.00	43.71	24.31	58.00	10.00	67.00
level of light intensity	190.57	225.61	90.00	36.00	680.00	213.71	160.88	147.00	69.00	540.00
duration of emergence	1271.43	328.64	1232.00	847.00	1800.00	1127.86	455.44	1129.00	238.00	1692.00
rate of emergence	2.21	0.79	2.26	1.14	3.19	2.29	0.76	2.50	0.94	3.26
degree of clustering	56.10	23.79	62.60	12.82	83.45	45.06	9.51	40.24	35.53	57.90
onset of emergence	412.43	660.64	540.00	−945.00	1081.00	17.00	474.93	−118.00	−600.00	960.00
end of emergence	1683.86	707.26	1892.00	546.00	2520.00	1144.86	357.17	1198.00	399.00	1572.00
median of emergence	679.29	754.22	878.00	−860.00	1505.00	265.79	463.49	190.00	−439.50	1087.00
exit preference	84.69	9.57	82.00	69.49	96.08	91.89	4.98	92.54	86.21	100.00
direction preference	61.48	21.58	53.85	32.50	93.02	66.46	23.23	57.14	37.10	100.00
	treatment 1997, $N = 7$					treatment 1998, $N = 7$				
number of emerged bats	46.57	16.57	57.00	20.00	63.00	43.71	13.09	41.00	26.00	63.00
level of light intensity	188.14	189.43	145.00	17.00	558.00	178.86	124.70	180.00	61.00	388.00
duration of emergence	1383.86	765.22	960.00	736.00	2460.00	1350.86	607.09	1260.00	447.00	2310.00
rate of emergence	2.65	1.58	2.19	0.50	4.85	2.31	1.15	1.96	1.03	4.43
degree of clustering	44.42	28.52	38.09	11.96	85.99	48.43	17.61	47.43	20.17	72.81
onset of emergence	190.29	1075.18	512.00	−1740.00	1440.00	138.14	484.32	−21.00	−463.00	840.00
end of emergence	1574.14	471.63	1626.00	720.00	2237.00	1489.00	486.93	1389.00	797.00	2214.00
median of emergence	621.86	941.20	798.00	−706.00	1697.00	414.79	455.78	218.00	5.50	1080.00
exit preference	87.77	6.54	89.74	79.66	98.25	87.21	6.98	88.89	75.61	94.44
direction preference	55.21	26.93	55.93	20.63	85.00	69.40	15.58	72.50	38.46	87.80

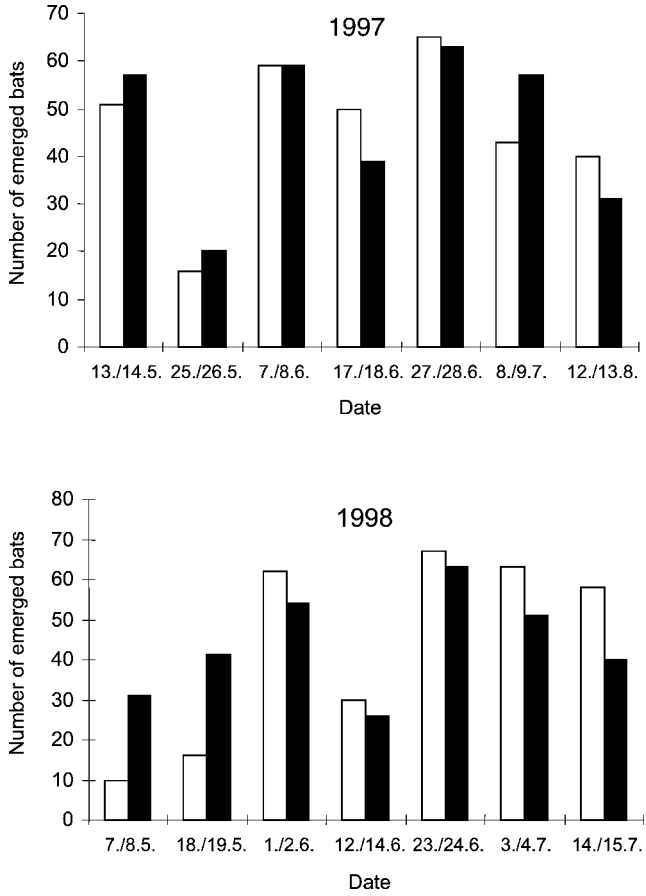


Fig. 1. Number of emerged bats on control (open bars) and treatment (closed bars) nights.

Reproductive period

Mann-Whitney tests showed no differences between years in any parameter of emergence (1997 vs. 1998, $N = 28$; control 1997 vs. control 1998, $N = 14$ and treatment 1997 vs. treatment 1998, $N = 14$). Nevertheless, a check of the graphs for each year (fig. 2) indicated an effect of year connected with reproductive season. Nonparametric 2-way ANOVA with year and reproductive period as categorical variables revealed significant variation by reproductive period in the number of emerged bats (Scheirer-Ray-Hare Test, $N = 28$, $p < 0.05$) and in the end of emergence (Scheirer-Ray-Hare Test, $N = 28$, $p < 0.05$). Year vs. reproduc-

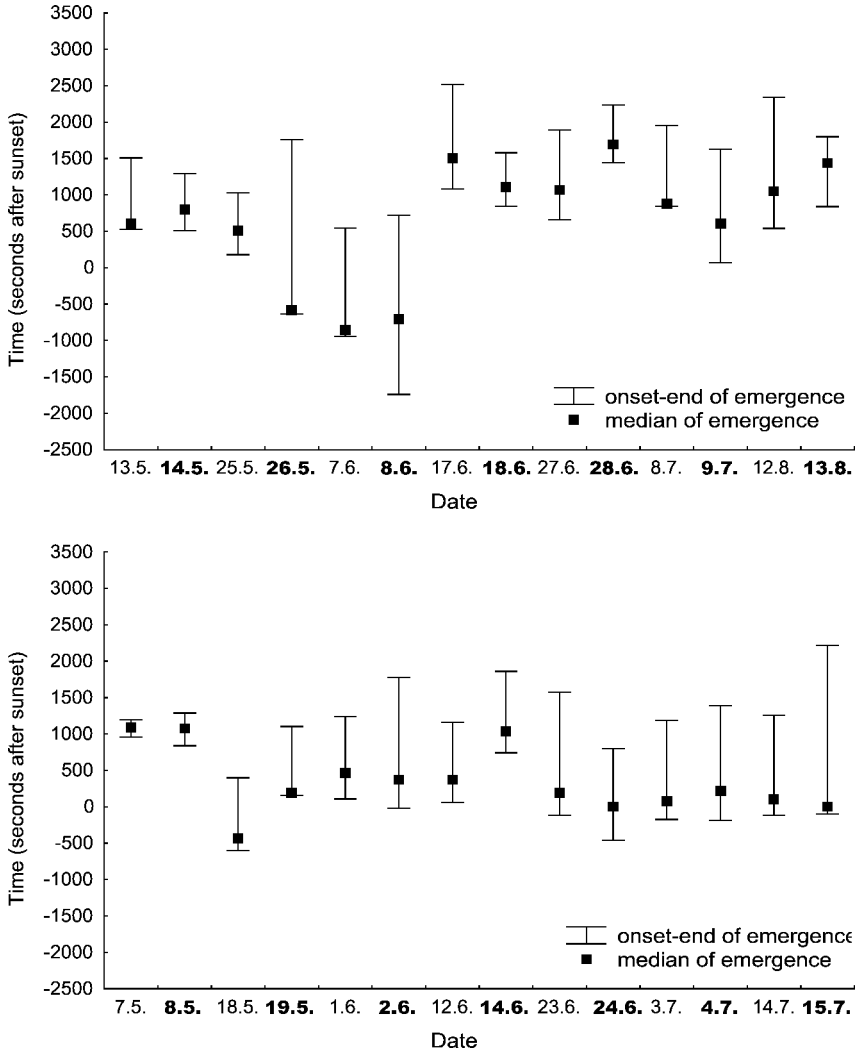


Fig. 2. Emergence activity of the colony of *Eptesicus serotinus* in 1997 (top) and 1998 (bottom). Treatment nights are indicated by bold figures.

tive period interactions were significant for the onset (Scheirer-Ray-Hare Test, $N = 28$, $p < 0.01$) and median of emergence (Scheirer-Ray-Hare Test, $N = 28$, $p < 0.01$). Because of these significant interactions we compared emergence parameters between the two periods of the breeding season for each year separately, by means of a Mann-Whitney test. In 1997, bats emerged later during lactation than during the gravidity period.

TABLE 2
Results of Mann-Whitney test by reproductive period.

Parameter	1997, $N = 14$			1998, $N = 14$		
	U	Z	p-level	U	Z	p-level
number of emerged bats	22.5	−0.194	0.846	6	−2.324	**0.020**
level of light intensity	10	−1.807	0.071	18	−0.775	0.439
duration of emergence	20	−0.516	0.606	5	−2.453	0.014
rate of emergence	19	−0.645	0.519	22	−0.258	0.796
onset of emergence	3	−2.711	**0.007**	6	−2.324	**0.020**
end of emergence	2	−2.840	**0.005**	19	−0.645	0.519
median of emergence	1	−2.969	**0.003**	7	−2.195	**0.028**
exit preference	12	−1.549	0.121	15	−1.162	0.245

In 1998, bats emerged earlier during lactation, moreover, they emerged in higher numbers and the emergence period was prolonged (fig. 2, table 2). In 1998 the number of emerged bats correlated with the duration of emergence ($N = 14$, $r_s = 0.603$, $p < 0.05$), but not in 1997 ($N = 14$, $r_s = 0.110$, NS). The corrected estimate of clustering did not vary between years (ANCOVA, $F_{1,20} = 0.143$, NS) or reproductive periods (ANCOVA, $F_{1,20} = 3.725$, NS).

Climatic factors

The number of emerged bats ($r_s = 0.670$, $p < 0.01$) and the rate of emergence ($r_s = 0.387$, $p < 0.05$) was significantly correlated with the factor scores of the first principal component of the PC analysis that was done on all weather variables. The first factor reflected mainly the effect of temperature parameters on expressed variability (table 3). The rate of emergence was dependent on the number of emerged bats ($r_s = 0.526$, $p < 0.01$). Mann-Whitney tests did not show any differences between two 'weather groups' (table 4) and there was no significant change in clustering (ANCOVA, $F_{1,20} = 2.209$, NS).

Predation risk

The presence of the predator model, a stuffed bird (barn owl or common kestrel) and its calls, did not affect any parameter of emergence (Wilcoxon matched pairs test, $N = 28$), nor did each of the models separately (Wilcoxon matched pairs test, $N = 14$). Degree of clustering did not increase due to predation risk (ANCOVA, barn owl: $F_{1,9} = 0.644$, NS; common kestrel: $F_{1,8} = 0.328$, NS, together: $F_{1,20} = 0.062$, NS). We found no variation in degree of clustering by predation risk and by

TABLE 3
Values of factor loadings for the principal components describing the variation in climatic variables.

	PC1	PC2	PC3
T_{21}	0.94	−0.25	−0.05
T_m	0.89	−0.35	−0.17
T_{min}	0.72	0.27	−0.52
H	−0.23	0.76	−0.51
WS	0.46	0.45	0.64
P	−0.56	−0.59	−0.29
% total variance	46.39	23.29	17.61

TABLE 4
Mean values of climatic factors and particular days for separated 'weather groups'. T — temperature, H — humidity, WS — wind speed, P — atmospheric pressure (recorded at 9 p.m.), T_m — mean day temperature, T_{min} — minimal day temperature, G — gravidity, L — lactation and postlactation, C — control, P — predator (treatment).

Group 1 ($N = 15$)
T = 14.86°C, H = 71.2%, WS = 1.53 m.s^{-1}, P = 987.63 hPa, T_m = 15.71°C, T_{min} = 8.43°C
25.5.97 (G, C), 26.5.97 (G, P), 7.6.97 (G, C), 27.6.97 (L, C), 8.7.97 (L, C), 7.5.98 (G, C), 8.5.98 (G, P), 18.5.98 (G, C), 19.5.98 (G, P), 1.6.98 (G, C), 2.6.98 (G, P), 12.6.98 (G, C), 14.6.98 (G, P), 3.7.98 (L, C), 15.7.98 (L, P)
Group 2 ($N = 13$)
T = 17.18°C, H = 76.92%, WS = 0.15 m.s^{-1}, P = 988.54 hPa, T_m = 18.43°C, T_{min} = 13.31°C
13.5.97 (G, C), 14.5.97 (G, P), 8.6.97 (G, P), 17.6.97 (L, C), 18.6.97 (L, P), 28.6.97 (L, P), 9.7.97 (L, P), 12.8.97 (L, C), 13.8.97 (L, P), 23.6.98 (L, C), 24.6.98 (L, P), 4.7.98 (L, P), 14.7.98 (L, C)

'weather group' (2-way ANCOVA, $F_{1,18} = 0.00$, NS; $F_{1,18} = 1.86$, NS; predation × 'weather group' interaction $F_{1,18} = 0.31$, NS) or by predation and by reproductive period (2-way ANCOVA, $F_{1,18} = 0.14$, NS; $F_{1,18} = 3.48$, NS; predation × reproductive period interaction $F_{1,18} = 0.31$, NS).

The non-parametric 2-way ANOVA, with predation and 'weather group' as categorical variables was run on all parameters (except degree of clus-

tering) but we revealed no variation in any parameter and the interactions of categorical variables were non-significant. Because of the interaction between the effect of year and reproductive periods (see above), we could not perform the set of 2-way ANOVAs with reproductive period and predation as categorical variables and we lacked data for 3-way analysis. Therefore we used Wilcoxon matched pairs tests for each parameter (except degree of clustering) to analyse the effect of predation risk in each reproductive period separately. There were no differences between control and treatment nights either during gravidity or during the period of lactation and postlactation.

On the other hand, we found that predation risk influenced relationships among emergence parameters (table 5). The most important was the fact that the degree of clustering correlated with the level of light intensity, with the onset, median, the duration and rate of emergence on treatment nights, while there was no correlation between degree of clustering and any parameter of emergence on control nights (table 5, fig. 3).

DISCUSSION

The pre-emergence behaviour observed in the present study, with bats vocalizing, crawling down and congregating near the exit holes, is consistent with previous observations on serotine bats (GLAS, 1980-1981; CATTO *et al.*, 1995; ROBINSON & STEBBINGS, 1997). Bats caught in the bucket traps during the experiments conducted by FENTON *et al.* (1994) and some of the ones taken by raptors uttered shrill screams, vocalisations audible to a human observer. As a consequence of these experiments, the number of emerged bats decreased, they emerged later and changed exit holes. Vocal communication prior to emergence may have an important function in colonial species and some kinds of distress calls could alert bats to predators (KUNZ, 1974; FENTON *et al.*, 1994). But screech calls of *Phyllostomus hastatus* were not given in appropriate contexts to warn conspecifics (WILKINSON & BOUGHMAN, 1998). The exact significance of distress calls is not clear and additional experiments are required.

Many factors influence emergence activity of bats especially from shelters of maternity colonies and it is difficult to assess the impact of only predation risk and to eliminate other factors. Therefore we considered also the effect of year, reproductive period and climatic factors and tried to find potential relationships among all factors.

We did not find differences in emergence parameters between 1997 and 1998, but the effect of reproductive season was opposite in each year. Early emergence during lactation, like in 1998 in this study, has

TABLE 5

Spearman's correlation coefficients between emergence parameters. Upper part of the table — control nights ($N = 14$, except of correlation with degree of clustering where $N = 11$), lower part of the table — treatment nights ($N = 14$, except of correlation with degree of clustering where $N = 12$), significant coefficients are indicated by bold: $^{*}p < 0.05$, $^{**}p < 0.01$.

	number of emerged bats	level of light intensity	duration of emergence	rate of emergence	degree of clustering	onset of emergence	end of emergence	median of emergence	exit preference	direction preference
number of emerged bats	—	0.132	**0.600***	**0.655***	−0.027	−0.293	−0.244	0.279	−0.262	−0.202
level of light intensity	0.110	—	0.042	−0.204	−0.036	**−0.789****	**−0.842****	**−0.697****	−0.284	0.319
duration of emergence	0.071	**0.824****	—	0.011	−0.191	−0.218	−0.160	0.437	−0.424	−0.433
rate of emergence	0.437	**−0.657***	**−0.829****	—	−0.055	0.055	0.059	0.204	0.222	0.029
degree of clustering	0.408	**0.860****	**0.741****	**−0.622***	—	0.255	0.209	−0.036	−0.473	0.100
onset of emergence	−0.170	**−0.801****	**−0.858****	**0.616***	**−0.823****	—	**0.982****	**0.697****	0.169	−0.642
end of emergence	−0.289	−0.209	0.051	−0.257	−0.441	0.400	—	**0.705****	0.143	−0.279
median of emergence	−0.106	**−0.807****	**−0.789****	**0.604***	**−0.769****	**0.948****	0.420	—	0.046	−0.319
exit preference	0.082	−0.286	−0.075	0.088	−0.007	0.306	0.460	0.519	—	0.354
direction preference	−0.346	0.064	−0.121	0.007	−0.063	−0.198	−0.442	−0.345	**0.607***	—

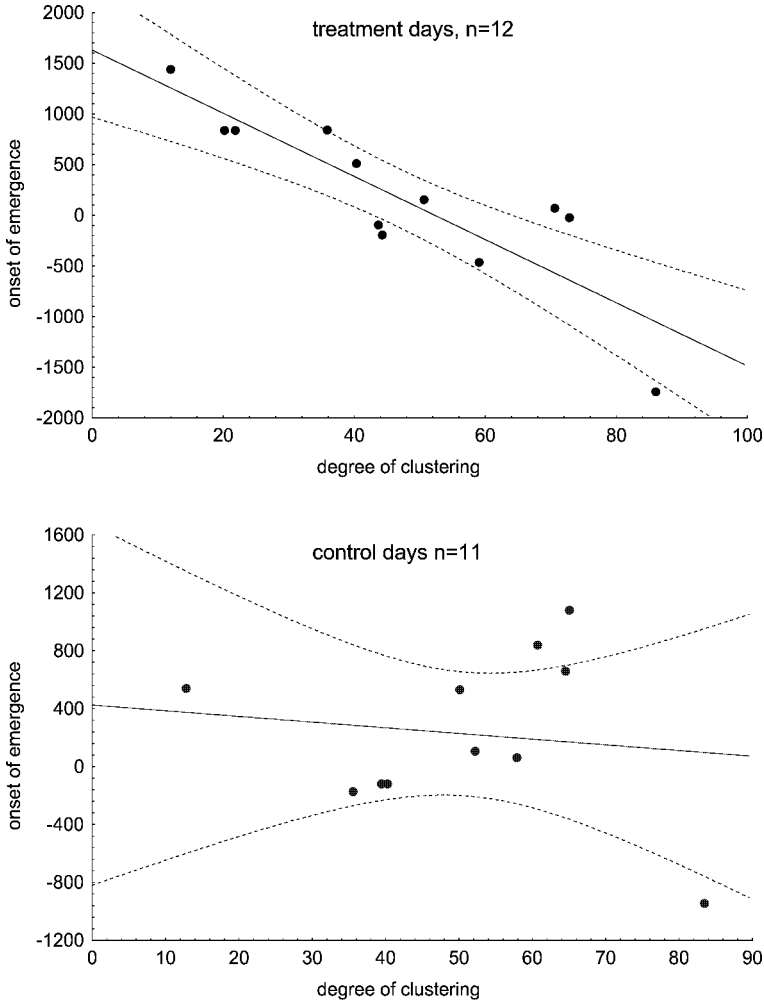


Fig. 3. Scatter plots of onset of emergence against degree of clustering.

been recorded for many bat species, *e.g.*, *Antrozous pallidus* (O'SHEA & VAUGHAN, 1977), *Tadarida pumila* (MCWILLIAM, 1989), *Nyctalus noctula* (JONES, 1995), *Nyctalus leisleri* (SHIEL & FAIRLEY, 1999), *Eptesicus nilsonii* (DUVERGÉ *et al.*, 2000) and also for *Eptesicus serotinus* (CATTO *et al.*, 1996; POKORNÝ, 1998). On the other hand, CATTO *et al.* (1995) did not find significant variation in emergence time throughout the breeding season at another maternity colony of *Eptesicus serotinus* close to the colony studied by CATTO *et al.* (1996).

Lactation is more energy demanding than pregnancy in bats (ANTHONY & KUNZ, 1977). Because the abundance of night-flying insects peaks after dusk and females must come back to suckle their young (SWIFT, 1980), bats emerge earlier to cover their increased energy demands. Bats in late pregnancy have much higher wing loading than during lactation, and their agility and manoeuvrability are reduced because of this handicap (HUGHES & RAYNER, 1991). In contract to the expectation, bats emerged later during lactation 1997 in comparison with gravidity 1997. Similar results were obtained for *Myotis velifer* (KUNZ, 1974) and *Rhinolophus ferrumequinum* (DUVERGÉ *et al*., 2000). The prediction that lactating bats should emerge earlier may be flawed since it was based on the implicit assumption that food availability remained constant throughout the season. The abundance of flying insect increased from May to July and August and this increase in food availability during lactation may therefore normally compensate for the increased energy demands, so that emerging earlier may not be necessary (DUVERGÉ *et al*., 2000). In addition, females may be prevented from emerging early by mother-infant interactions after parturition (KUNZ, 1974). Our results thus show that there may be intra-roost differences in the effect of reproductive season, which could be related to different food availability in each year. Prolongation of emergence during lactation could be caused by social changes in the colony. We observed juveniles departing after mothers with visible delay. A similar pattern was observed in other bats including *Myotis velifer* (KUNZ, 1974) and *Eptesicus nilsonii* and *Rhinolophus ferrumequinum* (DUVERGÉ *et al.*, 2000). O'SHEA & VAUGHAN (1977) predicated that during the period when *Antrozous pallidus* were beginning to fly, the bats were probably the most vulnerable to predation. Young bats may emerge later than adult because their flight is still slow and the potential cost (predation) of emergence is high. Moreover, young bats may still be suckling and the potential benefit of early emergence may be relatively low (DUVERGÉ *et al*., 2000). We considered that bats could apprehend predation risk differently during the breeding season, but these assumptions were not confirmed by our results.

Low temperatures reduced the number of emerged bats in our study. The roost might be inappropriate for bats during cold periods and they probably used another temporary roost, which we were unable to locate. HORÁČEK (1980) indicated that each maternity colony of *Eptesicus serotinus* has a few roosts, between which they alternate. Low ambient temperatures have been demonstrated to delay, reduce or prevent activity of bats due to its effect on the abundance of insects (O'SHEA & VAUGHAN, 1977; ERKERT, 1982; CATTO *et al*., 1995; RYDELL *et al*., 1996). Bats also reduced their activity on nights with low relative humidity to avoid excess

water loss because of extremes in vapour pressure deficits during flight (ADAM *et al*., 1994). Only strong wind and heavy precipitation seems to inhibit or extremely delay an emergence (ERKERT, 1982; MCWILLIAM, 1989; CATTO *et al*., 1996). Bats emerged earlier on nights with higher atmospheric pressure (NEGRO *et al*., 1992). A negative influence of cool periods on the rate of emergence in *Anthrozous pallidus* was demonstrated by O'SHEA & VAUGHAN (1977).

NEGRO *et al*. (1992), who recorded winter predation by *Falco tinnunculus* on *Pipistrellus pipistrellus*, found that kestrels did not chase bats during poor weather. Therefore we assume that bats are under higher predation risk during fine weather, and their perception of predation risk could depend on weather conditions. This hypothesis was not confirmed. Nevertheless we should remark that the influence of some climatic factors may not show up in our study because there were no extreme values of any climatic factor.

There are a few observations of direct changes in emergence or return behaviour obviously due to a predator. The activity of *Otus asio* near the colony of *Myotis lucifugus* apparently caused a temporary decline in the size of the bat colony (BARCLAY *et al*., 1982). *Myotis myotis* changed the emergence exits to avoid the attacks of *Strix aluco* (GÜTTINGER, 1990) and the colony left the roost earlier compared with other years (GÜTTINGER, pers. comm.). Colonies of *Pteronotus fuliginosus* use natural corridors for their evening emergence and predation by *Falco columbarius* induced the disappearance of an more exposed flyway (RODRÍGUEZ-DURÁN & LEWIS, 1985). We did not find any differences between values of emergence parameters for control and treatment nights for the barn owl or the common kestrel model. However, we discovered changes in relationships among emergence parameters probably due to predation risk.

The function of the clustering behaviour has been the topic of recent debate. There are three main hypotheses concerning the functional significance of this behaviour. A 'Bottle neck effect' is used to explain clustering behaviour as an artefact of large numbers of animals moving through a small constriction (BULLOCK, COMBES & EALES, 1987; KALCOUNIS & BRIGHAM, 1994), but when the statistical artefact of emergence size is removed, large emergences are actually less clustered than smaller ones (SPEAKMAN *et al*., 1999). In larger emergences the large number of bats crowding to get out through the emergence hole may push bats out of the roost and they are not able to co-ordinate their behaviour with other animals (SPEAKMAN & TALLACH, 1998; SPEAKMAN *et al*., 1999). The 'Information transfer' hypothesis suggests that bats may emerge in synchrony to co-ordinate their foraging behaviour (WILKINSON, 1992; WILKINSON & BOUGHMAN, 1998). Finally, clustering may represent

a selfish 'anti-predator strategy' (after HAMILTON, 1971); individuals emerging in groups decrease their own probability of being predated.

Previous experiments with plastic or stuffed owls did not reveal any significant effect on the extent of clustering (KALCOUNIS & BRIGHAM, 1994; SPEAKMAN *et al*., 1992). However, in Africa *Tadarida pumila* switched emergence behaviour from random to clustered with the arrival of *Macheiramphus alcinus* at the colony site (MCWILLIAM, 1989). FENTON *et al*. (1994), who studied predated colonies of *Tadarida pumila* and *Tadarida condylura*, emphasized the importance of clustering behaviour as a defensive strategy. At the maternity colonies of *Pipistrellus pipistrellus* in Scotland the extent of clustering behaviour was the greatest during the first half of emergence, when light levels were high, suggesting an anti-predation function (SPEAKMAN *et al*., 1995). Early emergence may increase the risk of being eaten by diurnal raptors (SPEAKMAN, 1991; SPEAKMAN, 1995; JONES & RYDELL, 1994; RYDELL *et al*., 1996). Our results also support the anti-predation function of clustering behaviour although emergence is clustered on both treatment and control nights and the degree of clustering did not differ between them. The anti-predation function of clustering was manifested on treatment nights by significant relationships between the degree of clustering and the onset, median, rate and duration of emergence and also the level of light intensity at the onset of emergence. There are many significant cross-correlation among these parameters, but the most important result is that bats emerging earlier and thereby increasing their risk to be attacked, increase their degree of clustering. The benefit of early emergence is that feeding areas can be reached before insect abundance declines as the evening progresses (RACEY & SWIFT, 1985).

If bats emerged early on treatment nights, they prolonged duration of their emergence, decreased the speed of emergence and, as we have mentioned, they increased the degree of clustering. This could be connected with an unpredictable emergence, which was suggested to be a defensive strategy of bats by FENTON *et al*. (1994). Bats may emerge erratically in groups with long gaps to confuse a predator. On the other hand, FENTON *et al*. (1994) found out that bats reduced the duration and increased the rate of emergence at colonies of less than 100 individuals. These changes in emergence behaviour decrease the risk of individual bats to be attacked by a hunting raptor as the handling time (time between the capture and the resumption of hunting) is stable.

Effective anti-predator defence mechanisms depend on efficient recognition of potential predators. The problem of the perception of a stuffed model and playbacked calls as potential threat and the ability of bats to discriminate playbacks from real calls, was debated by KALCOUNIS &

BRIGHAM (1994) and SPEAKMAN *et al*. (1992). Territorial calls are the most obvious indication of the presence of owls. Playback of recorded tawny owl (*Strix aluco*) calls were sufficient to induce antipredator defence behaviour in desert rodents and increased their corticosterone levels (HENDRIE *et al*., 1998; EILAM *et al*., 1999). The different relationships between emergence parameters on treatment and control nights indicated most probably that bats perceived the territorial calls and the bird models as predation threat. The relationships between the degree of clustering and other parameters were not recorded on control nights. However, there might be other stimuli to trigger a clear antipredator response, *e.g.*, movement of a bird or vocalisation of an attacked bat (FENTON *et al*., 1994). *Falco tinnunculus* flying into a stream of emerging bats caused a following break of emergence (ŘEHÁK, pers. comm.). It was surprising that no behavioural changes were detected in *Acomys cahirinus* after exposure to owl calls, despite increased cortisol levels, which are indicative of stress. EILAM *et al*. (1999) explained this by the fact that *Acomys cahirinus* forage in habitats relatively protected from aerial predation. Also bats avoid avian predation by their nocturnality (SPEAKMAN, 1995), so they may not require strong anti-predator strategies. Experiments with stress hormones and predation risk in bats are needed to clarify this situation.

ACKNOWLEDGEMENT

This study was financially supported by the Grant Agency of the Academy of Sciences of the Czech Republic (Grant No. C6087502). We are grateful to the householders Marešovi who allowed us access to their properties to count bats. We are also very obliged to M. Pokorný and H. Berková for their help during the field work and long discussions about the problem under the study. Our colleagues J. Gaisler, M. Reichard, J. Bryja and Z. Řehák made many helpful comments on the manuscript.

REFERENCES

ADAM M.D., M.J. LACKI & L.G. SHOEMAKER, 1994. Influence of environmental conditions on flight activity of *Plecotus townsendii virginianus*. Brimleyana **21**: 77-85.

ALLEN, G.M., 1939. *Bats*. Dover Publications, Harvard University Press, New York.

ANTHONY, E.L.P. & T.H. KUNZ, 1977. Feeding strategies of the little brown bat, *Myotis lucifugus*, in southern New Hampshire. Ecology **58**: 775-786.

BARCLAY, R.M.R., C.E. THOMSON & F.J.S. PHELAN, 1982. Screech owl, *Otus asio*, attempting to capture little brown bats, *Myotis lucifugus*, at a colony. Can. Field-Nat. **96**: 205-206.

BAUER, K., 1956. Schleiereule (*Tyto alba* Scop.) als Fledermausjäger. J. of Orn. **97**: 335-340.

BROWN, L.H., E.K. URBAN & K. NEWMAN (Eds), 1983. *The Birds of Africa. Vol. 1: Ostrich to Birds of Prey*. Academic Press, London & New York.

BULLOCK, D.J., B.A. COMBES & L.A. EALES, 1987. Analysis of the timing and pattern of emergence of the pipistrelle bat (*Pipistrellus pipistrellus*). J. Zool. (Lond.) **211**: 267-274.

CADE, T.J., 1987. *The falcons of the world*. Reprint. Comstock/Cornell University Press, Ithaca & New York.

CATTO, C.M.C., P.A. RACEY & P.J. STEPHENSON, 1995. Activity patterns of the serotine bat (*Eptesicus serotinus*) at a roost in southern England. J. Zool. (Lond.) **235**: 635-644.

CATTO, C.M.C., A.M. HUTSON, P.A. RACEY & P.J. STEPHENSON, 1996. Foraging behaviour and habitat use of the serotine bat (*Eptesicus serotinus*) in southern England. J. Zool. (Lond.) **238**: 623-633.

DYTHAM, C., 1999. *Choosing and using statistics: Biologist's Guide*. Blackwell Science, London.

EILAM, D., T. DAYAN, S. BEN-ELIYAHU, I. SCHULMAN, G. SCHEFER & C.A. HENDRIE, 1999. Differential behavioural and hormonal responses of voles and spiny mice to owl calls. Anim. Behav. **58**: 1085-1093.

ERKERT, H.G., 1982. Ecological aspects of bat activity rhythms. In: T.H. Kunz (Ed.): *Ecology of bats*: 201-242. Plenum Press, New York.

FENTON, M.B., I.L. RAUTENBACH, S.E. SMITH, C.M. SWANEPOEL, J. GROSELL & J. VAN JAARSVELD, 1994. Raptors and bats: threats and opportunities. Anim. Behav. **48**: 9-18.

GERHARDT, R.P., 1994. The food habits of sympatric *Ciccaba* owls in northern Guatemala. J. Field Ornithol. **65**: 258-262.

GÜTTINGER, R., 1990. Mausohren leben gefährlich. Fledermaus-Anzeiger, Regionalbeilage für die Kantone SG/AI/AR. September 1990.

GILLETTE, D.D. & J.D. KIMBROUGH, 1970. Chiropteran mortality. In: B.H. Slaughter & D.W. Walton (Eds): *About bats*: 262-281. Dallas Southern Methodist University Press, Dallas.

GLAS, G.H., 1980-81. Activities of serotine bats (*Eptesicus serotinus*) in a nursing-roost. Myotis **18-19**: 194-168.

HAMILTON, W.D., 1971. Geometry of the selfish herd. J. theor. Biol. **31**: 295-311.

HILL, J.E. & J.D. SMITH, 1984. *Bats: a natural history*. British Museum (Natural History), London.

HORÁČEK, I., 1980-81. Comparative notes on the population structure in several European bats species. Myotis **18-19**: 48-53.

HUGHES, P.M. & J.M.V. RAYNER, 1993. Addition of artificial loads to long-eared bats *Plecotus auritus*: handicapping flight performance. J. Exp. Biol. **161**: 285-298.

IBÁÑEZ, C., C. RAMO & B. BUSTO, 1992. Notes on food habits of the black and white owl. Condor **94**: 529-531.

JENKINS, E.V., T. LAINE, S.E. MOORGAN, K.R. COLE & J.R. SPEAKMAN, 1998. Roost selection in the pipistrelle bat, *Pipistrellus pipistrellus*, in northeast Scotland. Anim. Behav. **36**: 909-917.

JONES, G., 1995. Flight performance, echolocation and foraging behaviour in noctule bats *Nyctalus noctula*. J. Zool. (Lond.) **237**: 303-312.

JONES, G. & J. RYDELL, 1994. Foraging strategy and predation risk as factors influencing emergence time in echolocating bats. Phil. Trans. R. Soc. Lond. **346**: 445-455.

KUNZ, T.H., 1974. Feeding ecology of a temperate insectivorous bat (*Myotis velifer*). Ecology **55**: 693-711.

KUNZ, T.H. (Ed.), 1982. *Ecology of bats*. Plenum Press, New York & London.

MCWILLIAM, A.N., 1989. Emergence behaviour of the bat *Tadarida* (*Chaerephon*) *pumila* (*Chiroptera*: *Mollosidae*) in Ghana, West Africa. J. Zool. (Lond.) **219**: 698-701.

NEGRO, J.J., C. IBÁÑEZ, J.L. PÉREZJORDÁ & M.J. DELARIVO, 1992. Winter predation by common kestrel *Falco tinnunculus* on Pipistrelle Bats *Pipistrellus pipistrellus* in Southern Spain. Bird Study **39**: 195-199.

OBUCH, J., 1992. Tawny Owl (*Strix aluco*) preying on bats. In: I. Horáček & V. Vohralík (Eds): *Prague Studies in Mammalogy*: 119-121. Charles University Press, Prague.

O'SHEA, T.J. & T.A. VAUGHAN, 1977. Nocturnal and seasonal activities of the pallid bat, *Antrozous pallidus*. J. Mamm. **58**: 269-284.

POKORNÝ, M., 1998. *Prostorová a časová aktivita netopýra večerního* (Eptesicus serotinus) *v zemědělské krajině* [Temporal and spatial activity of serotine bat (*Eptesicus serotinus*) in the agrocoenoses]. MSc thesis, Masaryk University, Brno, Czech Republic.

RACEY, P.A. & S. SWIFT, 1985. Feeding ecology of *Pipistrellus pipistrellus* (*Chiroptera: Vespertilionidae*) during pregnancy and lactation. I. Foraging behaviour. J. Anim. Ecol. **54**: 205-215.

ROBINSON, M.F. & R.E. STEBBINGS, 1997. Activity of the Serotine bat, *Eptesicus serotinus*, in England. Myotis **35**: 5-16.

RODRÍGUEZ-DÚRAN, A. & A.L. LEWIS, 1985. Seasonal predation by merlins on sooty mustached bats in western Puerto Rico. Biotropica **17**: 71-74.

RYDELL, J. & J.R. SPEAKMAN, 1994. Evolution of nocturnality in bats: Potential competitors and predators during their early history. Biol. J. Linn. Soc. **53**: 183-191.

RYDELL, J., A. ENTWISTLE & P.A. RACEY, 1996. Timing of foraging flights of three species of bats in relation to insect activity and predation risk. Oikos **76**: 242-252.

SCHOBER, W. & E. GRIMMBERGER, 1993. *Bats of Britain and Europe*. Kosmos Verlag, Stuttgart.

SHIEL, C.B. & J.S. FAIRLEY, 1999. Evening emergence of two nursery colonies of Leisler's bat (*Nyctalus leisleri*) in Ireland. J. Zool. (Lond.) **247**: 439-447.

SPEAKMAN, J.R., 1991. The impact of predation by birds on bat population in the British Isles. Mammal Rev. **21**: 123-142.

SPEAKMAN, J.R., 1995. Chiropteran nocturnality. Symp. Zool. Soc. Lond. **67**: 187-201.

SPEAKMAN, J.R., D.J. BULLOCK, L.A. EALES & P.A. RACEY, 1992. A problem defining temporal pattern in animal behaviour: clustering in the emergence behaviour of bats from maternity roosts. Anim. Behav. **43**: 491-500.

SPEAKMAN, J.R., R.E. STONE & J.E. KERSLAKE, 1995. Temporal patterns of Pipistelle bats, *Pipistrellus pipistrellus*, from maternity colonies are consistent with anti-predator response. Anim. Behav. **50**: 1147-1156.

SPEAKMAN, J.R. & N. TALLACH, 1998. Do emerging pipistrelle bats lose control of their timing due to 'crowd pressure'? J. Zool. (Lond.) **246**: 445-448.

SPEAKMAN, J.R., N. IRWIN & R. STONE, 1999. Effect of roost size on the emergence behaviour of pipistrelle bats. Anim. Behav. **58**: 787-795.

SWIFT, S.M., 1980. Activity patterns of Pipistrelle bats (*Pipistrellus pipistrellus*) in north-east Scotland. J. Zool. (Lond.) **190**: 285-295.

VERBOOM, B., 1998. *The use of edge habitats by commuting and foraging bats*. IBN Scientific Contributions 10, DLO Institute for Forestry and Nature Research (IBN-DLO), Wageningen.

WILKINSON, G.S., 1992. Information transfer at evening bat colonies. Anim. Behav. **44**: 501-518.

WILKINSON, G.S. & J.W. BOUGHMAN, 1998. Social calls coordinate foraging in greater spear-nosed bats. Anim. Behav. **55**: 337-350.

Antipredační chování během výletové a návratové aktivity

Petrželková, K. & Zukal, J. (2003) Does a live barn owl (Tyto alba) affect emergence behavior of serotine bats (*Eptesicus serotinus*)? Acta Chiropterologica 5, 177-184.

Acta Chiropterologica, 5(2): 177–184, 2003
PL ISSN 1508-1109 © Museum and Institute of Zoology PAS

Does a live barn owl (*Tyto alba*) affect emergence behavior of serotine bats (*Eptesicus serotinus*)?

KLÁRA J. PETRŽELKOVÁ[1, 2] and JAN ZUKAL[1, 2]

[1]*Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, Brno CZ-60365, Czech Republic*; *E-mail*: *petrzel@sci.muni.cz*
[2]*Department of Zoology and Ecology, Masaryk University, Kotlářská 2, Brno CZ-61137, Czech Republic*

We studied the impact of predation risk on emergence behavior of a maternity colony of *Eptesicus serotinus*. Observations were made during sets of three consecutive nights — control, treatment and post-treatment. On treatment nights, a trained individual of barn owl (*Tyto alba*) was displayed during the emergence of the colony. Presence of the owl did not induce any significant change in the emergence parameters with exception of the degree of clustering. In pregnancy bats increased their clustering during treatment and post-treatment nights. The presence of the owl induced changes in relationships among emergence parameters. If bats emerged earlier when predation risk supposed to be higher, they increased their degree of clustering to decrease the individuals' probability of being attacked. We conclude that clustering in emergence is an important anti-predation strategy.

Key words: *Eptesicus serotinus, Tyto alba,* emergence, predation risk, reproduction

INTRODUCTION

A bat colony represents a large potential food source concentrated in a small area, and thus may attract predators. Although bats are known to fall prey to a large diversity of predators (Gillette and Kimbourgh, 1970), much reported predation appears to be opportunistic. Only for *Macheiramphus alcinus*, *Falco rufigularis* and *Strix nigrolineata* are bats a major component of the diet (Cade, 1987; Gerhardt, 1994). However, some individuals of other avian predators can specialize in preying on bats, e.g., *Tyto alba* (Bauer, 1956; Romano *et al*., 1999; authors' unpublished data), *Strix aluco* (Obuch, 1992; 1998) or *Falco tinnunculus* (Negro *et al*., 1992; Lee and Kuo, 2001). Speakman (1991) estimated that predation by avian predators accounts for about 11% of the annual mortality of British bats despite the apparent low representation of bats in the diets of predatory birds.

Bats seem to be most vulnerable to predation during their predictable evening emergence and morning return, when they are concentrated near their roost from which they must emerge/enter through sites with limited access (Barclay *et al*., 1982; Erkert, 1982; Negro *et al*., 1992; Roberts *et al*., 1997; Lee and Kuo, 2001). The factors regulating the timing of nocturnal activity are of endogenous (hormonal) as well as exogenous (light, temperature, etc.) origin (reviewed by Erkert, 1982). Colony size, reproductive status and level of predation risk can also contribute to variation in timing of emergence (Jones and Rydell,

1994; Speakman *et al*., 1995; Catto *et al*., 1996).

Emergence consists of groups of bats leaving the roost together, interspersed with periods when few or no individuals leave. This behavior has been called 'outburst activity' or 'clustering' (Speakman *et al*., 1992). Two hypotheses concerning the functional significance of clustering are considered (Speakman *et al*., 1999). It may be an anti-predator behavior (Hamilton, 1971) or bats may emerge together to co-ordinate their foraging behavior (Wilkinson, 1992; Wilkinson and Boughman, 1998). The idea that clustering simply reflected an artefact of the movement of a large number of animals through a restricted hole (bottleneck effect — Bullock *et al*., 1987) was rejected by Speakman *et al*. (1999), who showed that bottlenecks disrupted clustering rather than promoted it.

Although many aspects of bat behavior are supposed to be affected by predation risk, there have been few attempts to demonstrate the influence of predation risk on emergence behavior. Speakman *et al*. (1992), Kalcounis and Brigham (1994) and Petrželková and Zukal (2001) used a predator model associated with or without recorded calls, to study the response to predation risk on emergence behavior. Effective anti-predator defense mechanisms depend on efficient recognition of potential predators. In all the above mentioned studies the potential perceived threat to bats of owl models/playbacks was discussed. To our knowledge only Fenton *et al*. (1994) observed anti-predator behavior of bat colonies to a real predator pressure.

In the present study, we studied the impact of the presence of a barn owl (*Tyto alba*) on the emergence behavior of a maternity colony of serotine bats (*Eptesicus serotinus*). Unlike comparable previous studies we used a live trained animal. Our aim was to investigate the responses of bats to a live moving animal rather than to a stuffed model associated with recorded calls (Petrželková and Zukal, 2001).

Materials and Methods

Study Colony

Field work was carried out from the beginning of May to the end of June in 2000 at a maternity colony of *E. serotinus* in Střelice (49°09'N, 16°30'E), a small village near Brno, Czech Republic. Bats roosted in the attic of a family house at the periphery of the village and emerged from up to five exit holes (crevices between roof tiles or between the roof molding and tiles). One exit hole was used more frequently (> 80% of colony members) than the others. The front part of the house faced a street (southwest) and the rear of the house, with exit holes, to a complex of gardens (northeast). The rear of the house was covered by walnut (*Juglans regia*) branches. Střelice is surrounded mainly by agricultural land with patches of woodland.

Bats usually start to establish a colony at this site at the end of April or beginning of May and leave the roost during July/August. However, in the year of this study the first bats appeared at the roost in the mid-April and departed at the end of June. Experiments began in the mid-May and last until beginning of July.

Data Collecting

Emergence activity was monitored every week, on three consecutive nights. The first night served as a control, with emergence activity recorded without the presence of the owl. During the second night, a trained owl was displayed during the emergence period. On the third night changes in emergence after the night when the predator was presented were recorded. Each observation started approximately 30 minutes before the expected onset of emergence period according to the time of sunset and lasted until no bat had emerged for 15 minutes. The barn owl used for the predator treatment was a 1-year male, which was always handled by a falconer. We chose a barn owl, because it preys on bats, including *Eptesicus serotinus* (Obuch, 1998), and because this species had been recorded at the locality (J. Vačkař, pers. com.). A stuffed specimen of barn owl was used in a previous study (Petrželková and Zukal, 2001). During predator treatment the falconer with the owl stood on the flat roof beneath the roost exit. The owl was flown tethered on a 60 cm long belt or sat on the falconer's raised hand, occasionally flapping its wings. When

flown the owl was at the same height or just beneath the stream of emerging bats. The emergence behavior of bats was recorded from the roof of an adjacent garage or, on the first and third nights when the falconer was absent on the same roof used by the falconer. Thus the presence of the observer/falconer served as a neutral stimulus.

All observations on emergence and return activity were recorded verbally on a dictaphone, with each emerging/returning individual recorded. Recordings were later transcribed with accuracy to one second using TIMER software (J. R. Speakman, School of Biological Sciences, University of Aberdeen, Scotland). The emergence model was characterized by the following parameters: 1) onset of emergence (O) — the time of the first bat to emerge, 2) median of emergence (M) — the time of the middle bat to emerge, 3) end of emergence (E) — the time of the last bat to emerge. All these parameters were given in seconds with respect to sunset. In addition we recorded 4) the duration of emergence (D) — length of the interval between the first and the last bat to emerge (in minutes), 5) rate of emergence (R) — number of bats emerging per minute, 6) degree of clustering (G) — expressed as adjusted *G*-value (see data analysis). The level of light intensity was measured at the onset of emergence (I) using a luxmeter (GOSEN) placed close to the roost of the colony. Because of the inaccessibility of the roost and because we did not want to disturb the colony, the reproductive stage of the females could not be directly reported. All bats left the roost immediately after first emergence of weaned bats and therefore the breeding season was divided only into two periods — 'gravidity' and 'lactation'. June 10th was defined as the transition date between gravidity and lactation based on studies on other colonies, according to the information from literature (Schober and Grimmberger, 1993; Catto *et al*., 1995), from previous studies on the colony under investigation (Petrželková and Zukal, 2001) and from a study of another maternity colony of *Eptesicus serotinus* near the study site (Pokorný, 1998) in previous years. We took account of the fact that bats established the colony sooner than in previous years. Air temperature was recorded at Troubsko, a village near the study site (2 km), at 9 p.m. and atmospheric pressure was recorded in Brno-Tuřany (ca. 15 km) at 9 p.m. Climatic information were supplied by the Hydrometeorological Institute in Brno, Czech Republic.

Data Analysis

The analysis of clustering behavior was performed by the behavioral temporal clustering analysis program CLUSTAN (J. R. Speakman). This software calculates an explicit comparison of the observed distribution of intervals between two consecutive individual emergence events to that expected if the emergence was random, and tests the significance of this difference using χ^2- or *G*-tests, depending on sample size. Clustering is indicated when there is over-representation of the shortest and the longest intervals in the sequence. *G*-values and χ^2-values are used as measures of clustering. If the values exceed the significance level then the distribution is clustered. However, it is necessary to adjust *G*-values and χ^2 according to method of Speakman *et al*. (1999) to eliminate the impact of sample size. Adjusted *G*-values were chosen to express the degree of clustering, because *G*-test is more robust to the small sample sizes (number of emerged bats). If the sample size is smaller than 30, CLUSTAN is not able to analyze clustering behavior.

To test whether temperature and atmospheric pressure differed significantly among the three days within each experimental set, we used repeated measures ANOVA with type of night (control, treatment, post-treatment) as a repeated factor. Repeated measures ANOVA with reproductive period as a categorical factor was used to test differences in emergence parameters among control night, treatment night and post-treatment night (repeated factor). Non-normal distributed variables were transformed by Box-Cox transformation. Spearman's correlation between adjusted *G*-values and other emergence parameters were performed to examine changes in relationships due to predation risk (Sokal and Rohlf, 1995).

Results

We observed emergence from the roost on total of 27 nights, but only 20 nights in seven sets were used for analyses. The remaining nights were single, and did not form complete sets. Adjusted *G*-values were counted only for 19 emergences. The number of bats emerging from the roost was stable after the establishment of the colony (median of number of emerging bats was 56) and the bats commenced leaving the roost immediately when their young began to fly.

There was no difference among days in temperatures (repeated measures ANOVA:

TABLE 1. Effect of the presence of the predator on emergence activity. Results of repeated measures ANOVA on emergence factors (see Materials and Methods for details). Abbreviations: RF — repeated factor (type of night), CF — categorical factor (reproductive period). $P \leq 0.05$ in bold

Emergence parameters	CF		RF		RF*CF	
	F	*P*	*F*	*P*	*F*	*P*
Onset of emergence (O)	0.122	0.75	1.538	0.27	0.056	0.95
Median of emergence (M)	0.638	0.47	2.037	0.19	0.021	0.98
End of emergence (E)	2.440	0.19	0.178	0.84	0.25	0.79
Degree of clustering (G)	0.007	0.94	6.914	**0.03**	5.458	**0.05**
Rate of emergence (R)	0.035	0.86	0.073	0.93	0.194	0.83
Duration of emergence (D)	3.948	0.12	0.573	0.59	0.395	0.69
Level of light intensity (I)	2.565	0.18	1.945	0.21	0.241	0.79

$F_{2, 10} = 0.639$, $P = 0.55$) or atmospheric pressure ($F_{2, 10} = 3.000$, $P = 0.13$) within experimental sets of nights. Therefore, we ignored the effects of temperature and atmospheric pressure among control, treatment and post-treatment nights within experimental sets.

The presence of the barn owl did not induce any changes in most of the emergence parameters. No effect was also detected on the post-treatment days (Table 1). However, repeated measures ANOVA revealed significant variations in adjusted *G*-values among days within experimental sets, and a repeated measures factor versus reproductive period interaction was significant (Table 1, Fig. 1).

We found out that the presence of the owl influenced relationships among emergence parameters. The most important was the fact that the adjusted *G*-values correlated with the onset and median time of emergence on treatment nights, while there was no correlation between adjusted *G*-values and any parameter of emergence on control nights. Strong but non-significant correlations were also found between adjusted *G*-values and the level of light intensity and between adjusted *G*-values and the end of emergence. On post-treatment nights adjusted *G*-values corre-lated with rate of emergence (Table 2).

DISCUSSION

Colonies of bats form resource patches that appear to offer excellent opportunities to predators. While concentrations of predators are commonly observed around the roost entrances of the enormous cave colonies of *Tadarida brasiliensis* (e.g., Lee and Kuo, 2001), at most bat colonies predators are seen rarely. However, predation may affect many aspects of bat behavior, like general coloniality and roost selection (Barclay *et al*., 1982; Fenton *et al*., 1994;

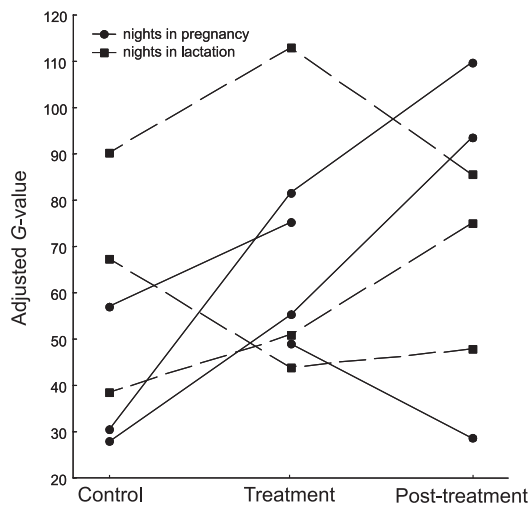


FIG. 1. Changes of adjusted *G*-values and type of night during control, treatment and post-treatment nights in respect to reproductive season

TABLE 2. Spearman's correlation coefficients between *G*-values and other emergence parameters. Control, treatment, post-treatment nights are treated separately. G — degree of clustering, O — onset of emergence, M — median of emergence, E — end of emergence, D — duration of emergence, R — rate of emergence, I — level of light intensity at the onset of emergence. Significant correlations ($P \leq 0.05$) in bold

Emergence parameter	Control		Treatment		Post-treatment	
	r	*P*	*r*	*P*	*r*	*P*
G & O	0.600	0.21	**-0.943**	**0.01**	0.143	0.79
G & M	0.486	0.33	**-0.886**	**0.02**	-0.029	0.96
G & E	0.486	0.33	-0.771	0.07	0.371	0.49
G & D	0.771	0.07	0.371	0.47	0.771	0.07
G & R	-0.257	0.62	0.029	0.96	**-0.886**	**0.02**
G & I	0.600	0.21	0.771	0.07	0.200	0.70

Jenkins *et al*., 1998), use of linear landscape elements (Verboom, 1998), and both emergence and return behavior (Fenton *et al*., 1994; Jones and Rydell, 1994; Duvergé *et al*., 2000; Petrželková and Zukal, 2001; Petrželková, 2003). Avian predation is supposed to be the most important factor influencing bat nocturnality (Rydell and Speakman, 1994; Speakman, 1995), though there is still a lack of a clear consensus.

Effective anti-predator defence mechanisms depend on efficient recognition of possible predators. Kalcounis and Brigham (1994) and Speakman *et al*. (1992) disputed that bats were able to distinguish models and playback calls of predators, used during their experiments, from real ones. Both authors presented plastic models of owls to emerging bats, Kalcounis and Brigham (1994) together with recorded owl calls. Our previous results indicated that *E. serotinus* perceived a stuffed owl and kestrel, along with playbacks of their calls, as a predation threat (Petrželková and Zukal, 2001). In the current study, *E. serotinus* responded to the live owl, which did not utter any calls. Therefore, we suppose bats could perceive a silhouette of an avian predator as a potential threat. Nevertheless, experiments with plastic owls have proven unsuccessful (Speakman *et al*., 1992; Kalcounis and Brigham, 1994), and we can speculate that bats may discriminate between a plastic (not stuffed) and a real owl. It is also possible that more than one stimulus are used by bats (silhouette plus movements or silhouette plus calls). Moreover, the vocalisation of an attacked bat could alert the rest of a bat colony (Fenton *et al*., 1994). In desert rodents, playbacks of calls of *Strix aluco* were sufficient to induce anti-predator defence behavior and increased corticosterone levels of rodents (Hendrie *et al*., 1998; Eilam *et al*., 1999).

There are a few observations which have recorded direct changes in emergence or return behavior directly in response to a predator. A part of a colony of *Myotis lucifugus* was reported to leave their roost in response to the activity of *Otus asio* nearby (Barclay *et al*., 1982). Also, *Myotis myotis* changed their emergence exit from a roost to avoid the attacks of *S. aluco* (Güttinger, 1990), with the colony abandoning its roost earlier than in previous years (R. Güttinger, pers. comm.). Attacks by *Falco columbarius* on emerging *Pteronotus fuliginosus* induced a disappearance of one of the emerging streams of the colony (Rodríguez-Durán and Lewis, 1985). Fenton *et al*. (1994) studying colonies of *Tadarida pumila* and *T. condylura* attacked by raptors, described several anti-predation strategies of bats. In colonies of less than 100 individuals, bats decreased their risk of attack by switching

roosts, and by adjusting the times and durations of emergence. Bats from larger colonies emerged earlier, despite the increased risk of predation by raptors. At all colonies, increasing numbers of bats decreased the risk of predator attack (Fenton *et al*., 1994).

Kalcounis and Brigham (1994) and Speakman *et al*. (1992) proposed that bats should increase their clustering in the presence of a predator to decrease their probability of attack. However, their experiments with models of owls did not reveal any significant effect of predation risk on the extent of clustering during emergence. Nevertheless, Speakman *et al*. (1992) discussed that development of clustering may be sufficient to act as an anti-predation device. In contrast, the results of our experiments indicate that in pregnancy, bats increased their clustering in the presence of the live owl and also on the night following the treatment. We speculate that this effect of the reproductive period might be connected with differences in other emergence parameters between pregnancy and lactation. In comparison with other reproductive periods, bats can emerge during pregnancy earlier (e.g., Jones, 1995; Shiel and Fairley, 1999) or later (Kunz, 1974; Duvergé *et al*., 2000). There could also be intra-colony differences in the effect of the reproductive period on emergence times, which could be related to different food availability in each year (Petrželková and Zukal, 2001).

As well as in our previous study (Petrželková and Zukal, 2001), predation risk induced changes in relationships among emergence parameters. When bats emerged early in the presence of owl, they increased their degree of clustering. Early emergence increases exposure to raptorial birds (Jones and Rydell, 1994; Rydell *et al*., 1996), but emerging late results in missing the peak abundance of aerial insects (Racey and Swift, 1985; Rydell *et al*., 1996). Therefore, we conclude that if bats emerged earlier and thus were exposed to increased potential predation pressure, they increased their degree of clustering to thereby decrease the individuals' probability of being attacked. This behavioural tactic may be expressed if bats perceive an actual risk of predation. The correlation between the degree of clustering and rate of emergence probably reflected the fact that faster emergence could disrupt clustering.

Ambient temperature and atmospheric pressure can affect emergence behaviour (e.g., Negro *et al*., 1992; Catto *et al*., 1995; Rydell *et al*., 1996). Our analyses showed no differences among nights within the experimental sets, so we neglected the effects of climatic factors on the perception of predation threat.

Tadarida pumila switched their emergence behaviour from random to clustered with the arrival of *Macheiramphus alcinus* at their roost site (McWilliam, 1989). With maternity colonies of *Pipistrellus pipistrellus* in Scotland, the extent of clustering behaviour was the greatest during the first half of emergence, when light levels were high, suggesting an anti-predation function (Speakman *et al*., 1995). Based on the results of our studies on bat anti-predation behaviour during emergence, we suggest that clustering during emergence is an important anti-predation strategy.

Acknowledgements

We are most thankful to the falconer V. Černý for his patience and goodwill, without his help this study could not have been completed. The Mares family kindly allowed us access to their property to study the bat colony. We are also obliged to M. Pokorný, H. Berková, K. Vošlajerová and M. Šugerková for their help during the field work, comments and discussions about our ideas. We would like to give our sincere gratitude to Prof. J. R. Speakman for his kind advices in planning the experiment and using CLUSTAN program. M. Reichard, J. Jarkovský and

Dr. C. Smith also made helpful comments on the manuscript.

Literature Cited

Barclay, R. M. R., C. E. Thomson, and F. J. S. Phelan. 1982. Screech owl, *Otus asio*, attempting to capture little brown bats, *Myotis lucifugus*, at a colony. The Canadian Field-Naturalist, 96: 205–206.

Bauer, K. 1956. Schleiereule (*Tyto alba* Scop.) als Fledermausjäger. Journal of Ornithology, 97: 335–340.

Bullock, D. J., B. A. Combes, L. A. Eales. 1987. Analysis of the timing and pattern of emergence of the pipistrelle bat (*Pipistrellus pipistrellus*). Journal of Zoology (London), 211: 267–274.

Cade, T. J. 1987. The falcons of the world. Comstock/Cornell University Press, Ithaca, 188 pp.

Catto, C. M. C., P. A. Racey, and P. J. Stephenson. 1995. Activity patterns of the serotine bat (*Eptesicus serotinus*) at a roost in southern England. Journal of Zoology (London), 235: 635–644.

Catto, C. M. C., A. M. Hutson, P. A. Racey, and P. J. Stephenson. 1996. Foraging behaviour and habitat use of the serotine bat (*Eptesicus serotinus*) in southern England. Journal of Zoology (London), 238: 623–633.

Duvergé, P. L., G. Jones, J. Rydell, and R. D. Ransome. 2000. Functional significance of emergence timing in bats. Ecography, 23: 32–40.

Eilam, D., T. Dayan, S. Ben-Eliyahu, I. Schulman, G. Schefer, and C. A. Hendrie. 1999. Differential behavioral and hormonal responses of voles and spiny mice to owl calls. Animal Behaviour, 58: 1085–1093.

Erkert, H. G. 1982. Ecological aspects of bat activity rhythms. Pp. 201–242, *in* Ecology of bats (T. H. Kunz, ed.). Plenum Press, New York, 425 pp.

Fenton, M. B., I. L. Rautenbach, S. E. Smith, C. M. Swanepoel, J. Grosell, and J. van Jaarsveld. 1994. Raptors and bats: threats and opportunities. Animal Behaviour, 48: 9–18.

Gerhardt, R. P. 1994. The food habits of sympatric *Ciccaba* owls in northern Guatemala. Journal of Field Ornithology, 65: 258–262.

Gillette, D. D., and J. D. Kimbrough. 1970. Chiropteran mortality. Pp. 262–281, *in* About bats (B. H. Slaughter and D. W. Walton, eds.). Southern Methodist University Press, Dallas, 313 pp.

Güttinger, R. 1990. Mausohren leben gefhrlich. Fledermaus-Anzeiger, Regionalbeilage für die Kantone SG/AI/AR. September 1990.

Hamilton, W. D. 1971. Geometry of the selfish herd. Journal of Theoretical Biology, 31: 295–311.

Hendrie, C. A., S. M. Weiss, and D. Eilam. 1998. Behavioral response of wild rodents to the calls of an owl: a comparative study. Journal of Zoology (London), 245: 439–446.

Jenkins, E. V., T. Laine, S. E. Morgan, K. R. Cole, and J. R. Speakman. 1998. Roost selection in the pipistrelle bat, *Pipistrellus pipistrellus*, in northeast Scotland. Animal Behaviour, 36: 909–917.

Jones, G. 1995. Flight performance, echolocation and foraging behavior in noctule bats *Nyctalus noctula*. Journal of Zoology, 237: 303–312.

Jones, G., and J. Rydell. 1994. Foraging strategy and predation risk as factors influencing emergence time in echolocating bats. Philosophical Transactions of the Royal Society of London B, 346: 445–455.

Kalcounis, M. C., and R. M. Brigham. 1994. Impact of predation risk on emergence by little brown bats, *Myotis lucifugus* (Chiroptera: Vespertilionidae), from a maternity colony. Ethology, 98: 201–209.

Kunz, T. H. 1974. Feeding ecology of a temperate insectivorous bat (*Myotis velifer*). Ecology, 55: 693–711.

Lee, Y. F., and Y. M. Kuo. 2001. Predation on Mexican free-tailed bats by peregrine falcons and red-tailed hawks. Journal of Raptor Research, 35: 115–123.

McWilliam, A. N. 1989. Emergence behavior of the bat *Tadarida* (*Chaerephon*) *pumila* (Chiroptera: Molossidae) in Ghana, West Africa. Journal of Zoology (London), 219: 698–701.

Negro, J. J., C. Ibáñez, J. L Pérezjordá, and M. J. Delarivo. 1992. Winter predation by common kestrel *Falco tinnunculus* on pipistrelle bats *Pipistrellus pipistrellus* in Southern Spain. Bird Study, 39: 195–199.

Obuch, J. 1992. Tawny owl (*Strix aluco*) preying on bats. Pp. 119–121, *in* Prague studies in mammalogy (I. Horaček and V. Vohralík, eds.). Charles University Press, Prague, 245 pp.

Obuch, J. 1998. The representation of bats (Chiroptera) in the diet of the owls (Strigiformes) in Slovakia. Vespertilio, 3: 65–75.

Petrželková, K. 2003. Impact of predation on bats. Ph.D. Thesis, Masaryk University, Brno, Czech Republic and Institute of Vertebrate Biology, Brno, Academy of Sciences of the Czech Republic, 93 pp.

Petrželková, K., and J. Zukal, 2001. Emergence behavior of the serotine bat (*Eptesicus serotinus*) under predation risk. Netherlands Journal of Zoology, 51: 395–414.

Pokorný, M. 1998. Prostorová a časová aktivita netopýra večerního (*Eptesicus serotinus*) v zemědělské krajině [Temporal and spatial activity of serotine bat (*Eptesicus serotinus*) in agrocoenoses]. M.A. Thesis, Masaryk University, Brno, Czech Republic, 91 pp. [In Czech].

Racey, P. A., and S. M. Swift. 1985. Feeding ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during pregnancy and lactation. I. Foraging behavior. Journal of Animal Ecology, 54: 205–215.

Roberts, K. J., F. D. Yancey, and C. Jones. 1997. Predation by great-horned owls on Brazilian free-tailed bats in north Texas. Texas Journal of Science, 49: 215–218.

Rodríguez-Dúran, A., and A. L. Lewis. 1985. Seasonal predation by merlins on sooty mustached bats in western Puerto Rico. Biotropica, 17: 71–74.

Romano, M. C., J. I. Maidagan, and E. F. Pire. 1999. Behavior and demography in an urban colony of *Tadarida brasiliensis* (Chiroptera: Molossidae) in Rosario, Argentina. Revista de Biologia Tropical, 47: 1121–1127.

Rydell, J., A. Entwistle, and P. A. Racey. 1996. Timing of foraging flights of three species of bats in relation to insect activity and predation risk. Oikos, 76: 242–252.

Schober, W., and E. Grimmberger. 1993. Bats of Britain and Europe. Kosmos Verlag, Stuttgart, 265 pp.

Shiel, C. B., and J. S. Fairley. 1999. Evening emergence of two nursery colonies of Leisler's bat (*Nyctalus leisleri*) in Ireland. Journal of Zoology (London), 247: 439–447.

Sokal, R. R., and F. J. Rohlf 1995. Biometry: the principles and practice of statistics in biological research. Third ed. W. H. Freeman and Company, New York , 887 pp.

Speakman, J. R. 1991. The impact of predation by birds on bat population in the British Isles. Mammal Review, 21: 123–142.

Speakman, J. R., D. J. Bullock, L. A. Eales, and P. A. Racey. 1992. A problem defining temporal pattern in animal behavior: clustering in the emergence behavior of bats from maternity roosts. Animal Behaviour, 43: 491–500.

Speakman, J. R., R. E. Stone, and J. E. Kerslake. 1995. Temporal patterns of pipistrelle bats, *Pipistrellus pipistrellus*, from maternity colonies are consistent with anti-predator response. Animal Behaviour, 50: 1147–1156.

Speakman, J. R., N. Irwin, and R. Stone. 1999. Effect of roost size on the emergence behavior of pipistrelle bats. Animal Behaviour, 58: 787–795.

Verboom, B. 1998. The use of edge habitats by commuting and foraging bats. IBN Scientific Contributions 10, DLO Institute for Forestry and Nature Research (IBN-DLO), Wageningen, 120 pp.

Wilkinson, G. S. 1992. Information transfer at evening bat colonies. Animal Behaviour, 44: 501–518.

Wilkinson, G. S., and J. W. Boughman. 1998. Social calls coordinate foraging in greater spear-nosed bats. Animal Behaviour, 55: 337–350.

Received 16 May 2003, accepted 20 August 2003

Antipredační chování během výletové a návratové aktivity

Petrželková, K., Obuch, J. & Zukal, J. (2004) Does the barn owl (*Tyto alba*) selectively predate individual great mouse-eared bats (*Myotis myotis*)?
Lynx (Praha) n.s., 35: 123-132.

Lynx (Praha), n. s., **35**: 123–132 (2004). ISSN 0024–7774

Does the barn owl (*Tyto alba*) selectively predate individual great mouse-eared bats (*Myotis myotis*)?

Loví sova pálená (*Tyto alba*) jedince netopýra velkého (*Myotis myotis*) výběrově?

Klára J. PETRŽELKOVÁ[1,2,4], Ján OBUCH[3] & Jan ZUKAL[1,2]

[1] Department of Zoology and Ecology, Masaryk University, Kotlařská 2, CZ–611 37, Brno, Czech Republic
[2] Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, CZ–603 65 Brno, Czech Republic
[3] Comenius University in Bratislava, Botanical garden, SK–038 15 Blatnica, Slovakia
[4] corresponding address: petrzelkova@ivb.cz

došlo 9. 11. 2004

Abstact. There is good evidence that owls prefer to prey on smaller and younger rodents, but nothing is known about possible selective predation on bats. We studied predation on the mouse-eared bat (*Myotis myotis*) by the barn owl (*Tyto alba*). A set of skulls of *Myotis myotis* from pellets of *Tyto alba* was compared with a control one (samples from museum collection) and it was found out that skulls from owl pellets were smaller. The differences were mainly in lengths of upper and lower toothrow and rostral breadths across upper teeth. Our results indicate that *Tyto alba* most probably prefer to prey on volant inexperienced yearlings which are easier to catch, whilst reaching almost adult size. Volant yearlings lack flying skills, they are conspicuous during the emergence and they often concentrate near the roost during their early practice flights, making them more vulnerable to owl predation then adults.

INTRODUCTION

Bats are usually a minor component of the diet of their avian predators, despite their tendency to form large and potentially vulnerable colonies. Only few tropical and subtropical birds specialize on bats, e.g. *Macheiramphus alcinus* (Accipitridae), *Falco rufigularis* (Falconidae) and *Strix nigrolineata* (Strigidae) (Cade 1987, Gerhart et al. 1994). However in other raptor and owl species particular individuals can specialize to prey on bats, e.g. *Tyto alba* (Bauer 1956, Romano et al. 1999, our unpublished data), *Strix aluco* (Obuch 1992) or *Falco tinnunculus* (Negro et al. 1992, Lee & Kuo 2001). Speakman (1991) estimated that predation by birds account for about 11% of the annual mortality of British bats despite the apparent low representation of bats in the diets of predatory birds. Obuch (1998) showed that owls in Slovakia catch more bats compared with e.g. Germany or Hungary. This could be promoted by more various natural conditions and higher occurrence of rocky and karstic regions in Slovakia, which are attractive for bats. A review of published results of owl pellet analyses in the Czech and Slovak Republics revealed that bats occurred in 39% of samples across all owl species. Most successful in catching bats was *Strix aluco*, bats occurred in 77% of the samples and consisted 4% of the diet (Petrželková & Zukal 1999).

Prey selection of bats by owls with regards to the prey size has not been closely studied yet. Previous studies were focused on bat species preference in owl diet (e.g. BEKKER & MOSTERT 1991, KOWALSKI & LESIŃSKI 1990, RUPRECHT 1979, OBUCH 1998) and there are indications that owls prefer bigger species (BEKKER & MOSTERT 1991, PETRŽELKOVÁ & ZUKAL 1999) and species hunting in an open habit (PEREZ-BARBERIA 1990).

We compared a set of skulls from pellets of *Tyto alba* with a control set of skulls from museum collection to test whether owls prefer any size/age group within a population of *Myotis myotis*.

MATERIALS AND METHODS

We examined 121 craniums (upper parts of skulls) and 142 mandibles of *Myotis myotis* from the pellets of *Tyto alba* collected at 7 localities in the Czech and Slovak Republic and 120 crania and 120 mandibles of the same species from 30 localities deposited in the collection at the Institute of Vertebrate Biology AS CR in Brno in the Czech Republic. Only at one locality – Ratková, Slovakia – were *Myotis blythii* registered with insignificant dominance (UHRIN et al. 2002). There were samples collected by the last author and he has high experiences with bone determination to be able excluded *Myotis blythii* from sample. We did not include museum specimens collected at hibernacula. We also excluded juvenile prevolant specimens from analyses, because the number of juveniles in the collections did not reflect the situation in the *Myotis myotis* population. Finally, we excluded the specimens with unclear origin to ensure that in the analysis will be not included specimens of *Myotis blythii*. We were aware of a possible bias in our control sample. Once prevolant individuals and possible *Myotis blythii* were excluded from the analyses, no size/age bias between sample of individuals in the collections and individuals in maternity colonies was detected. Thus we believe that our sample presents a valid control.

The following skull measurements were taken from each cranium: greates length of skull (LCr), condylobasal length (LCb), zygomatic breadth (LaZ), breadth of interorbital constriction (LaI), infraorbital breadth (LaInf), breadth of braincase (LaN), lengths of upper tooth-row (IM^3, CM^3, P^4M^3, M^1M^3, CP^4, P^2P^3), rostral breadth across upper canines (CC), upper premolars (P^4P^4) and molars (M^3M^3). Mandibular measurements included: mandible length (LMd), coronoid process height (ACr) and lengths of lower toothrows (IM_3, CM_3, P_4M_3, M_1M_3, CP_4). Dental measurements had to be taken from alveoli on both pellet and collection material, because skulls from pellets often had teeth out. Skulls from owl pellets were often damaged so we could not take all measurements on some of them. Caliper and digital image analyses (MicroImage 3.0) were used for measuring.

Comparisons between 'owl' and 'control' groups were carried out separately for craniums and mandibles. Firstly we compared single measurements by univariate statistical methods. Kolgomorov-Smirnov tests were used to test if the distributions did not differ significantly, while Mann-Whitney U-tests were used to compare medians of the groups. Further, we performed two principal component analyses. Thereafter we compared factor scores between 'owl' and 'control' group.

RESULTS

Using univariate tests we found significant differences between 'owl' and 'control' groups mainly in lengths of upper and lower toothrow and rostral breadths across upper teeth (CC, P^4P^4, M^3M^3, IM^3, CM^3, P^4M^3, M^1M^3, CP^4, P^2P^3, LCb, IM^3, CM^3, P^4M^3, M_1M_3, CP_4 – Tab. 1). Skulls from collections were larger in all these measurements then the skulls from owl pellets (Fig. 1).

First component (PC1-u) of the PCA carried out on all cranial measurements was driven mainly by lengths of toothrow (IM^3, CM^3, P^4M^3, M^1M^3, CP^4) and rostral breadths across teeth (CC, P^4P^4, M^3M^3) but also by LCb. Second principal component (PC2-u) mostly correlated

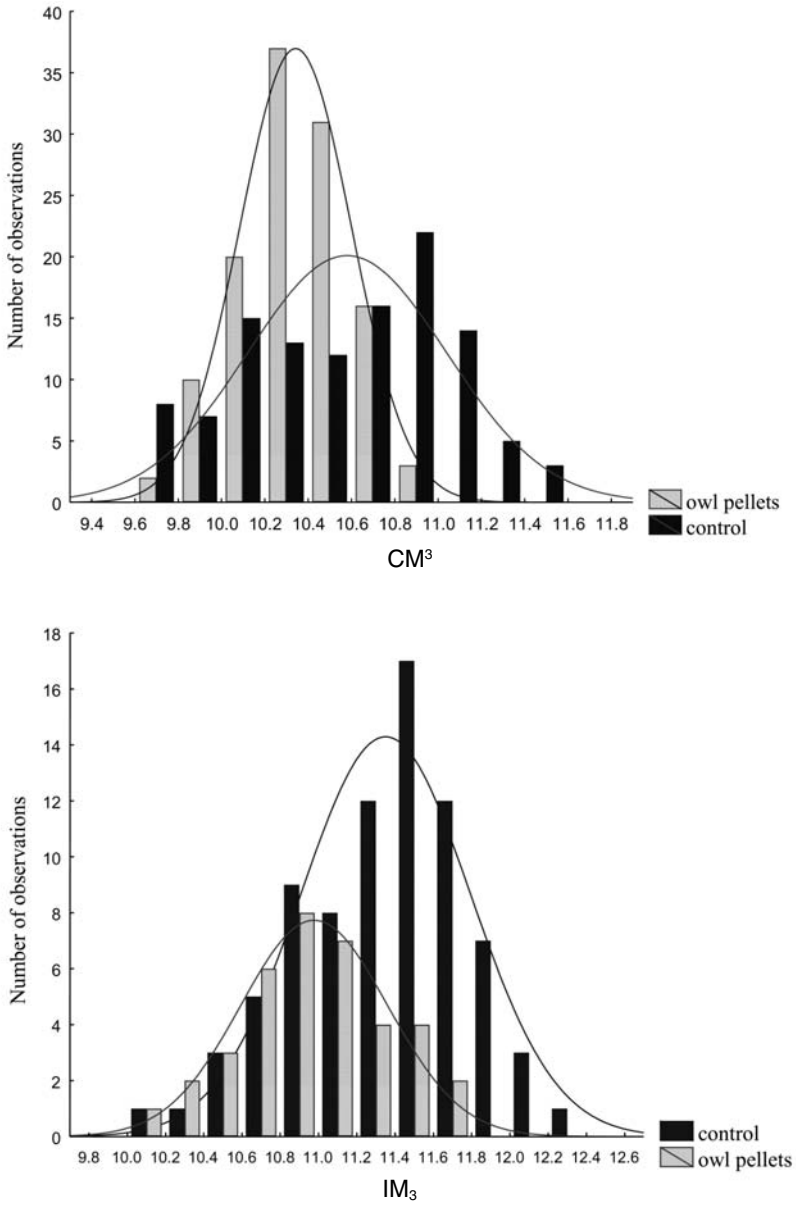


Fig. 1. Length-frequency distribution of CM^3 (above) and IM_3 (below) from control group and from owl pellets with a significant difference.
Obr. 1. Histogram hodnot rozměrů CM^3 (nahoře) a IM_3 (dole) u kontrolní skupiny lebek a u skupiny lebek z vývržků. Vysvětlivky: number of observations – počet pozorování, control – kontrolní soubor, owl pellets – soví vývržky.

Tab. 1. Medians and results of Mann-Whithey tests and Kolgomorov-Smirnov tests on (a) cranial and (b) mandibular measurements. C – control, O – owl pellets, MW – Mann-Whitney test, KS – Kolgomorov--Smirnov test

Tab. 1. Mediány a výsledky Mann-Whitney a Kolgomorov-Smirnov testů vypočtených na rozměrech (a) horní, (b) dolní čelisti. C – kontrolní soubor, O – soví vývržky, MW – Mann-Whitney test, KS – Kolgomorov-Smirnov test, p-level – hladina významnosti

	median C	median O	Z	MW p-level	KS p-level
LaInf	6.42	6.47	–1.91	0.056	> .10
LaI	5.24	5.23	–1.54	0.125	> .10
LaZ	15.02	15.11	–0.86	0.389	> .10
LaN	10.86	10.91	–1.58	0.114	> .10
CC	6.21	6.11	4.41	**<.001**	**< .001**
P^4P^4	7.71	7.76	1.73	0.083	**< .05**
M^3M^3	10.12	10.03	2.99	**0.003**	**< .005**
IM^3	11.40	10.98	5.68	**<.001**	**< .001**
CM^3	9.97	9.68	5.40	**<.001**	**< .001**
$P4M^3$	7.39	7.27	3.97	**<.001**	**< .001**
$M1M^3$	5.75	5.78	1.13	0.258	> .10
CP^4	4.37	4.17	5.72	**<.001**	**< .001**
P^2P^3	1.47	1.40	5.83	**<.001**	**< .001**
LCr	23.55	23.63	1.33	0.184	> .10
LCb	22.47	22.43	2.08	**0.037**	> .10
LMd	17.92	17.87	–0.26	0.798	> .10
ACr	6.03	6.07	1.07	0.282	> .10
IM_3	11.96	11.65	–3.78	**<.001**	**< .001**
CM_3	10.65	10.34	–4.55	**<.001**	**< .001**
P_4M_3	7.81	7.66	–3.51	**<.001**	**< .001**
M_1M_3	6.31	6.17	–4.26	**<.001**	**< .001**
CP_4	4.37	4.14	–8.37	**<.001**	**< .001**

mainly with LaZ and LaN, also with LCb and LCr (Tab. 2a). First component accounts for 45.3% of the variation and the second for 14.3%. The graph of PC1-u and PC2-u indicated that owl selected a group within a bat population (Tab. 2a). Significant differences between PC1-u factor scores of 'owl' and 'control' groups (Mann-Whitney test, Z= –2.3, p=0.02) proved that skulls from owl pellets were smaller, but differences between PC2-u showed opposite trend (Mann-Whitney test, Z= –2.28, p=0.02) (see also Tab. 2a). However PC2-u explained less of variability in comparison with PC2-u and LaZ, LaN, LCb and LCr also contribute to the PC1-u considerably (Tab. 2a).

First principal component (PC1-l) of the PCA performed on all mandibular measurements reflected variability in lengths of lower toothrow (IM_3, CM_3, P_4M_3, M_1M_3, CP_4). Second factor (PC2-l) correlated mainly with LMd and ACr (Tab. 2b). First axis explains 66.3% of the variation and the second 20.7%. The graph of PC1-l and PC2-l again showed that owl selected a particular group within a population of *Myotis myotis* (Tab. 2b). Mann Whitney U-tests on the factor scores for PC1-l demonstrated that skulls from pellets were smaller (Z= –4.6, p< 0.01) while differences between 'owl' and 'control' group in factor scores of PC2-l showed an opposite trend

(Mann-Whitney, Z= 2.73, p< 0.01). However PC2-l did not extract a lot of variability and LMd and ACr which mainly affected PC2-l correlated also with PC1-l considerably (Tab. 2b).

DISCUSSION

It is widely accepted that vertebrate predators typically capture substandard individuals (e.g. the young, weak, sick, aged, and inexperienced) from prey population in higher than expected proportions (Begon et al. 1990). Ideally, individuals captured by a predator should be characterized in terms of a variety of traits that could influence their vulnerability to predation (Temple 1987). Predators forage optimally and they prefer prey which gives them the highest rate of energy return (Krebs & Davis 1987). Numerous studies have shown that owls preferred younger (and lighter) rodents than those typically found in population (Beacham 1979, Marti & Hougue 1979, Longland & Jenkins 1987, Dickman et al. 1991), but data on potential differential predation of size/age classes of bats by owls are missing. However Hartley & Hustler (1993) demonstrated that a pair of bat hawk *Machaeramphus alcinus* took advantage of easily-caught

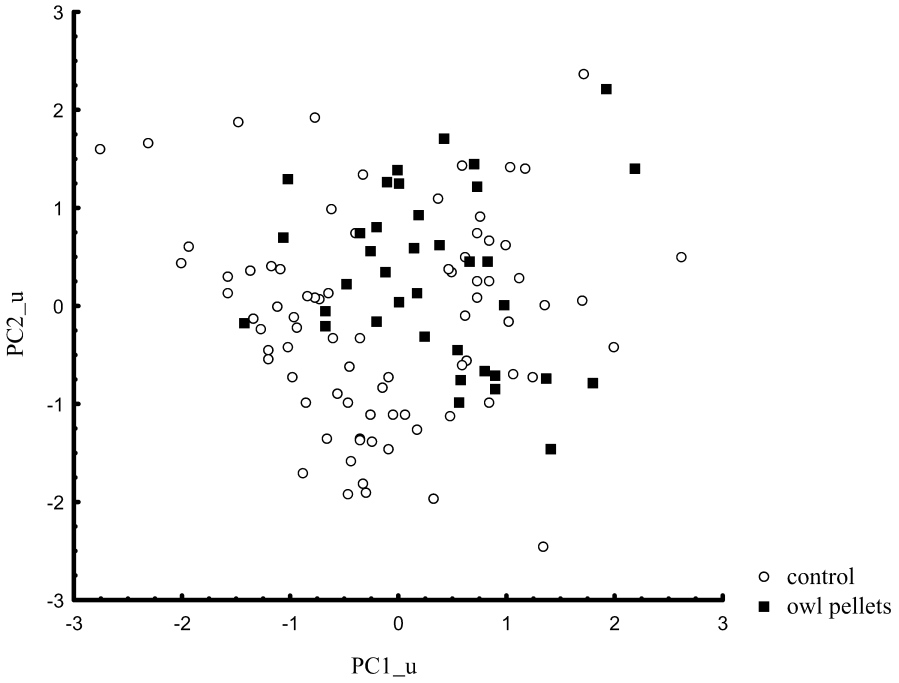


Fig. 2. Results of the PCA on cranial measurements.
Obr. 2. Výsledky analýzy hlavních komponent (PCA) provedené na rozměrech horní čelisti. Vysvětlivky viz obr. 1.

Tab. 2. Results of the PCA on cranial (a), and mandibular measurements (b). Factor coordinates based on correlations
Tab. 2. Výsledky analýzy hlavních komponent (PCA) provedené na rozměrech horní (a) a dolní čelisti (b). Korelace proměnných s hlavními komponentami

(a)	PC1-u	PC2-u	(b)	PC1-l	PC2-l
LaInf	–0.31	0.45	LMd	–0.55	**-0.73**
LaI	–0.11	0.40	ACr	–0.37	**-0.83**
LaZ	–0.44	**0.64**	IM_3	**–0.96**	0.07
LaN	–0.24	**0.60**	CM_3	**–0.98**	0.14
LCr	–0.57	0.57	P_4M_3	**–0.92**	0.28
LCb	**–0.60**	0.52	M_1M_3	**–0.88**	0.33
CC	**–0.83**	0.03	CP_4	**–0.83**	-0.04
P^4P^4	**–0.77**	<–0.01			
M^3M^3	**–0.86**	–0.09			
IM^3	**–0.89**	–0.24			
CM^3	**–0.91**	–0.29			
P^4M^3	**–0.87**	–0.30			
M^1M^3	**–0.75**	–0.29			
CP^4	**–0.74**	–0.17			
P^2P^3	–0.49	–0.22			

pregnant female bats in order to attain breeding condition, whereas their fledglings took advantage of recently independent but naive juvenile bats.

We found out that analyzed skulls from owl pellets were smaller, mainly in lengths of upper and lower toothrow and rostral breadths across teeth. *Myotis myotis* shows age variability in most of the cranial measurements, but the differences are most apparent in rostral breadths across teeth (Benda 1993). The growth of skull is probably finished at the age of 2 or 3 years, although the greatest changes had occurred in the first year (Benda 1993, 1994). According to Benda (1993), geographical variability in cranial measurements of *Myotis myotis* within Czech and Slovak Republic could be neglected. Idem sexual dimorphism is not important from the point of population or species variability. Therefore we conclude that our results could indicate that *Tyto alba* preferred to prey on inexperienced yearlings which were easier to catch whilst were almost as big as adults.

Bats are captured by owls probably mainly during the periods of emergence or return from roosts (Barclay et al. 1982, Roberts et al. 1997, Gerhardt et al. 1994, Hoetker & Gobalet 1999, Romano et al. 1999), but owls are in general not adapted for catching bats (Cramp 1989). The relative benefits of capturing substandard individuals are greatest just when a predator is attacking a species of prey which is typically difficult to capture and kill (Temple 1987).

Both prevolant and newly volant bats are especially susceptible to predation. Flightless young, if not protected by physical barriers, may be taken directly by a variety of predators that range from cockroaches (Wilson 1971) and snakes (Rice 1957) to birds and mammals (Allen 1939, Gillette & Kimbrough 1970). Although *Tyto alba* often shares the roost with a bat colony, this owl hunts for bats in flight and picking hanging bats inside the roost is probably very exceptional (Bauer 1956, König 1961, Ruprecht 1979). Newly volant young are unskilled flyers and often concentrate near the roost during early practice flights (Kunz 1974b, Bradburry

1977, Buchler 1980). At such times these slow-flying bats with poor maneuverable abilities are especially vulnerable. Kunz (1974a) discussed that more then half of all *Eptesicus fuscus* caught by an individual of *Tyto alba* were young of the year. Also *Antrozous pallidus* seems to be most vulnerable to predation when bats are beginning to fly. These young bats are extremely clumsy and frequent collisions with walls, other bats and the observer were noted. Skulls of young *Antrozous pallidus* were found in the pellets of *Bubo virginianus* (O'Shea & Vaughan 1977). Roost selection is an important factor in determining the survival of juvenile bats. The young of species that roost in relatively exposed places are especially vulnerable to predation during emergence (Humphrey 1975).

However owls are supposed to be able to catch bats also during their foraging (Morrison 1978, Verboom 1998, Law & Lean 1999, Vargas et al. 2002) and we cannot exclude this for *Tyto alba*. In general, *Tyto alba* has two foraging periods a night split by a resting pause around midnight (Erkert 1969). Thus, foraging activity of bats and owls may partly overlap (Cramp 1989, Audet 1990). Some individuals of *Tyto alba* can hunt before sunset or after sunrise (Cramp 1989) which enable them to prey upon emerging/returning bats. Both *Myotis myotis* and *Tyto alba* prefer to forage on open habitats including meadows, and fields (Cramp 1989,

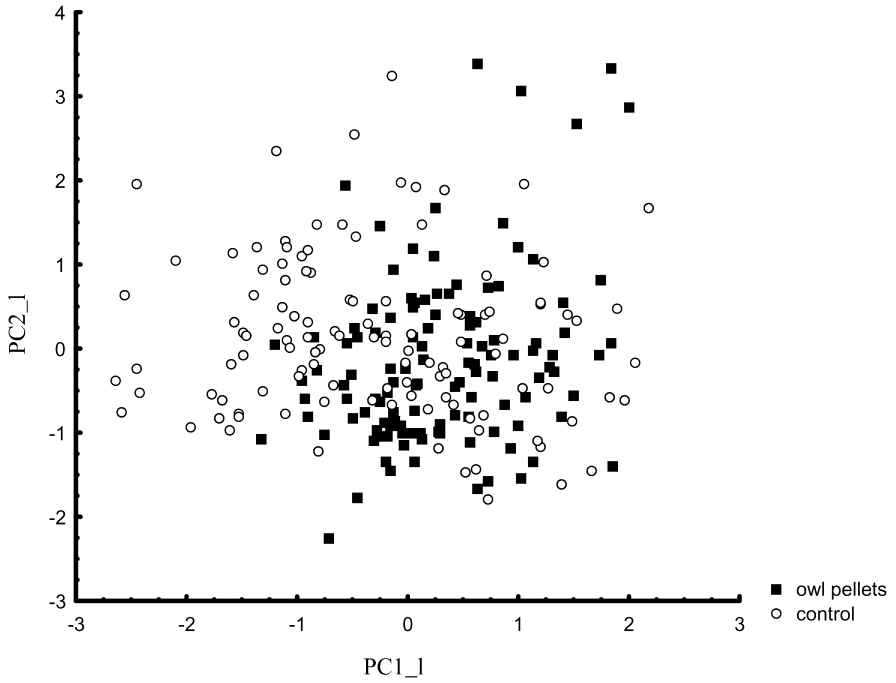


Fig. 3. Results of the PCA on mandibular measurements.
Obr. 3. Výsledky analýzy hlavních komponent (PCA) provedené na rozměrech dolní čelisti. Vysvětlivky viz obr. 1.

SCHOBER & GRIMMBERGER 1993, ARLETTAZ 1996). *Tyto alba* hunts by dropping to ground (CRAMP 1989) so we can speculate that the owl may catch *Myotis myotis* while gleaning a prey on the soil surface (ARLETTAZ 1996). But bats could be also aerial-pursuit like birds (CRAMP 1989). Fledging is a critical period for the survival of young bats and in *Myotis myotis* mothers are not overtly involved in acquisition of foraging skills by its young (AUDET 1990). At this time young bats can be easier prey because of their undeveloped foraging skills and lack of experience.

We also speculate that some individuals of *Tyto alba* may prey on bat colonies only or more intensively in postlactation period to exploit a source of easily capture young inexperienced bats. Therefore a bigger proportion of newly volant bats in owl diet could be pronounced by the departure of adult females in the end of summer (STEBBINGS 1968, KUNZ 1974a, SWIFT 1980). Next research on this problem is required to compare the proportion of particular age classes of bats in different reproductive periods.

ACKNOWLEDGEMENTS

We are grateful to our colleagues Marcel UHRIN, Ervin HAPL and Lucia BOBÁKOVÁ from the Muráňská planina National Park and to Pavel PŘIBYL who assisted in collecting of an important part of the owl pellets for analyses. We are also obligated to Petr BENDA, Jiří JARKOVSKÝ, Martin REICHARD, Martin POKORNÝ, Kateřina VOŠLAJEROVÁ and Hana BERKOVÁ for their helpful comments and long discussions about the problem under the study. We would like to thank to Iain MACKIE for the improvement of the manuscript. This study was funded by grant from the Grant Agency of the Czech Republic No. 206/01/1555.

SOUHRN

Sovy selektivně loví menší a mladší hlodavce, ovšem neexistují důkazy o selektivní predaci sov na netopýrech. V této práci jsme se zabývali predací netopýra velkého (*Myotis myotis*) sovou pálenou (*Tyto alba*). Soubor lebek *Myotis myotis* z vývržků *Tyto alba* byl srovnán s kontrolním souborem lebek z muzejních sbírek a bylo zjištěno, že lebky pocházející ze sovích vývržků jsou menší. Rozdíly byly nejvíce zřetelné u délek zubních řad v dolní a horní čelisti a v šířkách rostra mezi zuby v horní čelisti. Naše výsledky naznačují, že sovy preferují tohoroční vzletná mláďata netopýrů, která jsou téměř stejně velká jako dospělci, ale nemají dostatek zkušeností a jsou tedy pro sovy snadnější kořistí. Tato mláďata jsou méně obratná v letu, jsou tudíž nápadná během výletu z kolonie a navíc se po výletu často soustřeďují v blízkosti úkrytu a jsou tedy snáze ulovitelná.

REFERENCES

ALLEN G. M., 1939: *Bats*. Harvard University Press, New York, 358 pp.

ARLETTAZ R., 1996: Feeding behaviour and foraging strategy of free-living mouse-eared bats, *Myotis myotis* and *Myotis blythii*. *Anim. Behav.*, **51**: 1–11

AUDET D., 1990: Foraging behavior and habitat use by a gleaning bat, *Myotis myotis* (Chiroptera: Vespertilionidae). *J. Mammal.*, **71**: 420–427.

BAUER K., 1956: Schleiereule (*Tyto alba* Scop.) als Fledermausjäger. *J. Ornithol.*, **97**: 335–340.

BARCLAY R. M. R., THOMSON C. E. & PHELAN F. J. S., 1982: Screech owl, *Otus asio*, attempting to capture little brown bats, *Myotis lucifugus*, at a colony. *Can. Field Natur.*, **96**: 205–206.

BEACHAM T. D., 1979: Selectivity of avian predation in declining population of the vole *Microtus townsendii*. *Can. J. Zool.*, **57**: 1767–1772.

BEGON M., HARPER J. L. & TOWNSEND C. R., 1990: *Ecology*: *individuals*, *populations and communities*. Blackwell Scientific Publications, Oxford, 945 pp.

Bekker J. P. & Mostert K., 1991: Predation on the bats in the Netherlands; facts and assumptions. *Myotis*, **29**: 91–96.
Benda P., 1993: *Morfometrická proměnlivost západopalearktických forem podrodu Myotis* [*Morphometrical variability of Western Palaearctic forms of subgenus Myotis*]. M.Sc. Thesis, Department of Zoology, Charles University, Prague, Czech Republic, 153 pp (in Czech).
Benda P., 1994: Biometrics of *Myotis myotis* and *Myotis blythi*: age variation and sexual dimorphism. *Folia Zool*., **43**: 297–306.
Bradbury J. W., 1977: Social organization and communication. Pp.: 1–72. In: Wimsatt W. A. (ed.): *Biology of Bats*. *Volume 3*. Academic Press, New York, 651 pp.
Buchler E. R., 1980: The development of flight, foraging and echolocation in the little brown bat (*Myotis lucifugus*). *Behav*. *Ecol*. *Sociobiol*., **6**: 211–218.
Cade T. J., 1987: *The Falcons of the World*. Cornell University Press, Ithaca and New York, 190 pp.
Cramp S. (ed.), 1989: *The Birds of the Western Paleartic*, *Volume IV*. Oxford University Press. Oxford, New York, 960 pp.
Dickman C. R., Predavec M. & Lynam A. J., 1991: Differential predation of size and sex classes of mice by the barn owl, *Tyto alba*. *Oikos*, **62**: 67–76.
Erkert H. G., 1969: Die Bedeutung des Lichtsinnes für Aktivität und Raumorientierung der Schleiereule (*Tyto alba guttata* Brehm). *Ztschr*. *Vergleich*. *Physiol*., **64**: 37–70.
Gerhardt R. P., Gerhardt D. M., Flatten C. J. & Gonzáles N. B., 1994: The food habits of sympatric *Ciccaba* owls in northern Guatemala. *J*. *Field Ornithol*., **65**: 258–262.
Gillette D. D. & Kimbrough J. D., 1970: Chiropteran mortality. Pp.: 262–281. In: Slaughter B. H. & Walton D. W. (eds.): *About Bats*. Dallas Southern Methodist University Press, Dallas, 339 pp.
Hartley R. & Hustler K., 1993: A less-then-annual cycle in a pair of African Bat Hawks *Machaeramphus alcinus*. *Ibis*, **135**: 456–458.
Hoetker G. M. & Gobalet K. W., 1999: Predation on Mexican free-tailed bats by burrowing owls in California. *J*. *Raptor Res*., **33**: 333–335.
Humphrey S. R., 1975: Nursery roosts and community diversity of Nearctic bats. *J*. *Mammal*., **56**: 321–326.
König C., 1961: Schleiereule, *Tyto a*. *alba* Scop., "schlägt" fliegende Fledermäuse. *Beitr*. *Vogelk*., **7**: 229–233.
Kowalski M. & Lesinski G., 1990: The food of the tawny owl (*Strix aluco*) from near a bat cave in Poland. *Bonn*. *Zool*. *Beitr*., **41**: 23–26.
Krebs J. R. & Davies N. B., 1987: *An Introduction to Behavioural Ecology*. Blackwell Scientific Publications, Oxford, 432 pp.
Kunz T. H., 1974a: Reproduction, growth and mortality of the vespertilionid bat, *Eptesicus fuscus*, in Kansas. *J*. *Mammal*., **55**: 1–13.
Kunz T. H., 1974b: Feeding ecology of a temperate insectivorous bat (*Myotis velifer*). *Ecology*, **55**: 693–711.
Law B. S. & Lean M., 1999: Common blossom bats (*Syconycteris australis*) as pollinators in fragmented Australian tropical rainforest. *Biol*. *Conserv*., **91**: 201–212.
Lee Y. F. & Kuo Y. M., 2001: Predation on Mexican free-tailed bats by peregrine falcons and red-tailed hawks. *J*. *Raptor Res*., **35**: 115–123.
Longland W. S. & Jenkins S. H., 1987: Sex and age affect vulnerability of desert rodents to owl predation. *J*. *Mammal*., **68**: 764–754.
Marti C. D. & Hogue J. G., 1979: Selection of prey by size in screech owls. *Auk*, **96**: 319–327.
Morrison D. W., 1978: Foraging ecology and energetics of the frugivorous bat *Artibeus jamaicensis*. *Ecology*, **59**: 716–723.
Negro J. J., Ibáñez C., Pérez-jordá J. L &. Delarivo M. J., 1992: Winter predation by common kestrel *Falco tinnunculus* on pipistrelle bats *Pipistrellus pipistrellus* in Southern Spain. *Bird Study*, **39**: 195–199.
Obuch J., 1992: Tawny Owl (*Strix aluco*) preying on bats. Pp.: 119–121. In: Horáček I. & Vohralík V. (eds.): *Prague Studies in Mammalogy*. Charles University Press, Prague, 245 pp.

OBUCH J., 1998: The representation of bats (Chiroptera) in the diet of the owls (Strigiformes) in Slovakia. *Vespertilio*, **3**: 65–75.
O'SHEA T. J. & VAUGHAN T. A., 1977: Nocturnal and seasonal activities of the pallid bat, *Antrozous pallidus*. *J. Mammal.*, **58**: 269–284.
PEREZ-BARBERIA F. J., 1990: Patrones de predation de la lechuza comun (*Tyto alba*) sobre murcielagos: Especializacion u oportunismo? *Rev. Biol. Univ. Oviedo*, **8**: 99–105.
PETRŽELKOVÁ K. & ZUKAL J., 1999: The emergence activity of serotine bat and its changes due to predation risk and climatic factors. Pp.: 48–49. In: *Abstracts of VIIth European Bat Research Symposium in Krakow, Poland*. PLATAN Publishing Hause, ISEA PAS, Krakow, 273 pp.
ROBERTS K. J., YANCEY F. D. & JONES C., 1997: Predation by great-horned owls on Brazilian free-tailed bats in north Texas. *Texas J. Sci.*, **49**: 215–218.
RICE D. W., 1957: Life history and ecology of *Myotis austroriparius*. *J. Mammal.*, **38**: 15–32.
ROMANO M. C., MAIDAGAN J. I. & PIRE E. F., 1999: Behavior and demography in an urban colony of *Tadarida brasiliensis* (Chiroptera: Molossidae) in Rosario, Argentina. *Rev. Biol. Trop.*, **47**: 1121–1127.
RUPRECHT A. L., 1979: Bats (Chiroptera) as constituents of the food of barn owls *Tyto alba* in Poland. *Ibis*, **121**: 489–494.
SCHOBER W. & GRIMMBERGER E., 1993: *Bats of Britain and Europe*. Kosmos Verlag, Stuttgart, 265 pp.
SPEAKMAN J. R., 1991: The impact of predation by birds on bat population in the British Isles. *Mammal Rev.*, **21**: 123–142.
STEBBINGS R. E., 1968: Measurement, composition and behaviour of a large colony of the bat *Pipistrellus pipistrellus*. *J. Zool., Lond.*, **156**: 15–33.
SWIFT S. M., 1980: Activity patterns of Pipistrelle bats (*Pipistrellus pipistrellus*) in north-east Scotland. *J. Zool., Lond.*, **190**: 285–295.
TEMPLE S. A., 1987: Do predators always capture substandard individuals disproportionately from prey population? *Ecology*, **68**: 669–674.
UHRIN M., BENDA P., OBUCH J. & URBAN P., 2002: Mammal fauna of the Drienčanský kras Karst Region and surrounding areas (central Slovakia). *Lynx, n. s.*, **33**: 193–247.
VARGAS J., LANDAETA C. & SIMONETTI J. A., 2002: Bats as prey of Barn Owls (*Tyto alba*) in a tropical savanna in Bolivia. *J. Raptor Res.*, **36**: 146–148.
VERBOOM B., 1998: *The use of edge habitats by commuting and foraging bats*. IBN Scientific Contributions 10, DLO Institute for Forestry and Nature Research (IBN-DLO), Wageningen, 120 pp.
WILSON D. E., 1971: Ecology of *Myotis nigricans* (Mammalia: Chiroptera) on Barro Colorado Island, Panama Canal Zone. *J. Zool., Lond.*, **162**: 1–13.

Antipredační chování během výletové a návratové aktivity

Petrželková, K., Downs, N.C., Zukal, J. & Racey, P.A. (2006) A comparison between emergence and return activity in pipistrelle bats *Pipistrellus pipistrellus* and *P. pygmaeus*. Acta Chiropterologica 8, 381-390.

Acta Chiropterologica, 8(2): 381–390, 2006
PL ISSN 1508-1109 © Museum and Institute of Zoology PAS

A comparison between emergence and return activity in pipistrelle bats *Pipistrellus pipistrellus* and *P. pygmaeus*

KLÁRA J. PETRŽELKOVÁ[1, 2, 3], NICHOLAS C. DOWNS[1], JAN ZUKAL[1, 2], and PAUL A. RACEY[1, 4]

[1]*School of Biological Sciences, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, United Kingdom*
[2]*Department of Zoology and Ecology, Masaryk University, Kotlářská 2, Brno CZ-61137, Czech Republic*
[3]*Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, Brno CZ- 60365, Czech Republic*
[4]*Corresponding author: E-mail: p.racey@abdn.ac.uk*

Bats may be vulnerable to predation during evening emergence and morning return to their roosts. Early emergence increases the risk of exposure to raptorial birds, but emerging late confers a risk of missing the dusk peak of aerial insects. Here, both emergence and return activity was studied in detail at the same roosts for the first time. We investigated six maternity colonies of pipistrelle bats (*Pipistrellus pipistrellus* and *P. pygmaeus*) in NE Scotland and recorded light levels and time of emergence and return of the bats with respect to sunset and sunrise on the same nights. Parameters of return activity generally occurred at lower light intensities than those of emergence. Therefore, the interval between dawn return and sunrise was generally longer than that between sunset and dusk emergence. Emergence and return were equal in duration. Bats clustered more on emergence in comparison with return during pregnancy and lactation, whereas during postlactation this trend was reversed.

Key words: *Pipistrellus pipistrellus, P. pygmaeus,* emergence, return, predation risk, temperature

INTRODUCTION

During the reproductive period, females of many bat species gather together in roosts to form maternity colonies, where the young are born and raised to independence. These colonies vary in size from less than ten to several thousand individuals, depending on the species involved. Each evening, bats emerge from their roost to forage and drink and typically they return before dawn (Kunz, 1982). In this paper, we use the terms 'emergence' for the collective departure from the roost at dusk and 'return' for the collective re-entry to the roost at dawn.

Factors regulating the timing of nocturnal activity are of endogenous as well as exogenous origin. Return behaviour of bats at maternity colonies has been relatively little studied in comparison with emergence behaviour (Voûte *et al.*, 1974; Erkert, 1978; Marimuthu, 1984; Isaac and Marimuthu, 1993; Lee and McCracken, 2001). Emergence time of a bat colony changes in parallel with that of sunset and the return parallels sunrise (e.g., Erkert, 1978), but cloud cover can affect emergence (Erkert, 1978; McAney and Fairley, 1988; Kunz and Anthony, 1996) and return behaviour (Erkert, 1978). Thus light levels influence the initiation and cessation of flight activities

(Erkert, 1982). A variety of other extrinsic factors may affect timing and patterns of nocturnal activities of bats (reviewed in Erkert, 1982). Colony size, reproductive status, and level of predation risk can also contribute to variation of emergence (Wilkinson, 1992; Jones and Rydell, 1994; Speakman *et al.*, 1995; Catto *et al.*, 1996; Kunz and Anthony, 1996) and probably also of return times.

The timing of evening emergence may have critical implications for fitness in bats. By emerging late bats risk missing the peak abundance of aerial insects (Racey and Swift, 1985; Rydell *et al*., 1996), but early emergence increase exposure to raptorial birds (Jones and Rydell, 1994; Rydell *et al.*, 1996). Some authors have indicated that returns to the roost probably occur at lower light intensities than emergences (Voûte *et al.*, 1974; Erkert, 1978; McAney and Fairley, 1988, 1990; Isaac and Marimuthu, 1993). However, there are other reports that bats return to roosts at the same (Morrison, 1978; Chase *et al*., 1991; Entwistle *et al.*, 1996) or even higher light intensities (Lee and McCracken, 2001).

Emergence usually consists of individual bats emerging in close succession, with longer gaps when few or no individuals leave. This behaviour has been termed 'outburst activity' or 'clustering'. An improved technique of log survivorship analysis has been applied to document clustering in emergence behaviour and software has been developed (CLUSTAN), which allows the appropriate analysis to be performed (Speakman *et al.*, 1992). The statistical artifact of the number of emerging bats has to be removed by the procedures outlined in Speakman *et al.* (1999). Two hypotheses concerning the functional significance of clustering have been considered (Speakman *et al.*, 1999). It may be an antipredator behaviour (after Hamilton, 1971) or bats may emerge together to co-ordinate their foraging behaviour (Wilkinson, 1992). Clustering behaviour during return to the roost has not been studied. However bats exhibit ritualized behaviours once they reach the roost, which often includes reconnaissance of the site, rallying, and several landing trials before entry (reviewed by Kunz, 1982). In this paper we use the term 'rallying' for this behaviour according to Vaughan and O'Shea (1976), despite some authors use of the term 'swarming' (e.g., Kunz, 1982).

We compared both emergence and return behaviour at maternity colonies of pipistrelle bats (*Pipistrellus pipistrellus*, *P. pygmaeus*) on the same nights. We hypothesized that bats exit and enter their roosts during comparable light intensities and thus the time parameters of emergence and return are identical in respect of sunset/sunrise. We predicted no difference between the duration of emergence and return and no difference between the degree of clustering during emergence and return. The possible effects of reproductive period, locality and temperature were also considered.

Materials and Methods

Study Site

Field work was carried out from mid-May to the end of August 2001 at six localities with maternity colonies of the sibling species *Pipistrellus pipistrellus* (two colonies) and *P. pygmaeus* (four colonies) near Aberdeen, Scotland, U.K. Because all roosts were inaccessible, the species were distinguished according to frequency of the echolocation calls using a bat detector (Pettersson D980, Elektronik AB, Uppsala, Sweden). We observed emergences and returns of bats from their roosts on 34 nights. Colony A roosted in the chimney of a house on the outskirts of Aberdeen, facing a relatively busy road, surrounded by trees and other houses; colony B roosted in the attic of a house on the periphery of a village, surrounded by trees growing along a stream and in an adjacent forest; colony C roosted in the loft of a house next to pastures and the roost opening was opposite a forest; colony D inhabited the turret of a tall house in a

village, surrounded by trees and facing an open field; colony E roosted in the attic of a single house in open country; and colony F in the attic of a single house, surrounded by trees (Table 1).

Roosts had one or two openings and observers had to be able to distinguish individual emerging or returning bats. At colony D, it was difficult to distinguish returning individuals because of the density of bats crowded around the roost opening; thus we recorded only the time and the light level at the end of the return and the colony was observed only during pregnancy.

Despite the fact that the observations described involved no disturbance to the bats, maternity colonies A, B and F vacated monitored roosts during the study. However, colonies A and B returned to these roosts later. We were unable to locate the alternate roosts of monitored colonies.

Data Collecting

Each observation started approximately 30 minutes before the expected onset of emergence according to the time of sunset. Activity around the roost was visually monitored for the entire night in order to record precisely the beginning of the collective return. Observations continued until no bat had returned for 15 minutes. A night vision scope was used at lower light levels. All data concerning emergence and return activity were spoken into a dictaphone and each emerging or returning individual was timed to the second. The tapes were later transcribed using program TIMER (J. R. Speakman, School of Biological Sciences, University of Aberdeen, Scotland). The emergence activity of the whole colony was compared to the return activity using the following parameters (all time parameters were given in respect to sunset or sunrise for a particular day):

Onset of Emergence (OE) versus End of Return (ER); Median of Emergence (ME) versus Median of Return (MR); End of Emergence (EE) versus Onset of Return (OR); Duration of Emergence (DE) versus Duration of Return (DR); Degree of clustering (*G*-value) in Emergence (GE) versus Degree of clustering in Return (GR) (for more details about *G*-values see below);

Light intensity was recorded automatically every minute throughout the night using a luxmeter TESTO 545 (Radiospares, UK) placed close to the roost opening. Data recording lasted during the period of observation. Light levels during emergence and return were compared: Light level at the Onset of Emergence (LOE) versus Light level at the End of Return (LER); Light level at Median of Emergence (LME) versus Light level at Median of Return (LMR); Light level at the End of Emergence (LEE) versus Light level at the Onset of Return (LOR).

Temperature was registered by Tinytag Temperature Dataloggers (Gemini Data Loggers, UK) placed close to the roost opening. The temperature at the onset of emergence (tOE), the temperature at the end of return (tER) and the difference between these values (tOE-tER) were used in analyses.

Reproductive periods were clearly delineated by the behaviour of the bats. During pregnancy the whole colony emerged and the majority of the bats remained out of the roost until the morning return. During lactation, females returned to the roost individually to suckle their young and re-emerged throughout the night. However, we were still able to discern a collective re-entry to the roost at dawn from individual returns. The beginning of the postlactation period was indicated by the first emergence of weaned bats. They were smaller in size and their flight behaviour was inexpert and clearly distinguishable from that of adults.

Data Analysis

The analysis of clustering behaviour was performed by the behavioural temporal clustering analysis program CLUSTAN (J. R. Speakman, School of Biological Sciences, University of Aberdeen,

TABLE 1. Description of localities under study. P.pyg. — *Pipistrellus pygmaeus*, P.pip. — *P. pipistrellus*; (min–max) — minimum and maximum number of bats emerging and returning; P — pregnancy, L — lactation, PL — postlactation

Colony	Latitude/ Longitude	Species	Number of nights	Number of bats (min–max)	Monitored reproductive periods	Habitat in vicinity of roost exit
A	57°13'/02°10'	P.pyg	7	27–108	P, PL	semi-open
B	57°04'/02°34'	P.pyg	7	71–603	P, L, PL	closed
C	57°24'/02°15'	P.pyg	4	92–330	P, L, PL	semi-open
D	57°14'/02°31'	P.pyg	4	280–555	P	semi-open
E	57°25'/02°08'	P.pip	11	39–106	P, L, PL	open
F	57°16'/02°29'	P.pip	1	23–24	P	closed

Scotland). This software calculates an explicit comparison of the observed distribution of intervals between two consecutive individual emergence events to that expected if the emergence was random, and tests the significance of this difference using χ^2 or *G* tests, depending on sample size. Clustering is indicated when there is over-representation of the shortest and the longest intervals in the sequence. *G*-values and χ^2-values are used as measures of clustering. If the values exceed the significance level, then the distribution is clustered. However, it is necessary to adjust *G*-values and χ^2 to eliminate the impact of sample size (Speakman *et al.*, 1999). Adjusted *G*-values were chosen to express the degree of clustering, because the *G*-test is more robust to small sample sizes (number of emerged bats). If the sample size is smaller than 30, CLUSTAN is not able to analyze clustering behaviour.

Differences between the corresponding parameters of the emergence and return for particular days were counted (OE-ER, ME-MR, EE-OR, DE-DR, GE-GR, LOE-LER, LME-LMR, LEE-LOR) and nested Mixed Model ANOVAs were run on these values. The fixed factor 'reproductive period' was nested in a random factor 'locality'. Variables were transformed by Box-Cox or by rank transformation (Sokal and Rohlf, 1995). To account for possible effects of species identity, separate analyses for both species (*P. pipistrellus*, *P. pygmaeus*) were also conducted. These analyses produced concordant results (data not shown).

The *t*-tests for dependent samples were conducted to test emergence parameters versus corresponding return parameters (time, light levels) for each colony separately (except *G*-values). To meet assumptions of parametric tests, log transformation was applied to variables. Bonferroni corrections (with added mean correlation between variables as a parameter) were used for *P*-values (Sankoh *et al.*, 1997). In addition, measure of effect size (Cohen's *d*) was estimated (Cohen, 1988). This index measures the magnitude of a treatment effect as the standardized difference between two means by comparing the overlap in the distribution between the two data sets independently of sample size. Effect sizes can also be interpreted in terms of the percent of nonoverlap of the treated group's scores with those of the untreated group. Large effect size ($d > 0.8$) indicates a nonoverlap of more than 47.4% in the two distributions. To avoid its overestimation, arising from a paired design, we calculated Cohen's *d* from original mean values and standard deviations rather than from the *t*-test value (Cohen, 1988).

G-values between emergence and return were compared using repeated measures ANOVA with a factor of 'reproductive status' for each colony separately. Variables were transformed by Box-Cox transformation (Sokal and Rohlf, 1995). Due to small number of observations it was necessary to pool the observations during pregnancy and lactation to carry out this analysis and only colonies A, B and E were included.

To examine the effect of temperature, Spearman's correlation between emergence/return parameters (excluding EE, LEE, OR, LOR) and temperature parameters was performed at each colony separately. Bonferroni corrections (with added mean correlation between variables as a parameter) were used for *P*-values (Sankoh *et al.*, 1997). All tests were performed in Statistica 6.0 (StatSoft, Inc. 1984–2001).

Results

The relationship between emergence and return activity did not change in respect to locality or reproductive period. Reproductive period significantly affected only differences in clustering (GE-GR) and there was no effect of locality (nested Mixed ANOVA Model, reproductive period: $F_{2, 28.7} = 4.18$, $P = 0.03$, locality: $F_{4, 25} = 0.69$, $P = 0.60$). Differences in *G*-values between emergence and return were highly positive in pregnancy ($n = 14$, $\bar{\times}$GE-GR = 16.96, SD = 39.15) and lactation ($n = 9$, $\bar{\times}$GE-GR = 40.76, SD = 61.77) implying higher *G*-values during emergence in comparison with return, whereas in the postlactation period the trend was opposite ($n = 10$, $\bar{\times}$GE-GR = -7.55, SD = 12.55).

Given the results above, we were able to compare emergence and return parameters (except for GE and GR) at particular colonies for all reproductive periods together. Clear results were demonstrated at colony E: return activity generally occurred at lower light intensities than emergence (Table 2a, Fig. 1a and 1b) and thus time parameters of dawn return (ER, MR, OR) with respect to sunrise were longer than that of emergence (OE, ME, EE) with respect to sunset (Fig. 1c and 1d). Only the duration of emergence and return was equal (Table 2a). The same trends were apparent at other

TABLE 2. Comparison of emergence and return parameters. Significant values are shown in bold

Comparison	Colony				
	A	B	C	D	E
a) Student *t*-test (*P*-values)					
Corrected alpha	< 0.026	< 0.018	< 0.026	< 0.028	< 0.018
Onset of Emergence (OE) vs End of Return (ER)	0.676	0.109	0.070	0.097	**<0.001**
Light level at OE vs Light level at ER	0.175	**0.026**	0.146	0.049	**<0.001**
Median of Emergence (ME) vs Median of Return (MR)	0.085	0.499	**0.015**	–	**<0.001**
Light level at ME vs Light level at MR	0.029	0.056	0.188	–	**<0.001**
End of Emergence (EE) vs Onset of Return (OR)	0.213	0.612	0.381	–	**<0.001**
Light level at EE vs Light level at OR	0.386	0.281	0.685	–	**0.005**
Duration of Emergence (DE) vs Duration of Return (DR)	0.188	0.174	0.628	–	0.837
b) Measure of effect size (Cohen's *d*)					
Onset of Emergence (OE) vs End of Return (ER)	0.263	0.636	**0.870**	**1.670**	**1.490**
Light level at OE vs Light level at ER	**0.840**	**1.138**	**1.312**	**2.203**	**1.407**
Median of Emergence (ME) vs Median of Return (MR)	**0.862**	0.013	**1.153**	–	**1.900**
Light level at ME vs Light level at MR	**0.930**	**1.046**	**1.020**	–	**1.351**
End of Emergence (EE) vs Onset of Return (OR)	0.693	0.136	0.382	–	**1.596**
Light level at EE vs Light level at OR	0.452	0.634	0.553	–	**1.378**
Duration of Emergence (DE) vs Duration of Return (DR)	0.766	0.625	0.332	–	0.073

colonies (Fig. 1), but results were significant only for LOE versus LER at colony B and ME versus MR at colony C (Table 2a). Notably, high values of Cohen's *d* indicated that ER and MR consistently occurred at lower light intensities than OE and ME at all other colonies; and that ER and MR with respect to sunrise were consistently longer than OE and ME with respect to sunset at selected colonies (see Table 2b).

At colony B bats clustered more on emergence in comparison with return during pregnancy and lactation, whereas during postlactation this trend was reversed (Table 3). Similar trends, but non-significant, were demonstrated also for colony A and C.

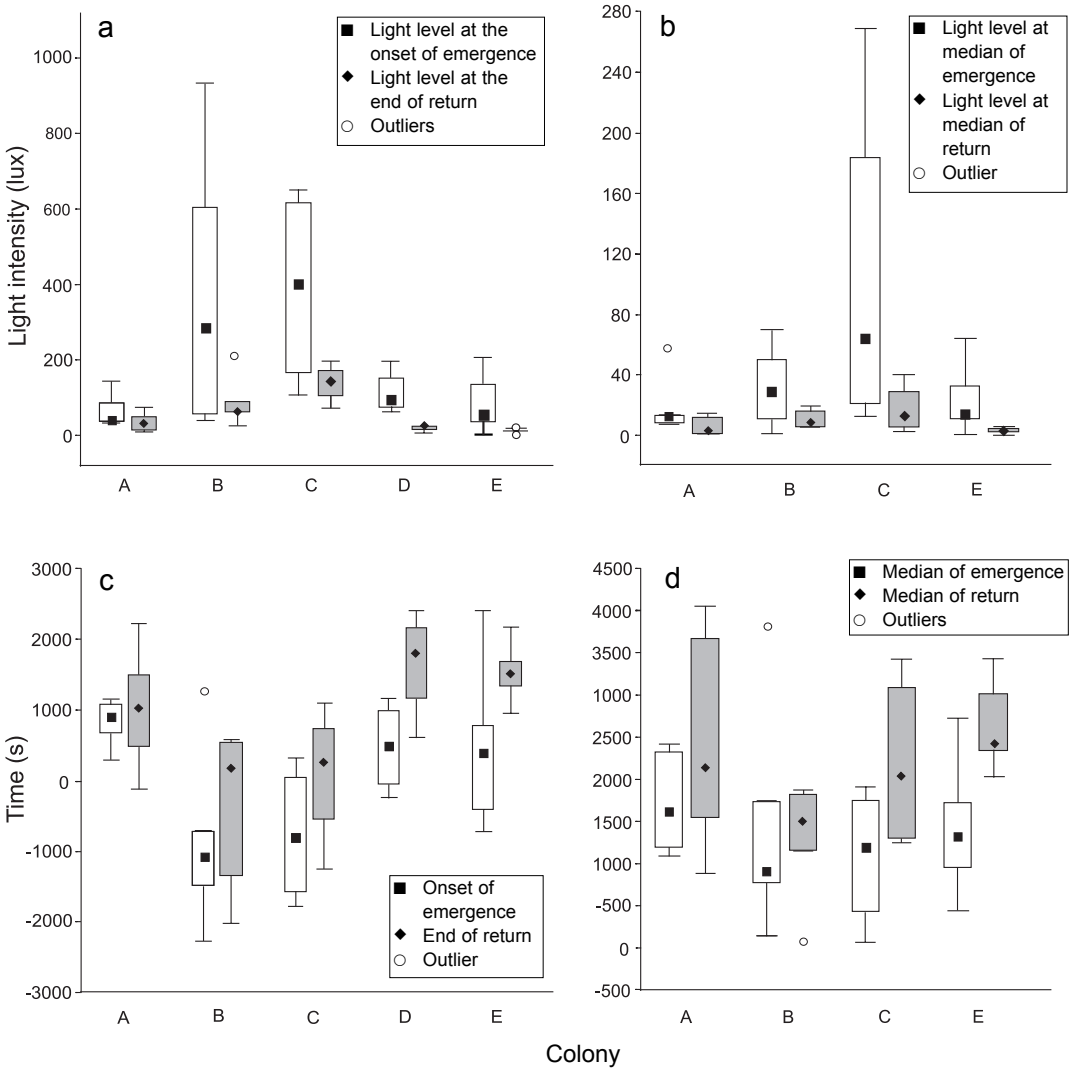


FIG. 1. Comparisons of emergence and return parameters at colonies of pipistrelle bats. Box plots with medians; boxes: 25, 75% quartiles; whiskers: non-outlier minima, non-outlier maxima; open boxes: emergence; hatched boxes: returns. Time refers to seconds before or after sunset or sunrise. a — the light intensity at the onset of emergence (LOE) and the light intensity at the end of return (LER), b — the light intensity at the median time of emergence (LME) and the light intensity at the median time of return (LMR), c — time of the onset of emergence (OE) and the end of return (ER), d — time of the median of emergence (ME) and the median of return (MR). Time is shown before and after sunset

TABLE 3. Results of repeated measures ANOVA that tested the effects of reproductive period on differences between degrees of clustering on emergence and on return. Factor of reproduction, repeated factor emergence/return (E/R) and their interaction are presented. $P < 0.05$ in bold

Effect	Colony					
	A		B		C	
	F-value	*P*-level	*F*-value	*P*-level	*F*-value	*P*-level
Reproduction	0.55	0.49	4.62	0.08	3.81	0.06
E/R	0.77	0.42	3.30	0.13	0.22	0.65
E/R*Reproduction	4.26	0.09	9.68	**0.03**	0.29	0.78

The effect of temperature differed among colonies. Changes in temperature during the night (tOE-tER) did not have any effect on emergence or return parameters. At colony A, bats emerged at lower light intensities when the temperature was higher, whereas during return, higher temperature led to prolongation of activity and bats returned later and at higher light intensities (Table 4). At colony B the effect of tER on return parameters was not statistically significant, but there was the same trend for ER, LER and LMR as at colony A (Table 4). At colony B there was no significant correlation between tOE and emergence parameters. However there were reverse trends for OE and LOE to those at colony A (Table 4). At colony E there was no effect of temperature on emergence or on return (Table 4).

Only the returning bats performed 'rallying behavior'. They congregated in front of roosts, flew about repeatedly and approached roost entrances several times before entering. Occasionally the approach behaviour included landing on the walls of the building near the entrance hole. Therefore the return seemed to be more conspicuous than the emergence.

DISCUSSION

By comparing emergence and return parameters of our studied colonies, it appears that bats return to the roost at lower light intensities than those measured during emergence. Consequently the time between dawn return and sunrise was longer than that between sunset and dusk emergence. The trend did not change with locality or

TABLE 4. Spearman's correlation coefficients between emergence parameters and temperature at the onset of emergences (tOE) and between return parameters and temperature at the end of return (tER). According to Bonferroni's adjustments, the alpha value is for each test is lowered to 0.03 for correlations of tOE with emergence parameters and to 0.04 for correlations of tER with return parameters. Significant coefficients are shown in bold. Onset of Emergence (OE), Light level at the Onset of Emergence (LOE), Median of Emergence (ME), Light level at Median of Emergence (LME), End of Return (ER), Light level at the End of Return (LER), Median of Return (MR), Light level at Median of Return (LMR)

Comparison	Colony		
	A	B	C
Onset of Emergence (OE) vs temperature at Onset of Return (OR)	0.50	-0.31	0.04
Light level at OE vs temperature at OR	**-0.81**	0.54	0.05
Median of Emergence (ME) vs temperature at OR	0.57	0.69	<0.01
Light level at ME vs temperature at OR	-0.70	0.03	0.25
End of Return (ER) vs temperature at ER	**-0.94**	-0.34	-0.10
Light level at ER vs temperature at ER	**0.81**	0.55	-0.21
Median of Return (MR) vs temperature at ER	**-0.81**	0.02	-0.20
Light level at MR vs temperature at ER	0.63	0.27	-0.08

reproductive period. This finding is consistent with the general rule postulated by Daan and Aschoff (1975) that the onset of activity in nocturnal mammals usually occurs at higher light intensities than its end. Previous authors mostly recorded only the time the first bat emerged and the last bat returned with respect to sunset/sunrise, or compared light levels when the first bat emerged with those when the last bat returned, which did not represent the behaviour of the whole colony. Voûte *et al.* (1974) showed that the time between the end of nocturnal activity and sunrise was generally longer than that between sunset and the onset of activity for six species of the genus *Myotis*. The same result was also obtained for *Rhinolophus hipposideros* (McAney and Fairley, 1988), *Nyctalus leisleri* (McAney and Fairley, 1990) and also for six species of tropical bats (Erkert, 1978; Isaac and Marimuthu, 1993). At the beginning of their nocturnal activity, bats are hungry and probably also thirsty so that the cost of missing foraging opportunities by emerging later probably outweighs the risk of predation. Conversely during their return, satiated bats prefer to be back in their roosts earlier, at lower light intensities, in comparison with emergence, and thus avoid predation.

Returns at lower light intensities allow 'rallying' behaviour to be performed before light levels increase to the point when predation by raptors is possible. Such behaviour during return has been recorded for several bat species, e.g., *Antrozous pallidus* (Vaughan and O'Shea, 1976), *Myotis dasycneme* (Vôute *et al.*, 1974), *M. nattereri* (Swift, 1997), *Pipistrellus hesperus* (Cross, 1965) and is also mentioned for *P. pipistrellus* (Stebbings, 1968; Swift, 1980). Nevertheless the function of this 'rallying' behaviour during return remains to be clarified. A similar time of return of all the bats using a roost might result in congestion at the roost entrances. However even single returning bats also showed this behaviour. 'Rallying' behaviour could inform bats about the location of the roost (Vaughan and O'Shea, 1976), may help to establish contact with conspecifics (Voûte *et al.*, 1974) or transfer information about foraging success (Wilkinson, 1992).

In our study, earlier return was clearest for colony E although the same trend was evident at the other colonies. Colony E was situated in the most open habitat. The roost openings were not sheltered and the number of potential predators at this area was highest (11 species, compared with 4–6 at the other roosts — Grampian Ringing Group, pers. comm.). Therefore the bats at this colony may be subject to higher predation risk than at the other colonies studied. Also there was no indication of the effect of temperature on emergence or return at colony E. Bats did not prolong their activity at higher temperatures in the morning, which showed that they probably did not respond to possibly increased foraging opportunities (Maier, 1992; Catto *et al.*, 1995) and preferred to return to the roost sooner rather than expose themselves to diurnal raptors. Non-significant results between some return and emergence parameters at other colonies may be a consequence of the smaller number of nights of observations. However, for all colonies, differences between light intensities have been acknowledged using Cohen's *d*. These results indicate that light levels better characterize emergence and return activities and use of light levels can better detect differences between emergence and return. Differences in habitats adjacent to the roosts, commuting distances, different predation risk and prey availability at the foraging areas for the studied colonies might also impact on the relationship between emergence and return behaviour of colonies. Surprisingly, there was no difference in the duration of the emergence and return period at any of our studied colonies.

The durations of emergence and return have not been compared previously, although casual observations of a bat maternity colony suggest that the return period is longer. This impression is reinforced by the 'rallying' behaviour before entry to the roost. Swift (1980, 1997) described gradual less synchronized return in *P. pipistrellus* and *M. nattereri* with the progress of reproduction, implying that it took longer.

During postlactation the degree of clustering at emergence was lower than at return, which could be explained by the fact that inexperienced newly volant bats do not emerge so synchronously (O'Shea and Vaughan, 1977; Duvergé *et al.*, 2000; Petrželková and Zukal, 2001). On the other hand during pregnancy and lactation, bats clustered more at emergence than at return. Clustering might compensate for higher predation risk during emergence at higher light levels. When *Eptesicus serotinus* emerged earlier and was thus exposed to increased potential predation pressure, the bats increased their degree of clustering which would decrease the probability of an individual being attacked (Petrželková and Zukal, 2001). However because 'rallying' behaviour occurs on return, clustering at this time may not have the same function as on emergence or its function may be attenuated.

Emergence of pipistrelle maternity colonies at dusk occurs at higher light levels than the return to the roosts during the early morning. The duration of emergence and return behaviour is equal and clustering behaviour in the evening and morning differs, depending on reproductive phase. Similar detailed studies are needed for other bat species to establish whether these findings represent a general phenomenon. More observations on the return behaviour of bat colonies are required and the functions of 'rallying' and clustering behaviour during return need to be explained.

Acknowledgements

We are grateful to all householders who allowed us access to their properties to observe bats. KJP would like to thank very much her field assistants Frederique Tholozan, Pascale Michel and Peter Dymond. Isobel Davidson helped to identify suitable bat roosts for our study. We are also very obliged to our colleagues John Speakman, Iain Mackie, Lumír Gvoždík, Martin Reichard, Adam Svobodník, Martin Pokorný and Hanka Berková for their helpful comments and long discussions about the problem under study. The observations reported in this paper involved no disturbance to the bats.

Literature Cited

Catto, C. M. C, P. A. Racey, and P. J. Stephenson. 1995. Activity patterns of the serotine bat (*Eptesicus serotinus*) at a roost in southern England. Journal of Zoology (London), 235: 635–644.

Catto, C. M. C, A. Hutson, P. A. Racey, and P. J. Stephenson. 1996. Foraging behaviour and habitat use of the serotine bat (*Eptesicus serotinus*) in southern England. Journal of Zoology (London), 238: 623–633.

Chase, J., M. Y. Small, E. A. Weiss, D. Sharma, and S. Sharma. 1991. Crepuscular activity of *Molossus molossus*. Journal of Mammalogy, 72: 414–418.

Cohen, J. 1988. Statistical power analysis for the behavioural sciences. 2nd ed. Lawrence Earlbaum Associates, Hillsdale, New Jersey, 567 pp.

Cross, S. P. 1965. Roosting habits of *Pipistrellus hesperus*. Journal of Mammalogy, 46: 270–279.

Daan, S., and J. Aschoff. 1975. Circadian rhythms of locomotor activity in captive birds and mammals: their variations with season and latitude. Oecologia, 18: 269–316.

Duvergé, P. L., G. Jones, J. Rydell, and R. D. Ransome. 2000. Functional significance of emergence timing in bats. Ecography, 23: 32–40.

Entwistle, A. C., P. A. Racey, and J. R. Speakman. 1996. Habitat exploitation by a gleaning bat, *Plecotus auritus*. Philosophical Transactions of the Royal Society of London, 351B: 921–931.

Erkert, H. G. 1978. Sunset-related timing of flight activity in neotropical bats. Oecologia, 37: 59–67.

Erkert, H. G. 1982. Ecological aspects of bat activity rhythms. Pp. 201–242, *in* Ecology of bats (T. H. Kunz, ed.). Plenum Press, New York, xiv + 425 pp.

Hamilton, W. D. 1971. Geometry of the selfish herd. Journal of Theoretical Biology, 31: 295–311.

Isaac, S. S., and G. Marimuthu. 1993. Early outflying and late homeflying in the Indian pygmy bat under natural conditions. Oecologia, 96: 426–430.

Jones, G., and J. Rydell. 1994. Foraging strategy and predation risk as factors influencing emergence time in echolocating bats. Philosophical Transactions of the Royal Society of London, 346B: 445–455

Kunz, T. H. 1982. Ecology of bats. Plenum Press, New York, xiv + 425 pp.

Kunz, T. H., and E. L. P. Anthony. 1996. Variation in nightly emergence behaviour in the little brown bat, *Myotis lucifugus* (Chiroptera: Vespertilionidae). Pp. 225–236, *in* Contributions in mammalogy. A memorial volume honoring J. Knox Jones, Jr. (H. H Genoways and R. J. Baker, eds.). Museum of Texas Tech University, Lubbock, 315 pp.

Lee, Y. F., and G. F. McCracken. 2001. Timing and variation in the emergence and return of Mexican free-tailed bats, *Tadarida brasiliensis mexicana*. Zoological Studies, 40: 309–316.

Maier, C. 1992. Activity patterns of pipistrelle bats (*Pipistrellus pipistrellus*) in Oxfordshire. Journal of Zoology (London), 228: 69–80.

Marimuthu, G. 1984. Seasonal changes in the precision of the circadian clock of a tropical bat under natural photoperiod. Oecologia, 61: 352–357.

McAney, C. M., and J. S. Fairley. 1988. Activity patterns of the lesser horseshoe bat *Rhinolophus hipposideros* at summer roosts. Journal of Zoology (London), 216: 325–328.

McAney, C. M., and J. S. Fairley. 1990. Activity of Leisler's bat *Nyctalus leisleri* (Kuhl, 1818) at a summer roost in Ireland. Myotis, 28: 83–92.

Morrison, D. W. 1978. Foraging ecology and energetics of the frugivorous bat *Artibeus jamaicensis*. Ecology, 59: 716–723.

O'Shea, T. J., and T. A., Vaughan. 1977. Nocturnal and seasonal activities of the pallid bat, *Antrozous pallidus*. Journal of Mammalogy, 58: 269–284.

Petrželková, K., and J. Zukal. 2001. Emergence behaviour of the serotine bat (*Eptesicus serotinus*) under predation risk. Netherlands Journal of Zoology, 51: 395–414.

Racey, P. A., and S. M. Swift. 1985. Feeding ecology of *Pipistrellus pipistrellus* (Chiroptera: Vespertilionidae) during pregnancy and lactation. I. Foraging behaviour. Journal of Animal Ecology, 54: 205–215.

Rydell, J., A. C. Entwistle, and P. A. Racey. 1996. Timing of foraging flights of three species of bats in relation to insect activity and predation risk. Oikos, 76: 243–252.

Sankoh, A. J., M. F. Huque, and S. D. Dubey. 1997. Some comments on frequently used multiple endpoint adjustments methods in clinical trials. Statistics in Medicine, 16: 2529–2542.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 3rd ed. W. H. Freeman and Company, New York, 887 pp.

Speakman, J. R., D. J. Bullock, L. A. Eales, and P. A. Racey. 1992. A problem defining a temporal pattern in animal behaviour: clustering in the emergence behaviour of bats from maternity roosts. Animal Behaviour, 43: 491–500.

Speakman, J. R., R. E. Stone, and J. E. Kerslake. 1995. Temporal patterns in the emergence behaviour of pipistrelle bats, *Pipistrellus pipistrellus*, from maternity colonies are consistent with an anti-predator response. Animal Behaviour, 50: 1147–1156.

Speakman, J. R., N. Irwin, and R. Stone. 1999. Effect of roost size on the emergence behaviour of pipistrelle bats. Animal Behaviour, 58: 787–795.

Stebbings, R. E. 1968. Measurement, composition and behaviour of a large colony of the bat *Pipistrellus pipistrellus*. Journal of Zoology (London), 156: 15–33.

Swift, S. M. 1980. Activity patterns of pipistrelle bats (*Pipistrellus pipistrellus*) in north-east Scotland. Journal of Zoology (London), 190: 285–295.

Swift, S. M. 1997. Roosting and foraging behaviour of Natterer's bats (*Myotis nattereri*) close to the northern border of their distribution. Journal of Zoology (London), 242: 375–384.

Vaughan, T. A., and T. J. O'Shea. 1976. Roosting ecology of the pallid bat *Antrozous pallidus*. Journal of Mammalogy, 57: 19–42.

Voûte, A. M., J. W. Sluiter, and M. P. Grimm. 1974. The influence of the natural light-dark cycle on the activity rhythm of pond bat (*Myotis dasycneme* Boie, 1825) during summer. Oecologia, 17: 221–243.

Wilkinson, G. S. 1992. Information transfer at evening bat colonies. Animal Behaviour, 44: 501–518.

Received 27 January 2006, accepted 01 June 2006

Těžké kovy a syndrom bílého nosu - potenciální hrozby pro populace letounů

Martínková, N., Bačkor, P., Bartonička, T., Blažková, P., Červený, J., Falteisek, L., Gaisler, J., Hanzal, V., Horáček, D., Hubálek, Z., Jahelková, H., Kolařík, M., Korytár, L., Kubátová, A., Lehotská, B., Lehotský, R., Lučan, R., Májek, O., Matějů, J., Řehák, Z., Šafář, J., Tájek, P., Tkadlec, E., Uhrin, M., Wagner, J., Weinfurtová, D., Zima, J., Zukal, J. & Horáček, I. (2010) Increasing Incidence of *Geomyces destructans* Fungus in Bats from the Czech Republic and Slovakia. PLoS One 5, e13853.

OPEN ACCESS Freely available online

PLoS one

Increasing Incidence of *Geomyces destructans* Fungus in Bats from the Czech Republic and Slovakia

Natália Martínková[1,2]*, Peter Bačkor[3], Tomáš Bartonička[4], Pavla Blažková[5,6], Jaroslav Červený[7], Lukáš Falteisek[8], Jiří Gaisler[4], Vladimír Hanzal[9], Daniel Horáček[10], Zdeněk Hubálek[1], Helena Jahelková[8], Miroslav Kolařík[11], L'uboš Korytár[12], Alena Kubátová[11], Blanka Lehotská[13], Roman Lehotský[14], Radek K. Lučan[8], Ondřej Májek[2], Jan Matějů[8,15], Zdeněk Řehák[4], Jiří Šafář[16], Přemysl Tájek[5], Emil Tkadlec[1,17], Marcel Uhrin[18], Josef Wagner[19], Dita Weinfurtová[8], Jan Zima[1], Jan Zukal[1,4], Ivan Horáček[8]

1 Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic, **2** Institute of Biostatistics and Analyses, Masaryk University, Brno, Czech Republic, **3** Department of Biology and Ecology, Matej Bel University, Banská Bystrica, Slovakia, **4** Department of Botany and Zoology, Masaryk University, Brno, Czech Republic, **5** Agency for Nature Conservation and Landscape Protection of the Czech Republic, Mariánské Lázně, Czech Republic, **6** Department of Zoology, University of South Bohemia, České Budějovice, Czech Republic, **7** Department of Forest Protection and Game Management, Czech University of Life Sciences Prague, Praha, Czech Republic, **8** Department of Zoology, Faculty of Science, Charles University in Prague, Praha, Czech Republic, **9** Agency for Nature Conservation and Landscape Protection of the Czech Republic, Praha, Czech Republic, **10** Sametová 721/18, Liberec, Czech Republic, **11** Department of Botany, Faculty of Science, Charles University in Prague, Praha, Czech Republic, **12** University of Veterinary Medicine and Pharmacy, Košice, Slovakia, **13** Department of Landscape Ecology, Comenius University, Bratislava, Slovakia, **14** Miniopterus - Principal Organization of the Slovak Union for Nature and Landscape Conservators, Bratislava, Slovakia, **15** Agency for Nature Conservation and Landscape Protection of the Czech Republic, Karlovy Vary, Czech Republic, **16** Agency of Nature Conservation and Landsape Protection of the Czech Republic, Olomouc, Czech Republic, **17** Department of Ecology and Environmental Science, Palacky University, Olomouc, Czech Republic, **18** Institute of Biology and Ecology, P. J. Šafárik University, Košice, Slovakia, **19** ZO ČSS 7-01 ORCUS Bohumín, Czech Speleological Society, Bohumín, Czech Republic

Abstract

Background: White-nose syndrome is a disease of hibernating insectivorous bats associated with the fungus *Geomyces destructans*. It first appeared in North America in 2006, where over a million bats died since then. In Europe, *G. destructans* was first identified in France in 2009. Its distribution, infection dynamics, and effects on hibernating bats in Europe are largely unknown.

Methodology/Principal Findings: We screened hibernacula in the Czech Republic and Slovakia for the presence of the fungus during the winter seasons of 2008/2009 and 2009/2010. In winter 2009/2010, we found infected bats in 76 out of 98 surveyed sites, in which the majority had been previously negative. A photographic record of over 6000 hibernating bats, taken since 1994, revealed bats with fungal growths since 1995; however, the incidence of such bats increased in *Myotis myotis* from 2% in 2007 to 14% by 2010. Microscopic, cultivation and molecular genetic evaluations confirmed the identity of the recently sampled fungus as *G. destructans*, and demonstrated its continuous distribution in the studied area. At the end of the hibernation season we recorded pathologic changes in the skin of the affected bats, from which the fungus was isolated. We registered no mass mortality caused by the fungus, and the recorded population decline in the last two years of the most affected species, *M. myotis*, is within the population trend prediction interval.

Conclusions/Significance: *G. destructans* was found to be widespread in the Czech Republic and Slovakia, with an epizootic incidence in bats during the most recent years. Further development of the situation urgently requires a detailed pan-European monitoring scheme.

Citation: Martínková N, Bačkor P, Bartonička T, Blažková P, Červený J, et al. (2010) Increasing Incidence of *Geomyces destructans* Fungus in Bats from the Czech Republic and Slovakia. PLoS ONE 5(11): e13853. doi:10.1371/journal.pone.0013853

Editor: Dee A. Carter, University of Sydney, Australia

Received June 24, 2010; **Accepted** October 17, 2010; **Published** November 5, 2010

Funding: This study was supported by grants MSM0021620828 and LC06073 provided by Ministry of Education, Youth and Sports of the Czech Republic. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: martinkova@ivb.cz

Introduction

White-nose syndrome (WNS) is an emerging infectious disease, affecting hibernating insectivorous bats [1]. Since its first known appearance in 2006, WNS has spread with each year into the underground hibernacula in the USA and Canada, and over one million deaths within populations of several bat species have been attributed to the disease [1,2]. The decline is severe enough to warrant a prediction that once common *Myotis lucifugus* might become locally extinct in less than two decades [2].

The most likely infectious agent of WNS is the newly described fungal species *Geomyces destructans* Blehert & Gargas, 2009 [3]. It is psychrophilic, and does not grow at temperatures higher than about 20°C. During hibernation, when the body temperature of bats drops to the ambient temperatures of the underground hibernacula, the fungal mycelia can grow upon the skin surfaces of

these animals [1,3]. The fungus invades the hair follicles and associated glands, or it breaks the epidermis of naked skin on the ears, muzzle, and wing membranes [1,4]. The specific etiology of the fungal infection is unknown, but the bats awaken from hibernation. As arousal from hibernation is energetically demanding, it is believed that WNS leads to a more rapid disappearance of fat reserves [1,4,5,6], deteriorating the body condition, and often to increased mortality due to starvation [7]. The bats prematurely emerge from the hibernacula and attempt to forage, which in winter conditions causes frostbite and subsequent necrosis of the wing membranes [8]. The fungal lesions co-infected with Gram-negative bacteria exhibit necrosis and pustules [5], furthering wing membrane damage and compromising flight abilities. Although not all of the details of the epizootics are fully understood [4,5,7,8,9,10], it is widely accepted that WNS poses a severe threat for the bat populations in North America [2,11]. The threat is likely to increase in the future, leading to local extinctions of the bats [2], and suggesting altering ecosystem dynamics [7,10].

The problem may expand onto one on a global scale, as *G. destructans* was reported in France in March 2009 [12], and from Germany, Switzerland, and Hungary in the winter of 2008/2009 [13]. Here, we show that the occurrence of *G. destructans* in Europe is not episodic, but it is locally widespread and could be associated with skin lesions. We believe that *G. destructans* has been present historically within Europe, but that the epizootic is currently (re-)starting, with marked local differences in both the intensity and the dynamics of the disease.

Results

Historical Record of Geomycosis

The compilation of photographs of hibernating bats revealed a white patch on the muzzle of a *M. myotis* individual on March 4, 1995 from the Zbojnícka Cave, Malé Karpaty, Slovakia (Table 1). A photograph from January 25, 1997 from the Javoříčské Caves in northern Moravia, Czech Republic depicts a *M. myotis* individual with a fungal growth typical of the *G. destructans* infection (Fig. 1A). Further records show sporadic images of randomly photographed affected *M. myotis* until 2007/2008, when the incidence of bats with white patches started to increase in several species (Table 1).

Recent Presence of Geomycosis

Targeted on-site inspections of WNS-like clinical signs (white fungal growths on a bat, loss of sheen on wing membranes, emaciated forearms or the whole body if the hair was wet - worded as and regarded as 'WNS-suspect' throughout the remainder of this text) commenced in 2008/2009, as a part of the regular bat census. Bats exhibiting white fungal growths on their muzzle and/or wings were found at 7 sites. In total, 6 bat species were affected; *M. myotis* (24 individuals), *M. blythii* (1), *M. brandtii* (1), *M. dasycneme* (1), *M. emarginatus* (1) and *M. mystacinus* (1). During regular monitoring through the most recent winter of 2009/2010 (January/February), WNS-suspect bats were found at 33 sites out of over 800 hibernacula in the Czech Republic (CZ) and Slovakia (SK), combined. Additionally, 98 sites were inspected again in late February and March, and the fungus was then sampled for cultivation, microscopic, and genetic analyses. During that time period, the incidence of WNS-suspect bats increased to 76 localities across CZ and SK (Fig. 2). Most often the WNS-suspect bats were *M. myotis* (375 individuals), but also included *M. blythii* (19), *M. dasycneme* (2), *M. bechsteinii* (1), *M. mystacinus* (1), and *M. nattereri* (1). Specific regions differed in the prevalence of WNS-suspect bats. The highest levels of infestations were concentrated in submountain humid to mesic regions, where 11 to 100% of *M. myotis* were WNS-suspect. Infestation was less frequent in the hibernacula within mountainous zones (Šumava Mts.: 5% WNS-suspect *M. myotis*; the majority of SK localities: 0–5%), and also in limestone regions (Bohemian: 3%, Moravian: 2%, and Slovak: 3% karsts).

Table 1. Summary of WNS-suspect bats from those photographed randomly (number of all photographed bats/number of photographed WNS-suspect bats) during the periods 1994–1998 and 2003–2010.

Winter period	1994/95	1995/96	1996/97	1997/98	2003/04	2004/05	2005/06	2006/07	2007/08	2008/09	2009/10
Species											
R. hipposideros	-	3/0	-	2/0	-	4/0	11/0	26/0	82/0	45/0	153/15
M. bechsteinii	-	-	-	-	1/0	2/0	-	-	3/0	3/0	8/0
M. brandtii	-	1/0	-	-	-	-	-	3/0	2/0	5/1	5/0
M. daubentonii	-	10/0	2/0	7/0	14/0	9/0	14/0	38/0	18/0	42/0	55/0
M. emarginatus	-	-	-	-	-	-	-	6/0	2/0	6/0	42/0
M. myotis	7/1	117/1	37/0	238/4	555/0	452/2	577/7	434/1	738/16	500/13	612/86
M. mystacinus	-	-	2/0	3/0	4/0	-	4/0	8/0	-	18/1	3/0
M. nattereri	-	3/0	6/0	3/0	5/0	7/0	2/0	15/0	28/0	25/1	20/0
E. nilssonii	-	3/0	-	12/0	4/0	5/0	6/0	11/1	7/0	4/0	10/1
E. serotinus	-	1/0	-	-	-	2/0	-	-	-	-	-
P. pipistrellus	-	-	-	-	-	-	2/0	-	-	-	437/0
N. noctula	-	-	-	-	-	-	-	-	-	-	2/0
V. murinus	-	-	-	-	1/0	-	-	1/0	-	-	-
B. barbastellus	-	148/0	-	5/0	8/0	11/0	12/0	122/0	55/0	205/0	22/0
P. auritus	-	4/0	2/0	7/0	15/0	4/0	8/0	9/0	12/0	16/0	26/0
P. austriacus	-	2/0	-	-	5/0	1/0	-	9/0	1/0	-	14/0

Photographs by T. Bartonička, L. Bufka, J. Červený, B. Lehotská, R. Lehotský, J. Matějů, J. Šafář, P. Tájek, J. Vogeltanz, and O. Vojtěch.
doi:10.1371/journal.pone.0013853.t001

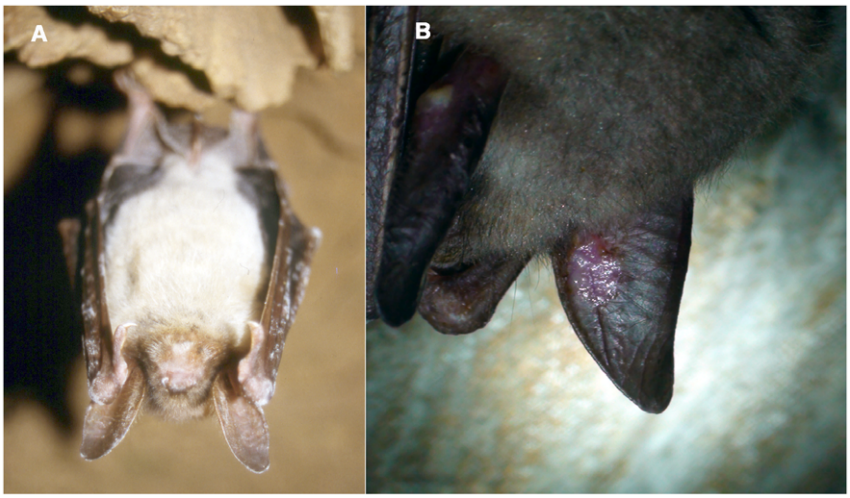


Figure 1. White-nose syndrome symptoms in the Czech Republic and Slovakia. (A) Hibernating *M. myotis* in the Javoříčské Caves, Czech Republic, photographed on 25 January 1997. Fungal growth was not identified. (Photo by Jiří Šafář) (B) Skin lesions on *M. myotis* from the Malá Amerika Mines, Karlštejn, Czech Republic, photographed on 16 March 2010. *G. destructans*, isolate number CCF3942, was isolated from the sample taken from the lesion. (Photo by Ivan Horáček).
doi:10.1371/journal.pone.0013853.g001

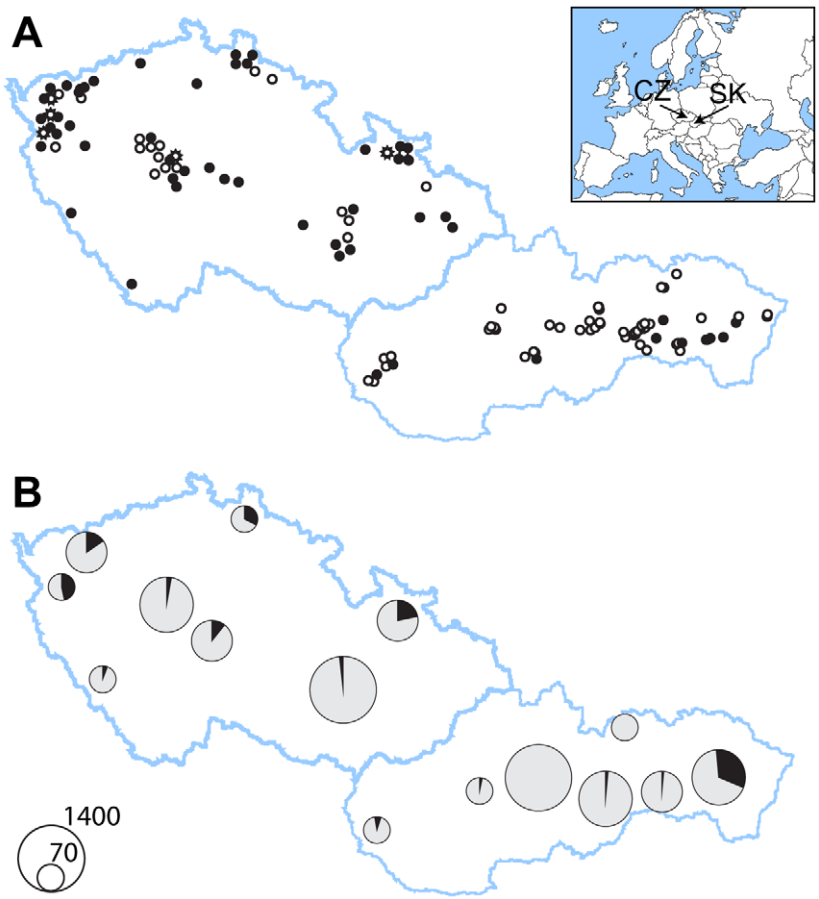


Figure 2. Occurrence of *Geomyces destructans* in the Czech Republic and Slovakia. (A) Distribution of WNS on the background of localities targeted for WNS screening. Some circles represent more than one hibernaculum. White circles -localities censused in 2009 and 2010; black circles - localities with WNS-suspect bats; stars - localities with photographic evidence of WNS in 2007 and 2008. (B) Prevalence of WNS-suspect individuals from *Myotis myotis* populations. Data pooled according to region; circle size is proportional to the population size.
doi:10.1371/journal.pone.0013853.g002

Four localities with WNS-suspect bats in Central Bohemia were visually checked every two weeks between late February and March 2010. We found decreasing percentages of individuals with fungal growth on muzzle and wings towards the end of their hibernation.

Occurrence of *Geomyces destructans*

We collected the fungus on swabs and transparent adhesive tape between February 2, 2010 and March 26, 2010. In total, we collected the fungus from 90 bats, where 58 samples were collected onto cotton swabs, 10 onto nylon swabs, and 20 onto adhesive tape, one on both a nylon swab and adhesive tape and one sample consisted of shed hair (Table S1). Direct microscopic observation of the adhesive tape samples and nylon swabs from the WNS-suspect bats (*M. myotis*) confirmed the presence of conidia and mycelia with morphology consistent with *G. destructans* on 22 bats (Fig. 3A). Out of the 48 cultures, we isolated *G. destructans* from 16 (Table S1, Fig. 3B, C); and of these, 6 originated from the nylon swabs, 9 from the cotton swabs, and 1 from the adhesive tape sample. The isolates showed microscopic features typical of *G. destructans* (according to [3]), *i. e.* branched conidiophores with intercalary, lateral and terminal arthroconidia, conidia with a truncate base, mostly 5.8–7.7×2.7–3.4 µm, young conidia ob-ovoid or cymbiform, mature conidia asymmetrical, crescent-like, curved (Fig. 3B). Colonies grow best on either malt extract or yeast and malt extract agars at 15°C (Fig. 3D). They grow slowly, reaching 18 mm after one month. The colonies were initially white, later pale brown to grey; the reverse uncoloured to brown or grey. These characteristics are similar to those previously described for isolates of *G. destructans* [3,12].

We isolated DNA from 59 fungus samples, and 32 sequences, 933 base-pairs in length, were deposited into GenBank (Accession Numbers: HM584948 - HM584979; Table S1). Twenty-eight sequences were identical to previously sequenced *G. destructans* isolates [3,9,12]. Four other sequences, 3 from samples collected from *M. myotis*, and 1 from *M. bechsteinii*, exhibited a single A→G substitution in the sequenced region, namely, at position 144 of the internal transcribed spacer 1 gene (*ITS1*); additionally, one of those samples contained both the A and G alleles. Other samples did not amplify in the PCR reaction, or the sequences represented different taxa (Table S1).

At least 6 individuals were without an apparent mycelia cover, but had conspicuous lesions on either their auricles or wing membranes (Fig. 1B). *G. destructans* isolate CCF3942, was isolated from a sample taken from the lesion, and identified both by direct microscopy and cultivation (Fig. 3A).

Population Size Trend of *Myotis myotis*

Both the CZ and SK populations of *M. myotis* have been continuously growing during the studied period (Fig. 4). The average annual realized growth rate per capita of the CZ population is 0.058 (95% CI -0.008 to 0.122), corresponding to an increase of about 6% per year. In SK, the average annual growth rate is 0.008 (95% CI -0.087 to 0.103), corresponding to an increase of about 1% per year. Since 2008, the numbers of CZ and SK populations have declined by 6% and 11%, respectively (the joint numbers declined by 8%). However, the declining numbers fall well within the prediction intervals calculated for 2009 and 2010 (Fig. 4). Hence, there is as yet no evidence for a change in the population trend (CZ: $p = 0.88$, SK: $p = 0.81$). These conclusions remained unchanged after input of the missing data (CZ: $p = 0.82$, SK: $p = 0.82$).

Discussion

We demonstrated that the fungus *G. destructans* is present in Central Europe, and that it is accompanied by aspects that might be suggestive of WNS (specifically white fungal growth on muzzle and wings, the skin lesions, loss of sheen on wing membranes, emaciated forearms or the whole body if the hair was wet). We have not conducted a histopathologic examination [4,5], as no animals were euthanized in the course of this study; however, *G. destructans* was isolated from a scarred ear of a *M. myotis* individual without any apparent fungal growth. The presence of *G. destructans* has been previously demonstrated [12,13], but the bat examined by Puechmaille et al. [12] was healthy, and Wibbelt et al. [13] reported a bat from Hungary with *G. destructans* growth to have survived until its next hibernation without any subsequent

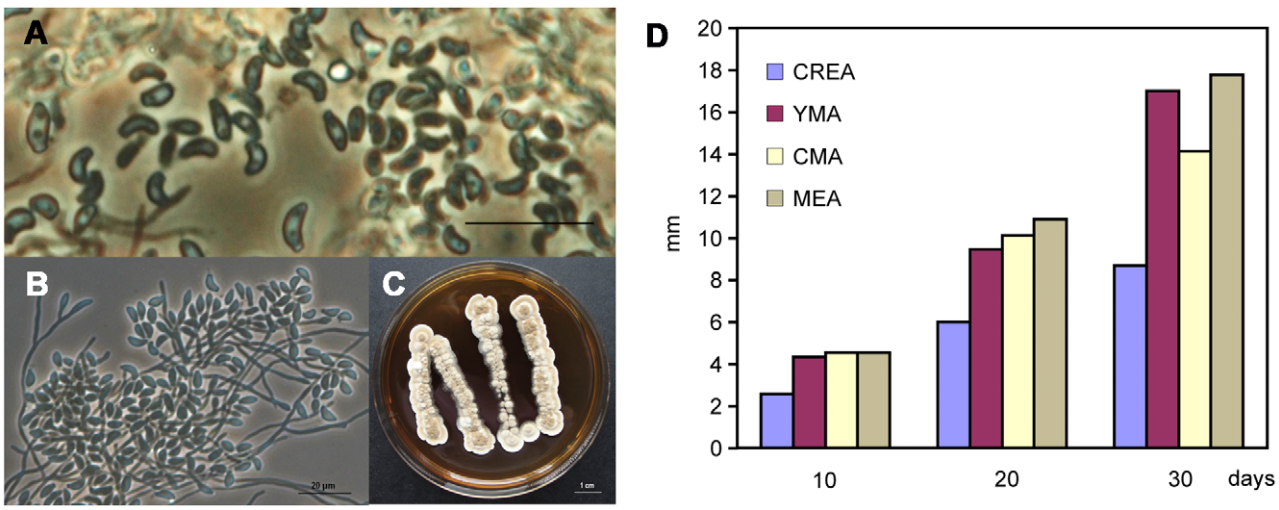


Figure 3. Spores and colonies of *G. destructans*. (A) Adhesive tape sample from the lesion of *M. myotis* photographed in Figure 1B, locality Malá Amerika Mines, Karlštejn, Czech Republic (Phase contrast). (B) *G. destructans* CCF3937. Conidiophores and arthroconidia (SDA, 14 days, 15°C, phase contrast). (C) Primary isolation of *G. destructans* CCF3942 (SDA, 1 month, 15°C). (D) Growth characteristics of *G. destructans* on four agar media at c. 15°C.

doi:10.1371/journal.pone.0013853.g003

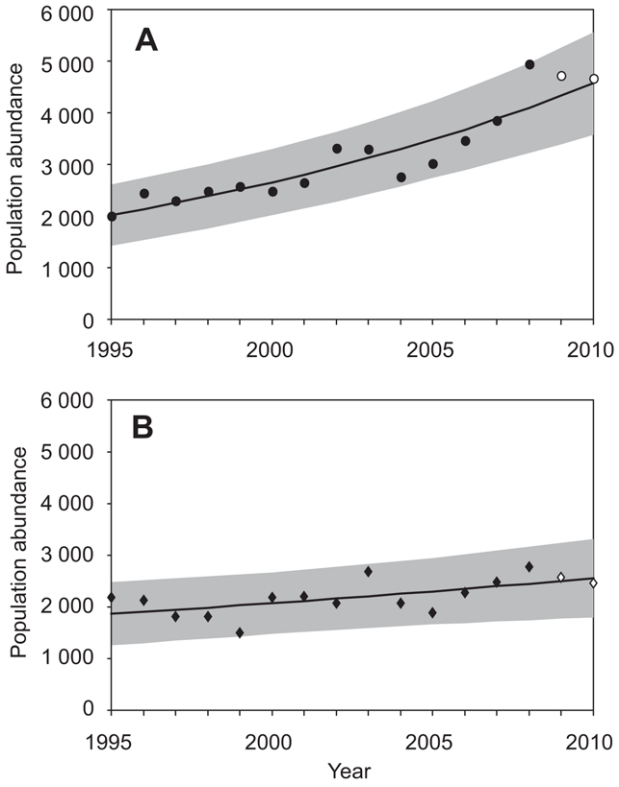


Figure 4. Upward population trends of hibernating *M. myotis*. In the Czech (A) and Slovak Republic (B) the trends were modelled over the period 1995–2010 by fitting Poisson regression allowing for over-dispersion in the data. The point prediction (solid line) and 95% prediction intervals (shaded area) are based on observations up to 2008 (solid symbols) and then extrapolated to 2009 and 2010. The open symbols represent observed data for 2009 and 2010.
doi:10.1371/journal.pone.0013853.g004

manifestation of the fungus. Neither study affirmed the presence of the disease, due to the absence of mass mortality in European bats; this contrasting with the disastrous population declines that have been seen in North America [1,2]. We have shown that the *G. destructans* infection in our study exhibited a marked difference in the possible impacts on the bat populations compared to the North American case. Long-term population census data indicate an increase in population size in *M. myotis* in the Czech Republic and Slovakia, followed by a minor decline in 2009 and 2010, but well within the prediction interval for new data. Consequently, future observations are necessary in order to decide on the causality between possible WNS and bat population trends in Europe. An association of this population size fluctuation with the emergence of *G. destructans* infection cannot be ruled out, however, at the moment, we treat the result with caution. Our population trend analysis showed that the decline likely either represents a natural population fluctuation. Further monitoring will be necessary for a more complete evaluation of this trend.

The incidence of white fungal patches, a clinical sign of WNS, in hibernating bats in CZ and SK, increased markedly in 2010, suggestive of an epizootic spread of the fungus. Seasonally, more WNS-suspect bats were found late in their hibernation; although, the fungal growths disappeared prior to their leaving the hibernacula. This is in accord with previous information that *G. destructans* grows slowly, and that visually apparent mycelia mostly develop in the late winter and early spring [1,9,12]. Direct observations of arousing bats suggested that the infected bats tend to groom and remove surface mycelia immediately after arousal. According to our data, sampling the fungus onto nylon swabs enabled successful cultivations, even from lesions without visible mycelia. Previous studies have shown that isolations of *G. destructans* cultures were relatively rare, despite the presence of fungal spores in the samples that were revealed microscopically [9,14]. Our results on a small sample size might help improve future sampling methodology to better facilitate the culture diagnostics of the pathogen.

Sequences of the *ITS1* gene showed for the first time to our knowledge polymorphism in the gene of *G. destructans*. In general, the *ITS* region has been used in WNS-related studies as a conservative marker that facilitates molecular identification of fungal species, similar in principle to DNA barcoding [1,3,9,12,13]. There are 33 sequences of the *G. destructans ITS* region in GenBank (retrieved on June 4, 2010), and all are identical. We have found four samples with a new allele. Genes encoding ribosomal RNA exhibit a low variability across large areas in fungi [15,16], so we can speculate that occurrence of *G. destructans* in Europe predates its presence in North America, as was suggested by [13]. Our inspection of the photographed bats with fungal growths since 1995 further supports this assumption. If *G. destructans* was historically present in Europe, why has it never been detected on a large scale before (on the other hand, see [17])? During more than four decades of continuous monitoring in CZ, we have only detected faint fungal-like growths on hibernating bats since the 1990s. Our microscopic and genetic analyses showed that such a faint sheen might represent a wide spectrum of organisms, including nematodes. While some photographs might be debatable, we believe that Figure 1A shows an infection of *G. destructans*. In Javoříčské caves in north-eastern part of CZ, where the earliest photographic record of infected *M. myotis* originated, the species is recently rare. Later photographs from the north-western part of CZ coincide with regions with multiple positive records from the winter 2008/2009, as well as the highest infestation in 2010.

These facts indirectly support the hypothesis, presented above, that *G. destructans* was a resident element in Europe prior to its first appearance in North America [13]. If that is the case, why does WNS not, and why in the recent past did it not, cause mass mortality in Europe? At the moment, we lack the data that would answer these questions unequivocally, but we agree with the hypothesis of Wibbelt et al. [13] that differences in clustering behaviour in the most affected species (*M. lucifugus* vs. *M. myotis*) during hibernation might play an important role.

Until now, no other agent except *G. destructans* has been consistently associated with WNS [1,3,4,5,9], and we can further assume that the proximate effects of the fungus result in increased arousal frequency, flight activity in and outside of the hibernacula, and secondary infections. The mass mortality accompanying WNS is present in North America, but not in Europe. Different strategies of hibernation in the European underground hibernacula and those in North America could magnify the final effects of a yet undefined causality chain of *G. destructans* infection that leads to fatal consequences. While in Europe bats tend to hibernate isolated or to form small clusters, in North America, some hibernacula are characterized by very large aggregations of hibernating bats, amounting to thousands of individuals. Within such large clusters, multiple appearances of infected bats, their repeated arousals, grooming, and temperature increase would lead to the disturbance of neighbouring animals, potentially spreading across the cluster, as in a shock wave. In addition to the behavioural disturbances, large clusters would be influenced by density-dependent disease transmission [18]. Seen from an

evolutionary perspective, WNS may act as a strong selection force that drives a change in hibernation strategy from hibernation in large clusters to a preference for less-populated hibernacula. This is the prevailing hibernation strategy in European *Myotis*. The hypothesis that this strategy was possibly selected for by previous mass mortality events, and the history of fungus-bat co-evolution [13] is indirectly supported by data on the historical occurrence of *M. bechsteinii*. In contrast to *M. myotis*, which first appeared in Central Europe in the Late Holocene, *M. bechsteinii* has been a constant element of the Mid-European interglacial communities since Early Pliocene. Mass accumulations of bat skeletal remains in European cave deposits of the Pleistocene and Pliocene age were often dominated by this species [19]. Currently, *M. bechsteinii* is a rare species that mostly avoids hibernation in caves and mines [20]. This suggests its regular hibernation in caves in the past with occasional mortality events. Assuming that some of the past mass mortality events in hibernacula could have been a result of a disease is not beyond the realm of possibility.

Unfortunately, the idea as to whether the disappearance of *M. bechsteinii* from caves was caused by recurring *G. destructans* infection, or a similar agent, is as yet merely speculation, and it might not be possible to reveal any hard facts supporting it. Nevertheless, the history of outburst events of *G. destructans*, environmental factors which could cause the outbursts, as well as the interactions between the fungus and hibernating bats are worthy of very detailed study. Further research of the ecological and genetic differentiation of hosts and pathogens might well provide crucial information for an assessment of the impacts of WNS (cf. [2]).

Conclusions

We have shown that *Geomyces destructans*, the suspected infectious agent of WNS, is present across CZ and SK, without distinctive areas of prevalence. The reported incidence of its occurrence has increased since 2008, but it has likely been present since 1995, at the very least. To date, mass mortality has not been recorded, and the population fluctuation of *M. myotis* observed in 2009 and 2010 cannot be causally linked to the emergence of the disease. Nevertheless, we assume that white-nose syndrome is present in Europe. Future research should be aimed at establishing the precise effects of the disease on bats in Europe, as well as to elucidate the possible reasons for its less-severe impacts on the continent, whether it turns out to be immunological resistance, disparity in hibernating behaviour, genetic differences and associated virulence between European and American isolates of the pathogen, or environmental factors affecting the fungal growth.

Materials and Methods

Material

We used nylon swabs (microRheologics, Brescia, Italy), cotton swabs, or transparent adhesive tape to collect the 90 samples of fungi from the muzzle and wing membranes of hibernating bats. The nylon swabs were used according to the manufacturer's recommendations. The cotton swabs were placed directly into 1.5 ml plastic tubes as per [12], and the adhesive tape was stuck onto microscopic slides as per [13]. In total, we collected 10 samples using the nylon swabs, 58 samples using the cotton swabs, 20 using the adhesive tape, and 1 using both the nylon swab and tape. One other sample consisted of shed hair.

We examined photographs of hibernating bats, taken prior to 2009, for the presence of white fungal patches. The database consisted of photographs from 1994–1998 and from 2003–2010.

Hibernacula Monitoring

The bat populations had been monitored in their underground hibernacula once a year, since 1969 [21]. The program currently consists of almost 900 sites [22,23,24]. In 2010, besides the standard census monitoring, 98 sites were repeatedly inspected in March. The animals were illuminated for a short time. The research adhered to the conditions of Permit #00356/KK/2008/AOPK for CZ, certificate of competency per Law No. 114/1992; for SK we employed Licence #2598/715/03-5.1pil, 5376/2009-2.1/jam, from the Ministry of Environment of the Slovak Republic, certificate of competency per Law No. 543/2002.

Fungal Cultures

We conducted a mycological examination of 48 nylon swabs, cotton swabs and adhesive tape samples from the WNS-suspect bats, from 19 localities in CZ and SK (Table S1). Of those, 15 samples exhibited distinctive spores of *G. destructans* under direct microscopic observation of the nylon swabs and adhesive tapes (Fig. 1A). We inoculated the fungal material from the swabs and tapes onto Sabouraud dextrose agar plates (SDA, [25]) and incubated them in the dark at two temperatures (c. 7°C and 15°C). After 14 or more days, we isolated the outgrowing colonies of *G. destructans* and any other organisms. We identified the isolates according to [3], based on their phenotypic characteristics. Seven isolates are deposited at the Culture Collection of Fungi (CCF), Charles University in Prague, and 3 additional isolates in the Czech Collection of Microorganisms (CCM) at Masaryk University in Brno (Table S1).

To assess the basic growth characteristics, we studied three isolates of *G. destructans* (CCF 3937, 3938, 3939) at c. 15°C on four different agar media: malt extract agar (MEA), corn meal agar (CMA), yeast and malt extract agar (YMA), and creatine sucrose agar (CREA; [25]). We measured the colonies after 10, 20, and 30 days.

DNA Sequencing

We isolated the fungal DNA directly from the 33 cotton swabs which were not used for the mycological examination, using a ZR Genomic DNA II kit (Zymo research, Orange, CA, USA), and 26 from adhesive tape and culture isolates, using a DNeasy Tissue Kit (Qiagen, Halden, Germany). In the initial screening, we amplified the genes encoding the partial SSU, the complete SSU intron, ITS1, 5.8S rRNA, ITS2, and the partial LSU, using universal fungal primers ITS-myko-F (5′-CAAACTTGGTCATTTAGAG-GAA-3′) and ITS-myko-R (5′-CCTCCGCTTATTGATATG-CT-3′). The PCR reactions, in a volume of 50 µl, consisted of 1× La buffer, 100 µM dNTPs, 50 pM of each primer, 1U LA DNA polymerase (Top-bio), and 2 µl DNA. Cycling conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 20 s, 55°C for 20 s, and 68°C for 1 min. We purified the PCR products from an agarose electrophoresis gel using a Zmoclean gel DNA recovery kit (Zymo Research).

To increase the specificity of the amplification, we further utilised primers designed for *G. desctructans* found in France: ITS-F (5′-TCCTCCGCTTATTGATATGC-3′) and ITS-R (5′-GGA-AGTAAAAGTCGTAACAAGG-3′) [12]. PCR reactions consisted of 1× Buffer, 100 µM dNTPs, 3 mM $MgCl_2$, 25 µM of each primer, 1 U Platinum *Taq* (Invitrogen, Carlsbad, CA, USA), and 2 µl DNA. Cycling conditions followed the touch-down protocol of [12]. The PCR reaction yielded single bands that were purified using PCR Purification Kit (Qiagen). All products were commercially sequenced from both directions, using BigDye® Terminator sequencing chemistry (Applied Biosystems, Foster City, CA, USA) on 3100-Avant Genetic Analyzer (Applied Biosystems) sequencers.

Data Analyses

We assembled the contigs in Aligner 3.5.6 (CodonCode, Dedham, MA, USA), and we identified the resulting sequences by comparing them to GenBank, using BLASTN 2.2 [26].

For the population trend analyses, we selected 106 hibernacula, with the most complete continuous records since 1995. The average annual realized growth rate per capita was estimated by regression through the origin, according to [27]. To test the hypothesis of significant changes in population size, we used data collected over years 1995 to 2008 from 106 hibernacula, and extrapolated the time trends to 2009 and 2010. We computed the prediction intervals, considering both the uncertainty about future count expectation and the random error of Poisson-distributed observations [28]. We included an estimation of the dispersion parameter to address the unexplained extra-Poisson variance. The model fitting and prediction was performed using Stata/IC 10.1 statistical software (StataCorp, College Station, TX, USA). To test the effect of the missing data, we reanalysed the dataset with the missing values input as a combination of the last observation carried forward and the next observation carried backward methods.

Supporting Information

Table S1 Material examined for *Geomyces destructans* presence found in the Czech Republic and Slovakia, host species, localities, direct microscopic examination, sequence Accession Numbers, and isolate numbers.
Found at: doi:10.1371/journal.pone.0013853.s001 (0.13 MB PDF)

Acknowledgments

We are grateful for contributions of Martin Ceľuch, Jaroslav Hromas, Peter Kaňuch, Ondřej Koukol, Miroslav Kovařík, and Peter Pjenčák who assisted with the project. Jaroslav Barva, Luděk Bufka, Jaroslav Vogeltanz, Oldřich Vojtěch and Petr Wolf provided their photographs for assessing the presence of fungal growth. The comments of Sébastien Puechmaille, Gudrun Wibbelt, Dee Carter, and an anonymous reviewer helped to improve a previous version of the manuscript, and we thank Winifred Frick, Marianne Moore, Paul Racey and Emma Teeling for their support.

Author Contributions

Conceived and designed the experiments: NM J. Zima IH. Performed the experiments: NM P. Blažková TB LF ZH MK ĽK AK. Analyzed the data: NM TB JČ AK OM ET MU IH. Contributed reagents/materials/analysis tools: NM PB TB PB JČ LF JG VH DH HJ BL RL RKL JM ZŘ JŠ PT MU JW DW JZ JZ IH. Wrote the paper: NM JG AK OM ET IH.

References

1. Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-Zier BM, et al. (2009) Bat white-nose syndrome: An emerging fungal pathogen? Science 323: 227–227.
2. Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, et al. (2010) An emerging disease causes regional population collapse of a common North American bat species. Science 329: 679–682.
3. Gargas A, Trest MT, Christensen M, Volk TJ, Blehert DS (2009) *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. Mycotaxon 108: 147–154.
4. Meteyer CU, Buckles EL, Blehert DS, Hicks AC, Green DE, et al. (2009) Histopathologic criteria to confirm white-nose syndrome in bats. J Vet Diagn Invest 21: 411–414.
5. Courtin F, Stone WB, Risatti G, Gilbert K, Van Kruiningen HJ (2010) Pathologic findings and liver elements in hibernating bats with white-nose syndrome. Vet Pathol 47: 214–219.
6. Boyles JG, Brack V (2009) Modeling survival rates of hibernating mammals with individual-based models of energy expenditure. J Mamm 90: 9–16.
7. Boyles JG, Willis CKR (2010) Could localized warm areas inside cold caves reduce mortality of hibernating bats affected by white-nose syndrome? Front Ecol Environ 8: 92–98.
8. Reichard JD, Kunz TH (2009) White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*). Acta Chiropterol 11: 457–464.
9. Chaturvedi V, Springer DJ, Behr MJ, Ramani R, Li XJ, et al. (2010) Morphological and molecular characterizations of psychrophilic fungus *Geomyces destructans* from New York bats with white nose syndrome (WNS). PLoS ONE 5(5): e10783. doi:10.1371/journal.pone.0010783.
10. Frick WF, Reynolds DS, Kunz TH (2010) Influence of climate and reproductive timing on demography of little brown myotis *Myotis lucifugus*. J Anim Ecol 79: 128–136.
11. White-Nose Syndrome Consortium (2010) The potential threat of white-nose syndrome to European bats: An action plan 10.
12. Puechmaille SJ, Verdeyroux P, Fuller H, Gouilh MA, Bekaert M, et al. (2010) White-nose syndrome fungus (*Geomyces destructans*) in bat, France. Emerg Infect Dis 16: 290–293.
13. Wibbelt G, Kurth A, Hellmann D, Weishaar M, Barlow A, et al. (2010) White-nose syndrome fungus (*Geomyces destructans*) in bats, Europe. Emerg Infect Dis 16: 1237–1242.
14. Lorch JM, Gargas A, Meteyer CU, Berlowski-Zier BM, Green DE, et al. (2010) Rapid polymerase chain reaction diagnosis of white-nose syndrome in bats. J Vet Diagn Invest 22: 224–230.
15. Fernandes EKK, Moraes AML, Pacheco RS, Rangel DEN, Miller MP, et al. (2009) Genetic diversity among Brazilian isolates of *Beauveria bassiana*: comparisons with non-Brazilian isolates and other *Beauveria* species. J Appl Microbiol 107: 760–774.
16. Fournier A, Widmer F, Enkerli J (2010) Development of a single-nucleotide polymorphism (SNP) assay for genotyping of *Pandora neoaphidis*. Fungal Biology 114: 498–506.
17. Feldmann R (1984) Teichfledermaus - *Myotis dasycneme* (Boie, 1825). Die Säugetiere Westfalens. Münster: Westfälisches Museum für Naturkunde. pp 107–111.
18. Fenton MB, Davison M, Kunz TH, McCracken GF, Racey PA, et al. (2006) Linking bats to emerging diseases. Science 311: 1098–1099.
19. Horáček I, Hanák V, Gaisler J (2000) The bats of the Palearctic region: A taxonomic and biogeographic review. In: Woloszyn BW, ed. Proceedings of the 8th European Bat Research Conference. Krakow: PAN. pp 11–157.
20. Hutson AM, Spitzenberger F, Tsytsulina K, Aulangier S, Juste J, et al. (2008) *Myotis bechsteinii*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.1. <www.iucnredlist.org>. Downloaded on 24 June 2010.
21. Gaisler J (1975) A quantitative study of some populations of bats in Czechoslovakia (Mammalia: Chiroptera). Acta Sci Nat Brno 9(5): 1–44.
22. Bauerová Z, Gaisler J, Kovařík M, Zima J (1989) Variation in numbers of hibernating bats in the Moravian Karst: Results of visual censuses in 1983-1987. In: Hanák V, Horáček I, Gaisler J, eds. European Bat Research 1987. Praha: Charles University Press. pp 499–505.
23. Horáček I (2001) Censusing bats in the underground hibernacula of the Czech Republic. Vespertilio 5: 3–5.
24. Uhrin M, Benda P, Obuch J, Urban P (2010) Changes in abundance of hibernating bats in central Slovakia (1992-2009). Biologia 65: 349–361.
25. Samson RA, Hoekstra ES, Frisvad JC (2004) Introduction to food- and airborne fungi. 7th Ed. Utrecht: CBS.
26. Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. J Comput Biol 7: 203–214.
27. Dennis B, Munholland PL, Scott JM (1991) Estimation of growth and extinction parameters for endangered species. Ecol Monogr 61: 115–143.
28. Hakulinen T, Dyba T (1994) Precision of incidence predictions based on Poisson distributed observations. Stat Med 13: 1513–1523.

Těžké kovy a syndrom bílého nosu - potenciální hrozby pro populace letounů

Pikula, J., Zukal, J., Adam, V., Banďouchová, H., Beklová, M., Hájková, P., Horáková, J., Kížek, R. & Valentíková, L. (2010) Heavy metals and metallothionein in vespertilionid bats foraging over aquatic habitats in the Czech Republic. Environmental Toxicology and Chemistry 29, 501-506.

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 29, No. 3, pp. 501–506, 2010

Printed in the USA
DOI: 10.1002/etc.80

First International Workshop on Aquatic Toxicology and Biomonitoring

HEAVY METALS AND METALLOTHIONEIN IN VESPERTILIONID BATS FORAGING OVER AQUATIC HABITATS IN THE CZECH REPUBLIC

JIRI PIKULA,*† JAN ZUKAL,‡ VOJTECH ADAM,§ HANA BANDOUCHOVA,† MIROSLAVA BEKLOVA,† PAVLINA HAJKOVA,‖ JANA HORAKOVA,† RENE KIZEK,§ and LUCIE VALENTIKOVA†

†University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, CZ-612 42 Brno, Czech Republic

‡Masaryk University, Kotlarska 2, CZ-611 37 Brno, Czech Republic

§Mendel University of Agriculture and Forestry, Zemedelska 1, CZ-613 00 Brno, Czech Republic

‖Zoo of the Capital City of Prague, Prague, Czech Republic

(*Submitted* 25 *August* 2008; *Returned for Revision* 29 *September* 2008; *Accepted* 11 *November* 2008)

Abstract—There has been growing interest in the study and conservation of bats throughout the world. Declines in their absolute numbers in recent decades are due, in part, to the fact that insectivorous bats may bioaccumulate toxic pollutants. The purpose of the present study was to quantify heavy metal concentrations in kidney, liver, and pectoral muscle samples in relation to metallothionein (MT) levels. In total, 106 bats belonging to 11 European species (i.e., *Myotis myotis*, *Myotis daubentonii*, *Myotis brandtii*, *Myotis nattereri*, *Myotis emarginatus*, *Myotis mystacinus*, *Pipistrellus pipistrellus*, *Pipistrellus nathusii*, *Pipistrellus pygmaeus*, *Nyctalus noctulla*, *Eptesicus serotinus*) were used for the study. The highest MT levels were found in *Pipistrellus pipistrellus*. High MT levels were also found in juvenile bats and aquatic-insect-foraging species. Cadmium was found only in the liver and kidney of *Myotis myotis*, except for a solitary finding in *Pipistrellus pipistrellus*. *Myotis myotis* juveniles had significantly higher liver and kidney Zn concentrations than adults. Interestingly, the liver Pb concentration was approximately two times higher in females than in males of *Myotis myotis*. The same gender difference was found for kidney Zn concentration in *Pipistrellus pipistrellus*. The present study confirms exposure of vespertilionid bats to toxic heavy metals (Pb, Cd) in the Czech Republic and provides data on the essential element Zn and the metal-binding protein MT in bats foraging over aquatic, aquatic and terrestrial, and terrestrial habitats. Environ. Toxicol. Chem. 2010;29:501–506. © 2009 SETAC

Keywords—Microchiroptera Insect foraging Metallic elements Bioaccumulation

INTRODUCTION

Interest in the study and conservation of bats throughout the world has been growing [1]. Research into the biology and ecology [2–5] as well as diseases [6] of bats in the Czech Republic has a long history of providing interesting results. Bat populations in many countries are declining rapidly, and the World Conservation Union classifies more than 22% of bat species as threatened and another 23% as almost threatened with extinction [7].

Bioaccumulation of insecticides and other pollutants is thought to be contributing to this decline [1,7,8]. Apart from agricultural chemicals, bats are threatened by chemical treatment of timber, human disturbance and destruction of roost sites through deforestation and reconstruction of buildings, and cats catching urban-roosting bats such as pipistrelles. Moreover, certain characteristics of bats, such as their small size and high longevity (20 years or more), make them suitable for use as indicators of general environmental conditions [1,9]. Microchiropteran bats of Central Europe are nocturnal obligate insectivores with no alternative feeding strategy [10]. Therefore, insects in their food chain are the only source of contaminants. It has been documented that the foraging habitats of bats may be contaminated by heavy metals [9,11–13]. It is also known that in many organisms the toxic effects of heavy metals may be reduced by binding to specific ligands and that, among these, metallothioneins (MTs) play a key role [14]. Levels of heavy metal ions are strictly controlled in all living organisms [15,16], because free ions can cause many serious problems, including oxidative stress or permanent signalling within the cell [17]. Cysteine-rich MTs [18] have a high affinity for various metal ions, which may represent up to 20% of the MT weight. Inducible MTs are the first detectable sign of exposure to heavy metals at the cellular level and may be used as biomarkers because there are probably constant levels of MTs in non-stressed cells [14,19,20]. Considerable differences in the content of MTs are related to species, tissues, age, nutrition, and other, unknown factors [21–26]. Although MTs have been identified in a broad range of species [18,27], there are no reports on MT levels in bats.

Central European vespertilionid bats employ two different foraging styles. Some of them hunt airborne prey, whereas the other group are gleaners [1]. Food composition and the resulting exposure to environmental pollution are also influenced by the habitats that different bat species use for insect foraging, i.e., aquatic only, aquatic and terrestrial, and terrestrial only, as well as the environmental conditions at sites of collection of bats. Given the few studies on heavy metals and the lack of data on

* To whom correspondence may be addressed (pikulaj@vfu.cz).

Presented at the 1st International Workshop on Aquatic Toxicology and Biomonitoring, Vodnany, Czech Republic, August 27–29, 2008.

Published online 10 December 2009 in Wiley InterScience (www.interscience.wiley.com).

MT levels in vespertilionid bats, the purpose of the present study was to analyze heavy metal concentrations in kidney, liver, and pectoral muscle samples as well as MT levels in a collection of 11 European bat species.

MATERIALS AND METHODS

Collection of samples

In total, 106 bats belonging to 11 European species, i.e., 36 mouse-eared bats (*Myotis myotis*), eight Daubenton's bats (*Myotis daubentonii*), one Brandt's bat (*Myotis brandtii*), one Natterer's bat *(Myotis nattereri*), one Geoffroy's bat (*Myotis emarginatus*), one whiskered bat *(Myotis mystacinus*), 24 pipistrelle bats *(Pipistrellus pipistrellus*), 18 Nathusius's pipistrelles (*Pipistrellus nathusii*), 12 soprano pipistrelles (*Pipistrellus pygmaeus*), three noctule bats (*Nyctalus noctulla*), and two serotine bats (*Eptesicus serotinus*), found dead or moribund from May to September 2007, were used for the present study. The same approach to sample collection has previously been used by other authors [9,11]. Given that bats are endangered species and are protected by law, no other approach is feasible. During autopsy, liver, kidney, and pectoral muscle samples were removed and kept at −80°C until analysis. During the initial inspection, bats were identified with respect to species, gender, and age (juveniles vs. adults, based on the condition of teeth and ossification of epiphyses).

Foraging habitats and styles of bats

From several studies [2,3,28–31] and a review [32] of habitat preference, foraging behavior, and diet composition, the bats were classified as aquatic-habitat foragers with aquatic insects prevailing in the diet (*Myotis daubentonii, Pipistrellus pipistrellus, Pipistrellus nathusii, Pipistrellus pygmaeus*), terrestrial- and aquatic-habitat foragers (*Myotis emarginatus, Myotis mystacinus, Myotis brandtii, Myotis nattereri, Nyctalus noctulla*), and those using only terrestrial habitats for hunting (*Myotis myotis, Eptesicus serotinus*). The bats came from three different habitats of the same agricultural landscape (Moravia, Czech Republic, central Europe). Bats were also grouped with respect to habitat preference, foraging behavior, and diet composition based on their origin from the three habitats.

Chemical analysis

Tissue levels of MT were determined by using electrochemical detection (adsorptive transfer stripping technique with differential pulse voltammetry, Brdicka reaction). The procedures employed have been described in greater detail elsewhere [27]. Metallothionein levels are given as micrograms per gram on a fresh weight basis. Relative standard deviation (SD) was below 5%. Recovery was tested by three additions of MT standard and varied from 95 to 100% [33]. The detection limit of MT was 2 pM [34].

Concentrations of heavy metals (Zn, Cd, and Pb) were detected in samples of liver, kidney, and pectoral muscles using differential pulse anodic stripping voltammetry as described previously [35,36]. Concentrations of heavy metals are given as free ions on a fresh weight basis (μg/g). Relative SD was below 8%. Recovery was tested by two additions of heavy metals and varied from 95 to 104%. The detection limits of Zn, Cd, and Pb were 20, 6, and 8 pM [37]. The decision to quantify Zn as an essential metallic element in tissues of bats was based on the recognized high affinity of MT for Zn [18], giving us a model of the relationship between MT and Zn levels. Unfortunately, because of the small body weight of vespertilionid bats (ranging from 4 to 20 g), it was not able to quantify the whole spectrum of heavy metals.

Statistical analysis

Because the data were not normally distributed and were low in numbers, nonparametric statistical analysis was used, and the results are presented as number of specimens, mean, median, and standard error. Statistica® for Windows® 7.0 (StatSoft) was employed to evaluate differences among groups using the Mann–Whitney U test. Values of $p < 0.05$ and $p < 0.01$ were considered statistically significant and highly significant, respectively, for all tests. Spearman rank order correlation analysis between MT and heavy metal levels was also employed.

RESULTS

Metallothionein levels

Table 1 presents the MT levels in liver, kidney, and pectoral muscles of the 11 bat species studied. The highest MT levels in liver, kidney, and pectoral muscles were found in the pipistrelle

Table 1. Liver, kidney, and pectoral muscle metallothionein levels (μg/g, analyzed on a fresh wt basis) of 11 vespertilionid species of bats[a]

		Liver			Kidney			Pectoral muscle		
Species	*n*	Mean	Median	Standard error	Mean	Median	Standard error	Mean	Median	Standard error
Myotis daubentonii	3/8/8	10.41	6.75	4.18	206.61	63.95	143.75	48.70	16.71	30.84
Pipistrellus pipistrellus	24/24/24	166.86	110.80	33.39	693.58	694.11	63.81	138.10	117.56	17.88
Pipistrellus nathusii	4/12/17	28.48	28.75	9.87	158.38	38.57	74.23	23.99	15.66	6.69
Pipistrellus pygmaeus	7/10/12	114.49	49.20	52.11	408.39	157.23	137.07	37.93	29.19	9.30
Myotis emarginatus	1/1/1	86.11	—	—	40.39	—	—	5.35	—	—
Myotis mystacinus	1/1/1	155.06	—	—	143.64	—	—	138.39	—	—
Myotis brandtii	1/1/1	18.09	—	—	50.22	—	—	35.53	—	—
Myotis nattereri	1/1/1	196.41	—	—	114.70	—	—	9.11	—	—
Nyctalus noctulla	3/3/3	142.84	90.45	87.11	166.28	20.85	150.66	19.03	20.54	1.66
Myotis myotis	35/35/35	127.36	64.75	22.74	162.83	99.66	24.57	95.27	68.65	13.00
Eptesicus serotinus	2/2/2	61.05	61.05	11.49	74.37	74.37	55.23	63.93	63.93	61.30

[a] n = Number of examined samples of liver/kidney/pectoral muscle; — = insufficient data to compute these values.

bat (*Pipistrellus pipistrellus*), and the lowest MT level was found in pectoral muscles in Nathusius's pipistrelle (*Pipistrellus nathusii*). There was, however, no general concentration gradient of median MT levels in the organs of the 11 species studied. In some species (*Myotis daubentonii, Pipistrellus pipistrellus, Myotis brandtii, Myotis myotis, Eptesicus serotinus*), the MT concentration gradient was kidney > muscle > liver. In *Pipistrellus nathusii* and *Pipistrellus pygmaeus*, the MT gradient was kidney > liver > muscle, whereas, in the remaining species (i.e., *Myotis emarginatus, Myotis mystacinus, Myotis nattereri, Nyctalus noctulla*), the gradient was liver > kidney > muscle.

Numerous data on MT levels in *Myotis myotis* (i.e., 35 specimens) allowed evaluation of gender and age differences. Interestingly, median MT levels in the liver and kidney of juveniles were nearly three times higher than those in adults. The difference was of statistical significance in the liver (20 juveniles, 15 adults, $p = 0.03$) and of high statistical significance in the kidney (20 juveniles, 15 adults, $p = 0.0002$). Gender comparison of MT levels in *Myotis myotis* revealed statistically significant differences only in pectoral muscles (8 males, 27 females, $p = 0.04$), with males having median MT levels approximately two times higher than females. In exploring the possibility of age and gender together, it was found that only young females ($n = 12$) had approximately three times higher kidney MT levels than adult females ($n = 15$) in *Myotis myotis* ($p = 0.04$). The pipistrelle bat (*Pipistrellus pipistrellus*) was another species in which numerous data allowed age and gender comparisons. In this bat species, MT levels in juvenile kidneys were significantly higher than in adults (10 juveniles, 14 adults, $p = 0.04$), and median liver MT levels in males were nearly three times higher than in females (7 males, 27 females, $p = 0.01$).

In considering the three ecological foraging groups of bats, i.e., aquatic only (A), aquatic and terrestrial (AT), and terrestrial only (T), there were statistically significant differences in kidney MT levels between A and AT as well as between A and T ($n_A = 54$, $n_{AT} = 7$, $p = 0.03$ and $n_A = 54$, $n_T = 37$, $p = 0.01$, respectively). Median kidney MT levels in the aquatic-insect-only-foraging group (A) were approximately 7.5 and 3.5 times higher than levels in the AT- and T-foraging groups, respectively. There was also a significant difference between pectoral muscle MT levels in bats from groups AT and T ($n_{AT} = 7$, $n_T = 37$, $p = 0.01$).

Heavy metals

Zinc and lead concentrations in liver, kidney, and pectoral muscle samples of 11 vespertilionid species of bats are shown in Tables 2 and 3, respectively. The highest individual values of Zn were found in the liver of *Pipistrellus pipistrellus*, in the kidney of *Pipistrellus pygmaeus*, and in the pectoral muscles of *Myotis myotis*. Individual Pb values were highest in the liver of *Eptesicus serotinus*, in the kidney of *Myotis myotis*, and in the pectoral muscles of *Myotis daubentonii*. For Cd concentrations in this collection of bats, there were only solitary findings of 0.01 μg/g in the liver of a female adult mouse-eared bat (*Myotis myotis,* $n = 33$) and in the kidney of a female juvenile pipistrelle (*Pipistrellus pipistrellus*, $n = 23$). Kidney samples of *Myotis myotis* positive for Cd originated from four adult females, one juvenile female, and one juvenile male ($n = 32$, mean = 0. 01, median = 0.00, SE = 0.009).

Evaluation of gender and age differences in concentrations of heavy metals of *Myotis myotis* revealed statistically significant differences in the liver Zn concentration of 19 juveniles and 14 adults ($p = 0.001$), with young bats having levels two times higher than adult levels. The kidney Zn concentration of 20 juveniles was approximately 1.5 times higher than of 12 adults ($p = 0.03$), whereas the muscle Pb concentration of 12 adults was approximately two times higher than that of 20 juveniles ($p = 0.01$). The only gender difference of statistical significance was that in liver Pb concentration, which was approximately two times higher in 26 females than in seven males ($p = 0.02$). No statistically significant age differences were found in *Pipistrellus pipistrellus*, and the only significant gender difference in this species was that in the kidney Zn concentration, which was approximately two times higher in 16 females than in seven males ($p = 0.03$). There were no statistically significant differences in concentrations of heavy metals (Zn, Cd, and Pb) in the liver, kidney, and pectoral muscles between the three ecological foraging groups of bats, A, AT, and T.

Correlation between metallothionein and heavy metals

Possible correlations between the MT level and tissue concentration of Zn, Cd, and Pb and the sum of Zn + Cd + Pb in *Myotis myotis* and *Pipistrellus pipistrellus* were evaluated for the whole species as well as for juveniles, adults, males, and females. Other evaluations concerned the three ecological foraging groups of bats, A, AT, and T. Statistically significant

Table 2. Liver, kidney, and pectoral muscle zinc concentrations (μg/g, analyzed as free ions on a fresh wt basis) of 11 vespertilionid species of bats[a]

		Liver			Kidney			Pectoral muscle		
Species	*n*	Mean	Median	Standard error	Mean	Median	Standard error	Mean	Median	Standard error
Myotis daubentonii	1/6/7	0.54	—	—	0.55	0.36	0.20	0.31	0.21	0.06
Pipistrellus pipistrellus	23/23/18	0.35	0.28	0.04	0.73	0.47	0.13	0.31	0.25	0.02
Pipistrellus nathusii	3/6/4	0.38	0.36	0.06	0.98	0.85	0.28	0.32	0.25	0.07
Pipistrellus pygmaeus	5/7/4	0.26	0.30	0.03	0.98	0.63	0.33	0.40	0.36	0.09
Myotis emarginatus	0/0/0	—	—	—	—	—	—	—	—	—
Myotis mystacinus	1/1/0	0.46	—	—	0.59	—	—	—	—	—
Myotis brandtii	1/1/1	0.50	—	—	0.29	—	—	0.69	—	—
Myotis nattereri	0/1/0	—	—	—	1.12	—	—	—	—	—
Nyctalus noctulla	2/3/0	0.27	0.27	0.05	0.67	0.30	0.40	—	—	—
Myotis myotis	33/32/32	0.42	0.34	0.03	0.43	0.41	0.02	0.35	0.30	0.02
Eptesicus serotinus	1/2/1	0.95	—	—	0.43	0.43	0.02	0.25	—	—

[a] *n* = Number of examined samples of liver/kidney/pectoral muscle; — = insufficient data to compute these values.

Table 3. Liver, kidney, and pectoral muscle lead concentrations (μg/g, analyzed as free ions on a fresh wt basis) of 11 vespertilionid species of bats[a]

		Liver			Kidney			Pectoral muscle		
Species	*n*	Mean	Median	Standard error	Mean	Median	Standard error	Mean	Median	Standard error
Myotis daubentonii	1/6/7	0.70	—	—	0.38	0.32	0.13	0.32	0.21	0.07
Pipistrellus pipistrellus	23/23/18	0.36	0.33	0.04	0.26	0.13	0.05	0.20	0.18	0.02
Pipistrellus nathusii	3/6/4	0.59	0.57	0.05	0.49	0.40	0.16	0.21	0.21	0.02
Pipistrellus pygmaeus	5/7/4	0.32	0.21	0.12	0.51	0.54	0.12	0.34	0.28	0.11
Myotis emarginatus	0/0/0	—	—	—	—	—	—	—	—	—
Myotis mystacinus	1/1/0	1.82	—	—	0.08	—	—	—	—	—
Myotis brandtii	1/1/1	0.37	—	—	0.40	—	—	0.35		—
Myotis nattereri	0/1/0	—	—	—	0.88	—	—	—	—	—
Nyctalus noctulla	2/3/0	0.71	0.14	0.01	0.60	0.60	0.17	—	—	—
Myotis myotis	33/32/32	0.14	0.38	0.05	0.30	0.30	0.04	0.20	0.18	0.01
Eptesicus serotinus	1/2/1	2.51	—	—	0.66	0.66	0.12	0.17	—	—

[a] n = Number of examined samples of liver/kidney/pectoral muscle; — = insufficient data to compute these values.

correlations were found in *Myotis myotis* between kidney MT and Zn concentration ($n = 32$, $R = 0.36$, $p = 0.04$), pectoral muscle Pb concentration ($n = 32$, $R = -0.36$, $p = 0.04$), pectoral muscle Zn concentration of juveniles ($n = 20$, $R = 0.49$, $p = 0.02$), liver Pb concentration of males ($n = 7$, $R = -0.86$, $p = 0.01$), pectoral muscle Pb concentration of males ($n = 7$, $R = -0.71$, $p = 0.04$), and kidney Zn concentration of females ($n = 24$, $R = 0.52$, $p = 0.006$). In *Pipistrellus pipistrellus*, statistically significant correlations were found between MT and kidney Pb concentration of males ($n = 7$, $R = -0.86$, $p = 0.01$) and pectoral muscle Pb concentration of females ($n = 12$, $R = -0.66$, $p = 0.02$). As far as the three ecological foraging groups of bats, A, AT, and T, are concerned, in the A (aquatic-insect-foraging) group, statistically significant correlations were found between liver, kidney, and pectoral muscle MT and Pb concentrations ($n = 31$, 41, and 33; $R = -0.43$, $R = -0.53$, and $R = -0.43$; $p = 0.01$, $p = 0.0004$, and $p = 0.01$, respectively). In the T (terrestrial-habitat-foraging) group of bats, statistically significant correlations were found between kidney MT and Zn, kidney Pb, and pectoral muscle Pb concentrations ($n = 34$, 34 and 34; $R = 0.34$, $R = -0.38$ and $R = -0.38$; $p = 0.04$, $p = 0.02$, and $p = 0.02$, respectively). Interestingly, as shown above, all statistically significant correlations between MT and Zn concentrations were positive, whereas they were negative between MT and Pb concentrations.

DISCUSSION

Variation in the levels of MT, Zn, Cd, and Pb in 11 vespertilionid bats from the Czech Republic was examined in this study. As with various other studies [8,9,11], the present study found mostly non-normal distribution of data in bats and had to use nonparametric statistical tests. No one has yet evaluated and reported MT levels in any bat species. With the exception of *Myotis daubentonii, Pipistrellus pipistrellus, Pipistrellus nathusii, Pipistrellus pygmaeus, Nyctalus noctulla, Myotis myotis*, and *Eptesicus serotinus*, however, the results are based on single specimens, so further studies on MT levels are needed in these species. On the other hand, numerous data on *Myotis myotis* and *Pipistrellus pipistrellus* even made it possible to evaluate gender and age differences. Vespertilionid bats have moderate or low hepatic MT levels compared with humans, monkeys, domestic animals, rodents, and lagomorphs [18]. In a previous paper [27] differences in MT levels in different animal species were examined with regard to their trophic level. All Central European bats, however, belong to the same insectivorous trophic guild. Therefore, another common grouping approach was used, based on habitat preference, foraging behavior, and diet composition to evaluate differences between aquatic-habitat-foraging bats with aquatic insects prevailing in their diet, terrestrial- as well as aquatic-habitat-foraging bats, and bats using only terrestrial habitats for hunting. Certainly, this is an artificial classification and may cause evaluation problems as a result of, for example, some overlap in foraging niches or low numbers of specimens in the intermediate group of bats foraging in both terrestrial and aquatic habitats. Nevertheless, it seems that aquatic-insect-foraging bats have higher levels of MT than bats from the other two groups. The results showingt higher MT levels in juvenile bats (*Myotis myotis* and *Pipistrellus pipistrellus*) compared with adults are consistent with the age-related changes described for human cells that are associated with down-regulation of MT in senescent cells and are independent of dietary Zn intake [38].

There are only a few studies on heavy metals in European bats [9,11,12], and, contrary to the present paper evaluating liver, kidney, and muscle, these only examined the kidney. Zinc, as an essential trace element, was the most abundant element in the liver, kidney, and muscles of all 11 bats. The higher Zn levels observed in juveniles of *Myotis myotis* and *Pipistrellus pipistrellus* are consistent with its physiological function in the growing organism and findings from Austrian bats [11]. In general, in the present study, MTs, as Zn-binding proteins, were positively correlated with Zn level but negatively correlated with Pb concentration. The negative correlation between MT and Pb levels may be a result of the lower affinity of MTs for Pb [36]. However, the counteracting effect of positive and negative correlations between MTs and Zn and lead may explain the lack of correlation between MTs and the sum of heavy metals in tissues. MT has been thought to be a suitable monitoring tool for detection of heavy metal pollution [36]. The sum of concentrations of Zn, Cd, and Pb was used to test the hypothesis that exposure to multiple heavy metals would result in higher MT levels. However, because of the lower affinity of MT for Pb and the negative correlation between MT and Pb levels, the use of MT levels as a biomarker of exposure to heavy metals may not be so straightforward in vespertilionid bats. It was found that Pb

bioaccumulates with age in *Myotis myotis* (two times higher levels in muscles of adults compared with juvenile mouse-eared bats). Higher liver Pb concentrations in females of *Myotis myotis* may be explained by mobilization of Pb, which behaves similarly to calcium in lactating females [13]. The significant gender and age differences in concentrations of Pb in *Myotis myotis* are unlike those found by some other authors [9,11], probably because of the low numbers of specimens of this species available to these researchers. In terms of Cd, there was only one single positive finding in a juvenile pipistrelle (*Pipistrellus pipistrellus*) even though more than half of this collection of bat species included adults and Cd is known to accumulate in the liver and kidney tissue during life [11]. This again conflicts with a published report demonstrating a significant difference between adult and juvenile pipistrelles [9]. Findings in the present study, however, are consistent with generally low Cd levels in aquatic insects, for which this bat species specializes [39]. On the other hand, there were higher levels and more frequent findings of Cd in the mouse-eared bat foraging in terrestrial habitats (forest), so it seems that Cd is more available in their trophic niche.

The two most extensive studies on heavy metals in European bats [9,11] used atomic absorption spectrophotometry to determine heavy metal concentrations with limits of detection in the smallest bat species of 1.86 mg/g dry weight for Hg, 1.24 mg/g dry weight for Pb, and 0.162 mg/g dry weight for Cd. The limit of detection of the method used in the present study, i.e., differential pulse anodic stripping voltammetry, is a few orders of magnitude lower than the above-mentioned method, down to nanograms per grams of dry weight for all metals analyzed (Cd, Zn, and Pb) [37,40,41]. A critical comparison between voltammetric and spectrometric determination of heavy metals has recently been published [42]. Voltammetric methods have not yet been employed for analysis of bat tissues. Furthermore, a report on ultratrace determination of heavy metals in environmental matrices by differential pulse anodic stripping voltammetry estimated detection limits for As(III), Se(IV), Cu(II), Pb(II), Cd(II), Zn(II), and Mn(II) as tens of picograms per milliliter of a sample [43]. Because of the higher sensitivity of this voltammetric method, the content of free heavy metal ions in bats could be determined. Given the physicochemical properties of heavy metal ions and their compounds, free heavy metal ions pose a threat inside the cell. These ions can create free radicals, which subsequently damage enzymatic systems, cell compartments, membranes, and even cell nuclei and nucleic acids [44,45]. The level of free heavy metal ions can be considered as a marker of acute oxidative stress induced by heavy metals.

Generally, kidney and liver concentrations of Pb above 10 μg/g on a wet weight basis are diagnostic for Pb toxicosis in animals [46] and the critical concentration for Cd is 20 to 30 μg/g [47]. Lead and Cd tissue concentrations in bats in the present study were lower than those diagnostic for toxicosis. For example, the highest liver Pb concentration of 1.77 μg/g and the highest kidney Cd concentration of 0.35 μg/g were found in *Myotis myotis*. Likewise, the maximum Zn concentration of 2.83 μg/g in kidneys of *Pipistrellus pipistrellus* is well below the range of 14 to 821 μg/g found in Austrian bats [11].

The present study confirms exposure of vespertilionid bats to toxic heavy metals in the Czech Republic and provides data on the essential element Zn and the metal-binding protein MT in 11 species of bats. Findings such as the gender- and age-related differences in MTs and heavy metals, the near-absence of Cd in aquatic-insect-foraging bats, and correlations between metallic elements and MTs are novel as well as very interesting and should encourage further investigations in this mammalian group.

Acknowledgement—The present study was supported by the Ministry of Education, Youth, and Sports of the Czech Republic, grant 6215712402.

REFERENCES

1. Fenton MB. 1997. Science and the conservation of bats. *J Mammal* 78:1–14.
2. Gaisler J, Kolibac J. 1992. Summer occurrence of bats in agrocoenoses. *Fol Zool* 41:19–27.
3. Gaisler J, Zukal J, Rehak Z, Homolka M. 1998. Habitat preference and flight activity of bats in a city. *J Zool Part 3* 244:439–445.
4. Gaisler J, Chytil J. 2002. Mark-recapture results and changes in bat abundance at the cave of Na Turoldu, Czech Republic. *Fol Zool* 51:1–10.
5. Zima J, Kovarik M, Gaisler J, Rehak Z, Zukal J. 1992. Dynamics of the number of bats hibernating in the Moravian Karst in 1983 to 1992. *Fol Zool* 43:109–119.
6. Hajkova P, Pikula J. 2007. Veterinary treatment of evening bats (Vespertilionidae) in the Czech Republic. *Vet Rec* 161:139–140.
7. Heard DJ. 2003. Chiroptera (bats). In Fowler ME, Miller RE, eds, *Zoo and Wild Animal Medicine*, 5th ed. Saunders, St. Louis, MO, USA, pp 315–333.
8. Luftl S, Freitag B, Deutz A, Steineck T, Tataruch F. 2005. Concentrations of organochlorine pesticides and PCBs in the liver of European bats (*Microchiroptera*). *Fresenius Environ Bull* 14:167–172.
9. Walker LA, Simpson VR, Rockett L, Wienburg CL, Shore RF. 2007. Heavy metal contamination in bats in Britain. *Environ Pollut* 148:483–490.
10. Routh A. 2003. Bats. In Mullineaux E, Best D, Cooper JE, eds, *BSAVA Manual of Wildlife Casualties*. BSAVA, Gloucester, UK, pp 95–108.
11. Luftl S, Freitag B, Deutz A, Tataruch F. 2003. Concentrations of heavy metals in European bats (*Microchiroptera*). *Fresenius Environ Bull* 12:353–358.
12. Streit B, Nagel A. 1993. Element assessment in tissue samples from European bats (*Microchiroptera*). *Fresenius Environ Bull* 2:162–167.
13. Streit B, Nagel A. 1993. Heavy metal transfer by lactation in a bat colony. *Fresenius Environ Bull* 2:168–173.
14. Fernandes D, Bebianno MJ, Porte C. 2008. Hepatic levels of metal and metallothioneins in two commercial fish species of the Northern Iberian shelf. *Sci Total Environ* 391:159–167.
15. Bremner I, Beattie JH. 1990. Metallothionein and the trace minerals. *Annu Rev Nutr* 10:63–83.
16. Brady FO. 1982. The physiological-function of metallothionein. *Trends Biochem Sci* 7:143–145.
17. Meneghini R. 1997. Iron homeostasis, oxidative stress, and DNA damage. *Free Radic Biol Med* 23:783–792.
18. Henry RB, Liu J, Choudhuri S, Klaassen CD. 1994. Species variation in hepatic metallothionein. *Toxicol Lett* 74:23–33.
19. Amiard JC, Amiard-Triquet C, Barka S, Pellerin J, Rainbow PS. 2006. Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. *Aquat Toxicol* 76:160–202.
20. Vanparys C, Dauwe T, Van Campenhout K, Bervoets L, De Coen W, Blust R, Eens M. 2008. Metallothioneins (MTs) and δ-aminolevulinic acid dehydratase (ALAd) as biomarkers of metal pollution in great tits (*Parus major*) along a pollution gradient. *Sci Total Environ* 401:184–193.
21. Miura N, Koizumi S. 2007. Heavy metal responses of the human metallothionein isoform genes. *Yakugaku Zasshi J Pharm Soc Jpn* 127:665–673.
22. Otsuka F, Ohno S, Suzuki K, Takahashi K, Ohsawa M, Koizumi S. 2007. Mechanism of metallothionein gene activation mediated by heavy-metal-dependent transcription factor MTF-1. *Yakugaku Zasshi J Pharm Soc Jpn* 127:675–684.

23. Itoh N, Kimura T. 2007. Cytokine-induced metallothionein expression and modulation of cytokine expression by metallothionein. *Yakugaku Zasshi J Pharm Soc Jpn* 127:685–694.
24. Min KS. 2007. The physiological significance of metallothionein in oxidative stress. *Yakugaku Zasshi J Pharm Soc Jpn* 127:695–702.
25. Sato M, Suzuki S. 2007. Endoplasmic reticulum stress and metallothionein. *Yakugaku Zasshi J Pharm Soc Jpn* 127:703–708.
26. Satoh M. 2007. Analysis of toxicity using metallothionein knockout mice. *Yakugaku Zasshi J Pharm Soc Jpn* 127:709–717.
27. Adam V, Beklova M, Pikula J, Hubalek J, Trnkova L, Kizek R. 2007. Shapes of differential pulse voltammograms and level of metallothionein in different animal species. *Sensors* 7:2419–2429.
28. Flavin DA, Biggane SS, Shiel CB, Smiddy P, Fairley JS. 2001. Analysis of the diet of Daubenton's bat *Myotis daubentonii* in Ireland. *Acta Theriol* 46:43–52.
29. Gajdosik M, Gaisler J. 2004. Diet of two *Eptesicus* bat species in Moravia (Czech Republic). *Fol Zool* 53:7–16.
30. Zukal J, Rehak Z. 2006. Flight activity and habitat preference of bats in a karstic area, as revealed by bat detectors. *Fol Zool* 55:273–281.
31. Bartonicka T, Rehak Z, Andreas M. 2008. Diet composition and foraging activity of *Pipistrellus pygmaeus* in a floodplain forest. *Biologia* 63:266–272.
32. Vaughan N. 1997. The diets of British bats (*Chiroptera*). *Mammal Rev* 27:77–94.
33. Fabrik I, Krizkova S, Huska D, Adam V, Hubalek J, Trnkova L, Eckschlager T, Kukacka J, Prusa R, Kizek R. 2008. Employment of electrochemical techniques for metallothionein determination in tumor cell lines and patients with a tumor disease. *Electroanalysis* 20:1521–1532.
34. Petrlova J, Potesil D, Mikelova R, Blastik O, Adam V, Trnkova L, Jelen F, Prusa R, Kukacka J, Kizek R. 2006. Attomole voltammetric determination of metallothionein. *Electrochim Acta* 51:5112–5119.
35. Krizkova S, Zitka O, Adam V, Beklova M, Horna A, Svobodova Z, Sures B, Trnkova L, Zeman L, Kizek R. 2007. Possibilities of electrochemical techniques in metallothionein and lead detection in fish tissues. *Czech J Anim Sci* 52:143–148.
36. Petrlova J, Krizkova S, Zitka O, Hubalek J, Prusa R, Adam V, Wang J, Beklova M, Sures B, Kizek R. 2007. Utilizing a chronopotentiometric sensor technique for metallothionein determination in fish tissues and their host parasites. *Sensors Actuators B Chem* 127:112–119.
37. Zehnalek J, Hanustiak P, Petrek J, Potesil D, Adam V, Havel L, Babula P, Kizek R. 2005. Simultaneous determination of copper, cadmium and lead in refined sugar and consumption sugar. *Listy Cukrov Reparske* 120:142–145.
38. Malavolta M, Cipriano C, Costarelli L, Giacconi R, Tesei S, Muti E, Piacenza F, Pierpaoli S, Larbi A, Pawelec G, Dedoussis G, Herbein G, Monti D, Jajte J, Rink L, Mocchegiani E. 2008. Metallothionein downregulation in very old age: A phenomenon associated with cellular senescence? *Rejuvenation Res* 11:455–459.
39. Goodyear KL, McNeill S. 1999. Bioaccumulation of heavy metals by aquatic macro-invertebrates of different feeding guilds: A review. *Sci Total Environ* 229:1–19.
40. Locatelli C. 2007. Voltammetric methods for the simultaneous determination of trace metals in foods, plant tissues and soils. *J Sci Food Agric* 87:305–312.
41. Wu Y, Li NB, Luo HQ. 2008. Simultaneous measurement of Pb, Cd and Zn using differential pulse anodic stripping voltammetry at a bismuth/poly(p-aminobenzene sulfonic acid) film electrode. *Sensors Actuators B Chem* 133:677–681.
42. Melucci D, Torsi G, Locatelli C. 2007. Analytical procedure for the simultaneous voltammetric determination of trace metals in food and environmental matrices. Critical comparison with atomic absorption spectroscopic measurements. *Ann Chim* 97:141–151.
43. Locatelli C, Torsi G. 2001. Voltammetric trace metal determinations by cathodic and anodic stripping voltammetry in environmental matrices in the presence of mutual interference. *J Electroanal Chem* 509:80–89.
44. Stohs SJ, Bagchi D. 1995. Oxidative mechanisms in the toxicity of metal-ions. *Free Radic Biol Med* 18:321–336.
45. Valko M, Morris H, Cronin MTD. 2005. Metals, toxicity and oxidative stress. *Curr Med Chem* 12:1161–1208.
46. Thompson LJ. 2007. Lead. In Gupta RC, ed, *Veterinary Toxicology*, 1st ed. Academic, New York, NY, USA, pp 438–441.
47. Chmielnicka J, Halatek T, Jedlinska U. 1989. Correlation of cadmium-induced nephropathy and metabolism of endogenous copper and zinc in rats. *Ecotoxicol Environ Saf* 18:268–276.

Těžké kovy a syndrom bílého nosu - potenciální hrozby pro populace letounů

Pikula, J., Banďouchová, H., Novotný, L., Meteyer, C.U., Zukal, J., Irwin, N.R., Zima, J. & Martínková, N. (2012) Histopathology Confirms White-Nose Syndrome in Bats in Europe. Journal of Wildlife Diseases 48, 207-211.

Journal of Wildlife Diseases, 48(1), 2012, pp. 207–211

Histopathology Confirms White-Nose Syndrome in Bats in Europe

Jiri Pikula,[1] Hana Bandouchova,[1] Ladislav Novotný,[1] Carol U. Meteyer,[2] Jan Zukal,[3,4] Nancy R. Irwin,[5] Jan Zima,[3] and Natália Martínková[3,6,7] [1] University of Veterinary and Pharmaceutical Sciences, Palackeho 1/3, 612 42 Brno, Czech Republic; [2] US Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711-6223, USA; [3] Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, v.v.i., Květná 8, Brno, 603 65 Czech Republic; [4] Department of Botany and Zoology, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic; [5] Department of Biology, York University, York YO10 5DD, UK; [6] Institute of Biostatistics and Analyses, Masaryk University, Kamenice 3, 625 00 Brno, Czech Republic; [7] Corresponding author (email: martinkova@ivb.cz)

ABSTRACT: White-nose syndrome, associated with the fungal skin infection geomycosis, caused regional population collapse in bats in North America. Our results, based on histopathology, show the presence of white-nose syndrome in Europe. Dermatohistopathology on two bats (*Myotis myotis*) found dead in March 2010 with geomycosis in the Czech Republic had characteristics resembling *Geomyces destructans* infection in bats confirmed with white-nose syndrome in US hibernacula. In addition, a live *M. myotis*, biopsied for histopathology during hibernation in April 2011, had typical fungal infection with cupping erosion and invasion of muzzle skin diagnostic for white-nose syndrome and conidiospores identical to *G. destructans* that were genetically confirmed as *G. destructans*.

Key words: *Geomyces destructans*, geomycosis, histopathology, *Myotis myotis*, white-nose syndrome.

White-nose syndrome (WNS) is an emerging disease, associated with the fungus *Geomyces destructans,* that is spreading among hibernating bats in North America (Blehert et al., 2009). Dermatohistopathology confirms WNS characterized by fungal invasion of living tissue and epidermal cupping erosions (Meteyer et al., 2009). Field clinical signs of WNS in bats include a suite of descriptors including white fungal growth on hairless parts of wings, muzzle, and ears, aberrant behavior in winter, loss of fat reserves, and increased mortality at affected sites (Foley et al., 2011). Mass mortality of bats can lead to regional population collapse and extinction (Frick et al., 2010).

In Europe, *G. destructans* was first identified morphologically from culture and sequencing of *ITS* and *SSU* rRNA regions from affected bats in 2008 (Wibbelt et al., 2010). Photographs of bats with white muzzles have been taken sporadically over several decades, but lately there has been an increase in these observations (Martínková et al., 2010). Recent genetic evidence confirms that *G. destructans* is widespread in Europe, but mass mortality has not been observed (Puechmaille et al., 2011b). There has been no histologic study of bats with geomycosis in Europe because bats may not be euthanatized without a permit, and detection of the fungus was therefore restricted to live bats without histopathologic examination. We present the histopathologic confirmation of WNS in bats from Europe.

A carcass of a greater mouse-eared bat (*Myotis myotis*) with white fungal patches on its muzzle was found in Stará Drátenická cave (Moravian Karst, Czech Republic) on 6 March 2010, during a winter survey. Conidiospores and hyphae morphologically identical to *G. destructans* were found on an adhesive tape sample taken from the bat in the cave (Fig. 1a), but cultures and PCR on this sample were negative (sample 5^t, Martínková et al., 2010). No other bats in the hibernaculum (29 bats of seven species) had signs of fungal infection.

Five greater mouse-eared adult bat carcasses were found 12 days after the survey on the floor of Byčí skála cave (Moravian Karst, Czech Republic; ~3 km from Stará Drátenická cave) approximately 40 m from the entrance. The carcasses were collected and frozen at −20 C for later examination. In 2010, 1,192 greater mouse-eared bats were counted in Byčí skála cave, hibernating alone or in clusters of around 30 individuals

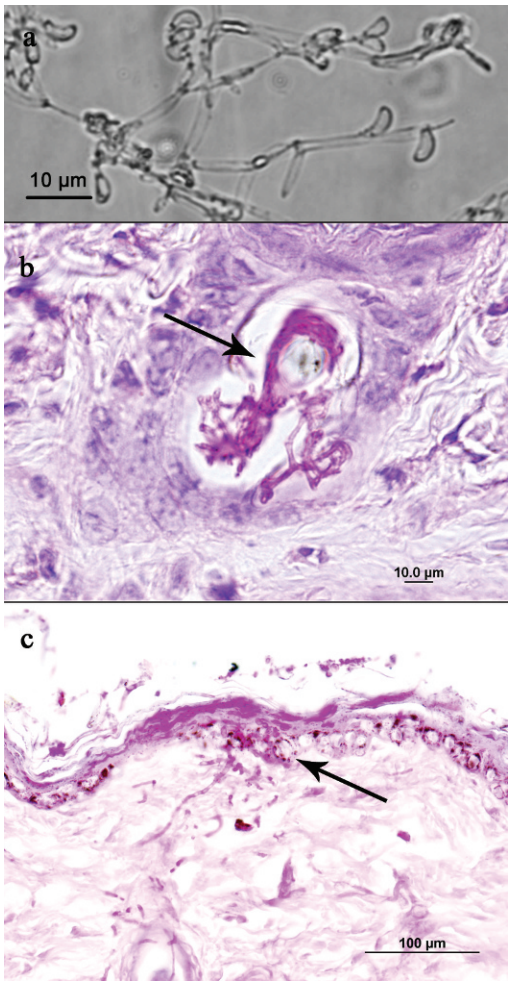


FIGURE 1. *Geomyces destructans* infection in bats (*Myotis myotis*) found dead in hibernacula in two caves in the Czech Republic. (a) Hyphae and spores morphologically identical to *G. destructans*, collected using adhesive tape from the muzzle of a bat from Stará Drátenická cave. (b) Fungal growth on the muzzle of a bat from Byčí skála cave resulting in colonization of the hair follicle (arrow). Periodic acid–Schiff (PAS) stain. (c) Fungal growth over epidermis of a bat from Byčí skála cave with hyphae obscuring the basement membrane and invading the superficial dermis (arrow). Fungal growth in the dermal tissue consisted of dichotomic branching hyphae, ~2 ìm in diameter, with occasional septa. PAS stain.

in crevices in high domes. In a previous study, *G. destructans* was genetically confirmed in two live bats found deeper in the cave than the carcasses discussed in this paper (Martínková et al., 2010).

Gross visual inspection of the six dead bats (one from Stará Drátenická cave and five from Byčí skála cave) in the laboratory showed no visible fungal growth or skin lesions, although fungal growth was present while the bat from Stará Drátenická cave remained on the cave wall. The body condition was poor to moderate with low adipose tissue stores. The mean body weight of the six bat cadavers was 18 g, which contrasts with live bats during hibernation that weigh about 26 g (Krapp, 2001). The upper jaw together with the skin, wing membranes (plagiopatagium), and lungs were dissected for histopathologic examination. Formalin-fixed samples containing bones of the upper jaw were decalcified using formic acid to facilitate preparation of sections. Wing membranes were rolled for paraffin embedding. Serial tissue sections of 5 µm were prepared and stained with periodic acid–Schiff stain. Internal organs were stained with hematoxylin and eosin.

No fungal infection was seen in the microscopic sections of wing skin from four bats, but fungal colonization of the skin of two bats from Stará Drátenická and Byčí skála caves was heavy. Although autolysis was present, organized hyphae were seen in all layers of epidermis of the muzzle and deep in the hair follicles (Fig. 1b). The hyphae penetrated the basement membrane of the epidermis with focal pigmentary incontinence and invaded the superficial and deep dermis and underlying connective tissue (Fig. 1c). Fungal hyphae and detritus were seen in the nasal cavity that may have been postmortem invasion. Hyphae were also present in the nasal cartilage perichondrium and endomysium of nasal muscles. No reactive inflammatory response was observed, except for sporadic small pustules containing hyphae in the dermal tissue. Lungs were congested and alveoli filled with slightly eosinophilic liquid, probably as a result of postmortem changes.

An 24-g, adult, male greater mouse-eared bat, hibernating in Byčí skála cave

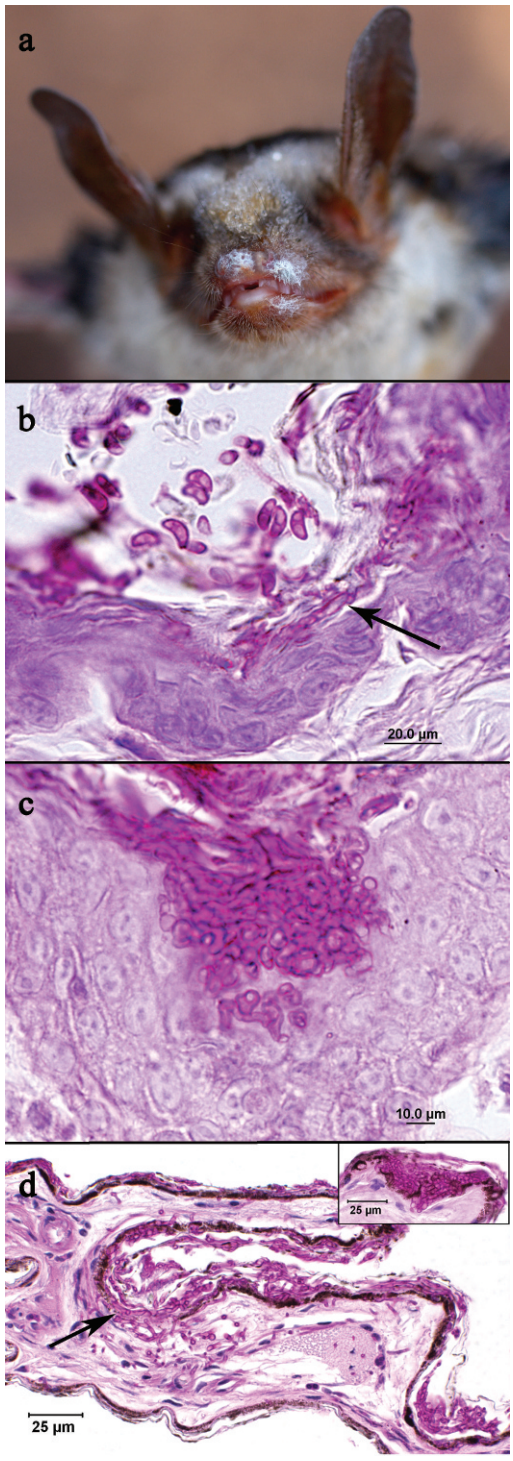


FIGURE 2. *Geomyces destructans* infection in a bat (*Myotis myotis*) from a hibernaculum in the Czech Republic, biopsied for histopathology, and comparison to the pathognomonic findings of white-nose syndrome (WNS) in the USA. (a) Male bat from the Byčí skála cave showing distinct fungal growth on the muzzle, around the nares as well as on the upper and lower lips. (b) Large numbers of curved conidia observed on the epidermis together with hyphae growing in stratum corneum and stratum basale (arrow). (c) A cuplike epidermal erosion in the nasal skin. (d) Section of wing membrane from a bat (*Myotis lucifugus*) with WNS collected in the USA. Inset shows typical cupping erosions diagnostic of WNS in the USA. The main image is a region of the same wing with superficial fungal colonization of the skin where it does not yet show the interface of dense fungi and cupping erosions but shows interruption of the basement membrane by fungal hyphae (arrow). Periodic acid–Schiff stain.

and showing visible fungal growth with brief illumination with a flashlight (Fig. 2a), was biopsied and euthanatized late in the hibernation period (4 April 2011). Clinical specimens collected from the muzzle using adhesive tape were positive for *G. destructans* conidiospores when examined by direct microscopy. The fungus was cultured on Sabouraud agar, and the isolate was genetically confirmed as *G. destructans* (EMBL-Bank accession number: HE588133). The sequence was identical to that previously reported from the Byčí skála cave (Martínková et al., 2010). Histopathologic findings on the skin of the muzzle were very similar to those observed in samples collected from bat cadavers as described above. Sections of the nasal skin and the wing membrane had large numbers of curved conidia associated with hyphae that were growing into the stratum corneum and through to the stratum basale (Fig. 2b). In contrast to the cadavers examined, dense aggregates of hyphae formed cupping erosions of the epidermis in the sampled euthanatized bat (Fig. 2c). Hyphae penetrated focally through the basement membrane to the dermis and deep into the hair follicles, also invading the associated sebaceous glands. There was lung congestion with large numbers of siderophages. There

were no pathologic findings in spleen, kidneys, heart, or liver.

The histopathology in the skin of this greater mouse-eared bat from Byčí skála cave (Fig. 2c) fulfilled the criteria currently used to confirm WNS in North America (Meteyer et al., 2009). The localized areas of dense hyphae with a discrete interface where the fungus forms cup-shaped erosions was present in the bat from the Czech Republic collected in 2011, and these findings are the current gold standard for diagnosing WNS. Between these localized areas of erosion, colonization of the superficial keratin by the fungal hyphae is also present in wing membranes from bats with white-nose syndrome in the USA (Fig. 2d).

Our results confirm the presence of WNS in Europe and demonstrate that *G. destructans* infection in hibernating bats in Europe can be associated with sporadic deaths that may remain unrecognized in the hibernaculum. Mortality rates are low and, to date, have not affected the long-term population size of greater mouse-eared bats (Martínková et al., 2010). With the increase and extent of *G. destructans* found in hibernacula in the Czech Republic and Slovakia in 2010, monitoring bat populations in subsequent years is even more critical. Histopathology of bats with geomycosis was identified as one of the key research priorities in the field in Europe (Puechmaille et al., 2011a). Following confirmation of WNS in Europe, further comparisons between North American and European *G. destructans* infections can enhance the understanding of the pathogenesis of geomycosis in bats (Cryan et al., 2010).

Material from the Byčí skála cave was provided by Vlastislav Káňa in 2010. We thank Paul A. Racey, Maja Rütten, and four anonymous reviewers for helpful comments on the manuscript. This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic (Projects MSM 6215712402, MSM 0021622416, LC06073). The research was conducted in accordance with following permits: 35335/ENV/06-2793/620/06, 00163/MK/2009, S01389/MK/2008,142/2010, 169/2011.

LITERATURE CITED

Blehert, D. S., A. C. Hicks, M. J. Behr, C. U. Meteyer, B. M. Berlowski-Zier, E. L. Buckels, J. T. H. Coleman, S. R. Darling, A. Gargas, R. Niver, J. C. Okoniewski, R. J. Rudd, and W. B. Stone. 2009. Bat white-nose syndrome: An emerging fungal pathogen? Science 323: 227.

Cryan, P. M., C. U. Meteyer, J. G. Boyles, and D. S. Blehert. 2010. Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. BMC Biology 8: 135.

Foley, J., D. Clifford, K. Castle, P. Cryan, and R. S. Ostfeld. 2011. Investigating and managing the rapid emergence of white-nose syndrome, a novel, fatal, infectious disease of hibernating bats. Conservation Biology 25: 223–231.

Frick, W. F., J. F. Pollock, A. C. Hicks, K. E. Langwig, D. S. Reynolds, G. G. Turner, C. M. Butchkoski, and T. H. Kunz. 2010. An emerging disease causes regional population collapse of a common North American bat species. Science 329: 679–682.

Krapp, F. 2001. Handbuch der Säugetiere Europas, Teil 1, Chiroptera 1. Aula Verlag, Wiebelsheim, Germany, 602 pp.

Martínková, N., P. Bačkor, T. Bartonička, P. Blažková, J. Červený, L. Falteisek, J. Gaisler, V. Hanzal, D. Horáček, Z. Hubálek, H. Jahelková, M. Kolařík, Korytár, A. Kubátová, B. Lehotská, R. Lehotský, R. K. Lučan, O. Májek, J. Matějů, Z. Řehák, J. Šafář, P. Tájek, E. Tkadlec, M. Uhrin, J. Wagner, D. Weinfurtová, J. Zima, J. Zukal, and I. Horáček. 2010. Increasing incidence of *Geomyces destructans* fungus in bats from the Czech Republic and Slovakia. PLoS ONE 5: e13853.

Meteyer, C. U., E. L. Buckles, D. S. Blehert, A. C. Hicks, D. E. Green, V. Shearn-Bochsler, N. J. Thomas, A. Gargas, and M. J. Behr. 2009. Histopathologic criteria to confirm white-nose syndrome in bats. Journal of Veterinary Diagnostics and Investigation 21: 411–414.

Puechmaille, S. J., W. F. Frick, T. H. Kunz, P. A. Racey, C. C. Voigt, G. Wibbelt, and E. C. Teeling. 2011a. White-nose syndrome: Is this emerging disease a threat to European bats? Trends in Ecology and Evolution, 26: 570–576.

———, G. Wibbelt, V. Korn, H. Fuller, F. Forget, K. Mühldorfer, A. Kurth, W. Bogdanowicz, C. Borel, T. Bosch, T. Cherezy, M. Drebet, T. Görföl, A.-J. Haarsma, F. Herhaus, G. Hallart, M. Hammer, C. Jungmann, Y. Le Bris, L. Lutsar, M. Masing, B. Mulkens, K. Passior, M. Starrach, A. Wojtaszewski, U. Zöphel, and E.

C. Teeling. 2011b. Pan-European distribution of white-nose syndrome fungus (*Geomyces destructans*) not associated with mass mortality. PLoS ONE 6: e19167.

Wibbelt, G., A. Kurth, D. Hellmann, M. Weishaar, A. Barlow, M. Veith, J. Prüger, T. Görföl, L. Grosche, F. Bontadina, U. Zöphel, H.-P. Seidl, P. M. Cryan, and D. S. Blehert. 2010. White-nose syndrome fungus (*Geomyces destructans*) in bats, Europe. Emerging Infectious Diseases 16: 1237–1243.

Submitted for publication 31 December 2010.
Accepted 12 September 2011.

Těžké kovy a syndrom bílého nosu - potenciální hrozby pro populace letounů

Zukal, J., Pikula, J. & H. Banďouchova (2012) Bats and toxic pollutants. Pp. 75-79 in: Flaquer, C. & Puig-Montserrat, X. eds. Proceedings of the International Symposium on the Importance of Bats as Bioindicators. Museum of Natural Sciences Edicions, Granollers.

Zukal, J., Pikula, J., H. Bandouchova (2012). Bats and toxic pollutants. Pp. 75-79 in Flaquer, C. & Puig-Montserrat, X. eds. Proceedings of the International Symposium on the Importance of Bats as Bioindicators. Museum of Natural Sciences Edicions, Granollers.

Bats and toxic pollutants

Jan Zukal[1], Jiri Pikula[2] and Hana Bandouchova[2]
[1] Institute of Vertebrate Biology AS CR v.v.i., Brno, the Czech Republic
[2] University of Veterinary and Pharmaceutical Sciences, Brno, the Czech Republic

Bats today face a number of major threats, with habitat disturbance and loss of roost sites (through deforestation and reconstruction of buildings) some of the more obvious. Less recognised or documented, however, are the detrimental effects due to toxicant exposure, which can be just as important but only become obvious once bat populations have started to decline. Toxicants come from a variety of sources, both natural and artificial, with agricultural and industrial chemicals some of the best known.

Like other wild animal taxa, bats are capable of indicating environmental quality. Rapid declines in the bat populations of many countries, however, have resulted in the classification of 24% of species as threatened and 21% as near threatened (Mickleburgh et al. 2002). Strict world-wide protection and conservation of most bat species prevents their use in standardised monitoring programmes for environmental contaminants, such as those undertaken with game animals. Further, the nocturnal and reclusive nature of these mammals makes recognition of die-offs more difficult than in other wild animals.

The study of bat toxicology, therefore, must be a multidisciplinary procedure with investigations based on analytical chemistry, biochemistry, statistical and mathematical modelling, and biological and ecological studies of the various species, including pathological and behavioural studies (Rattner 2009).

Toxic pollutants

Although humans have lived on earth for tens of thousands of years, it is only during the last two centuries that dramatic changes in the use of natural resources and energy, with resultant changes in economic systems, have led to exponential growth in the human population. One of the most important human impacts on natural processes and living organisms began with the increase in extraction, refining and processing of fossil fuels for the petrochemical industry at the beginning of the 20th century. Since that time, chemical compounds have contaminated all parts

of environment, and pollution by toxic elements continues to increase to the present day.

Many different types of contaminants are now present in the environment, ranging from synthetic chemicals (which would not be present in the environment without human intervention) to increased levels of trace metals that are required for life (Melancon 2003). These can be classified under various criteria, including origin, effect, property or degradability. Concerns range from possible harmful effects on flora and fauna (e.g. changes in growth rate, hormonal changes, immune system damage, or carcinogenicity) to possible harm caused to humans from consuming contaminated organisms. Notable chemical contaminants include four main groups: 1) heavy metals, 2) persistent organic pollutants, 3) environmentally persistent pharmaceutical pollutants, and 4) volatile organic compounds. To date, only the first two groups have been studied in any detail in bats.

Heavy metals

Heavy metals occur naturally in the environment and, therefore, there is always a natural background concentration in soils, rocks, sediments, water and in living organisms, with concentrations varying greatly. Anthropogenic pollution results in higher concentrations of these metals relative to the normal background values. Municipal and industrial waste and fossil fuels are especially likely to contain heavy metals.

The eleven elements of highest concern within the European Community are arsenic, cadmium, cobalt, chromium, copper, mercury, manganese, nickel, lead, tin and thallium. All are hazardous to health or the environment, with lead, mercury, arsenic and cadmium among the most hazardous.

Persistent organic pollutants

Persistent organic pollutants are organic compounds of natural or anthropogenic origin that resist photolytic, chemical and/or biological degradation (UNEP, 1999). They are semi-volatile, allowing them to be transported long distances from their original source via water and the atmosphere, meaning that they can reach regions where they have never been used or produced. Persistent organic pollutants are toxic, chemically stable and tend to concentrate in living organisms through the process of bioaccumulation due to their high lipid solubility. As fish, predatory birds and mammals (including bats and humans) are high up in the food chain, they accumulate the greatest concentrations, mainly in fatty tissues. Almost all of these chemicals are produced by humans through industrial processing; natural sources being very scarce.

Three main groups are differentiated: 1) pesticides (e.g. DDT and its analogues, HCH compounds, cyclodienes, toxaphene and compounds with caged structures), 2) industrial chemicals (e.g. PCBs and hexabromobiphenyl), and 3) by-products (e.g. dioxin, furan and PAHs). Persistent organic pollutants are used in industrial processes and the production of a wide range of products (e.g. solvents and pharmaceuticals). Others are still widely used as pesticides (Jones & de Voogt 1999). Persistent organic pollutants are of high concern as some have been identified as carcinogenic, mutagenic and teratogenic, and many are recognised as exerting sub-lethal effects.

Bats as bioindicators

Bats are among the most diverse and widespread mammal species on Earth. Approximately 1,200 chiropteran species are known and they are found on all continents (except Antarctica) inhabiting a wide variety of ecological niches (Nowak 1994). Bats also display a high number of roosting and feeding specialisations and play key functional roles in ecosystems, acting as plant pollinators, seed dispersers and predators of insects, including harmful forest and agricultural pests (Kunz et al. 2011). Most bat species are listed under the International Union for the Conservation of Nature's Red List of Threatened Species (IUCN 2010) and are of global conservation concern (Micklenburg et al. 2002).

In light of their diversity and importance, bats have enormous potential as biodiversity, ecological and environmental indicators. Jones et al. (2009) summarised a number of general parameters that make bats ideal indicators of human-induced climate change and habitat quality (Tab. 1). Insectivorous bats in particular have been used in wildlife toxicology studies as they have a number of characteristics that make them suitable for use as indicators of general environmental conditions (Tab. 2).

Insectivorous bat species are the primary consumers of nocturnal insects and food composition, and subsequent exposure to environmental pollution, is influenced by the habitats that different bat species use for insect foraging (e.g. aquatic, aquatic and terrestrial, or terrestrial only) as well as the environmental conditions at the sites the bats were collected

from. Aquatic habitats (e.g. rivers, lakes and canals) are favoured as they often attract a rich supply of insects, though emerging aquatic insects may provide contaminant subsidies. Some species have adapted well to urban environments and can be found feeding (e.g. around streetlamps) within major agglomerations with high contaminant pollution (Gaisler et al. 1998).

Like other insectivorous mammals and birds, bats receive higher contaminant residues in their diets than herbivores due to food chain build-up. Moreover, the high metabolic rate of bats (bats consume 40–100% of their body mass each night) connected with flight (several km per night) and their associated small size demands greater rates of food intake than less active or larger mammals. Greater food intake thus increases the amount of contaminant available for concentration in fat.

Bats tend to have low reproductive rates and long life spans of up to 30 years (Racey & Entwistle 2000). Bats, therefore, may be subject to long-term accumulation of toxic contaminants and large concentrated doses of lipophilic contaminants may be transferred to offspring in milk. Moreover, bats are at risk of contaminant residue mobilisation as fat is absorbed and energy utilised during hibernation (Thomas et al. 1990).

Monitoring toxic contaminants in bats

There have been a number of reports published on the adverse effects of natural toxins on bats, including a description of mass mortality associated with a cyanobacterial bloom (Pybus et al. 1986) and negative physiological effects on flight performance and echolocation following ethanol ingestion by fruit bats (Sánchez et al. 2010). Reports on the effects of anthropogenic pollutants on Holarctic bats, however, are far more frequent (O'Shea & Johnson 2009). The results of these studies, which have used a variety of methods, all strongly implicate bioaccumulation of insecticides and other pollutants as contributing to the recent decline in bats.

In Europe, for example, Luftl et al. (2003) and Walker et al. (2007), have used standard residue analysis to assess heavy metals in the livers and kidneys of dead or debilitated bats in Austria and Britain, respectively. In the Czech Republic, Pikula et al. (2010) have confirmed the susceptibility of vespertilionid bats to the toxic heavy metals lead and cadmium and provided data on the essential element zinc. They also examined responses of bats foraging over aquatic, aquatic and terrestrial, and terrestrial habitats to heavy metals through evaluation of the metal-binding protein metallothionein. In southern Brazil, Zocche et al. (2010) have observed adverse effects of exposure to heavy metals in a coal mining area, using the Comet assay to assess DNA damage in blood cells of insectivorous bats.

An important recent study on the effects and responses of toxic contaminants has highlighted high intestinal permeability as a means for passive absorption through cell walls in bats (Caviedes-Vidal et al. 2008). While this is a less selective system for nutrient absorption than the more common carrier-mediated system, it may compensate the bat for its relatively less intestinal tissue. Paracellular absorption, however, also allows toxins to be readily absorbed from plant and animal material through the intestinal lumen and, therefore, increases the susceptibility of bats to toxins in the diet.

The wide use of organophosphate-based pesticides in agriculture makes exposure of humans and animals unavoidable and can result in both acute effects and chronic damage to the nervous system (Stephens et al. 1995). Sub-lethal exposure to pesticides over longer periods, however, can also be an important source of adverse effects. While detoxification via cytochrome P450 enzyme systems can ameliorate such effects, this uses energy that may then be lacking for other functions.

Monitoring and evaluation of bat activity represents an alternative approach to examining the responses of bats to environmental pollution. For example, significant differences have been observed in both bat diversity and activity between areas of mixed coniferous forest exposed to different degrees of air pollution (Rachwald et al. 2004); while Vaughan et al. (1996) have shown how a decline in river water quality affects the foraging behaviour of Pipistrellus pipistrellus and Myotis spp. bats, with both overall activity and foraging activity reduced downstream of a sewage output.

Other methods include mark-recapture techniques, used by Frick et al. (2007) to obtain data on the effects of a major pesticide spill on annual survival and population growth of Myotis yumanensis in the USA; and comparing foraging activity at sites of pesticide application with data on insect contamination to estimate exposure of bats to pesticides (Stahlschmidt and Bruhl 2012).

One problem that many of these studies face is the fact that, under environmental conditions, bats can be exposed to multiple stressors at the same time, including natural toxins, anthropogenic pollutants and infectious agents.

Given that contaminants frequently occur as mixtures in nature, ecotoxicology must also take into account possible synergistic effects between pesticides and natural stressors (Relyea & Hoverman 2006) and chronic, low-level exposure (Sanderson & Solomon 2009) with additive or jointly independent actions (Kortenkamp et al. 2007). Further, a number of epizootic infectious diseases have been noted as more severe in areas contaminated by environmental pollutants, demonstrating the possibility of population level effects associated with contaminant-induced immunosuppression.

Perspectives and advice for future bat studies

In many instances, human risk assessments do not adequately protect other biota. There is no doubt, therefore, that it will be necessary to study both classical and new environmental pollutants in bats in the future. The main purpose of these studies must be to assess the potential risk of toxicants for bats in order to enhance their future protection. As such, researchers should bear in mind the following advice:

1. All studies should comply with national and international nature conservation legislation and laws for the protection of animals against cruelty;

2. The 3Rs method should be used whenever possible (i.e. Replacement, Refinement and Reduction);

3. If experimental work is necessary to evaluate responses of bats to toxicants, consider using bats from wildlife rehabilitation centres. These may have been permanently handicapped and, therefore, cannot be returned to the wild;

4. When planning collection of bats from the wild, power analysis should be conducted to estimate the sample size providing sufficient statistical power and significance;

5. Always use non-destructive and non-invasive sampling procedures;

6. If possible, make use of samples collected from natural die-off, i.e. specimens found dead or moribund;

7. Obtain samples from bats originally delivered for different analyses, such as rabies or white-nose syndrome testing;

8. Do not discard bat cadavers after obtaining samples for your particular study, they may be useful for future studies;

9. Cooperate with specialists from different fields in order to obtain the widest range of analysis and views;

10. Employ progressive analytical techniques and modern instrumentation with the lowest detection limits;

11. Employ techniques allowing analysis of very small samples;

12. Increase passive collection of data and samples for contaminant analysis and its effects during other projects;

13. Identify exposure biomarkers and correlate levels with toxin content in tissue, thereby allowing non-destructive intra-vital diagnoses;

14. When handling bats, bear in mind that they may be reservoirs for zoonotic agents;

15. Encourage further detailed ecotoxicological investigations into this interesting mammalian group.

Acknowledgments

We received support from the Grant Agency of the Czech Republic (grant No. 506/12/1064) and the Institute of Vertebrate Biology AS CR (RVO: 68081766). Authors thank Kevin Roche for his correction of the English text.

References

- **Caviedes-Vidal,** E., Karasov, W.H., Chediack, J.G., Fasulo, V., Cruz-Neto, A.P., Otani, L., 2008: Paracellular Absorption: A Bat Breaks the Mammal Paradigm. PLoS ONE, 3: e1425.
- **Frick**, W.F., Rainey, W.E., Pierson, E.D., 2007: Potential Effects of Environmental Contamination on Yuma Myotis Demography and Population Growth. Ecological Applications, 17: 1213 – 1222.
- **Gaisler**, J., Zukal, J., Rehak, Z., Homolka, M., 1998: Habitat preference and flight activity of bats in a city. J Zool, 244: 439 – 445.
- **International** Union for the Conservation of Nature, 2010: IUCN Red List of Threatened Species, version 2010.3.
- **Jones**, G., Jacobs, D.S., Kunz, T.H., Willig, M.R., Racey, P.A., 2009: Carpe noctem: the importance of bats as bioindicators. Endang Species Res, 8: 93 – 115.
- **Jones**, K,C., de Voogt, P., 1999: Persistent organic pollutants (POPS); state of the science. Environmental Pollution, 100: 209 – 221.
- **Kortenkamp**, A., Faust, M., Scholze, M., Backhaus, T., 2007: Low-level exposure to multiple chemicals: reason for human health concerns? Environ Health Persp, 115 (Suppl 1): 106 – 114.
- **Kunz**, T.H., de Torrez, E.B., Bauer, D., Lobova, T., Fleming, T. H., 2011: Ecosystem services provided by bats. Ann N Y Acad Sci, 1223: 1 – 38.
- **Luftl**, S., Freitag, B., Deutz, A., Tataruch, F., 2003: Concentrations of Heavy Metals in European Bats (Microchiroptera). Fresenius Environmental Bulletin, 12: 353 – 358.
- **Mickleburgh**, S. P., Hutson, A.M., Racey, P.A., 2002: A review of the global conservation status of bats. Oryx, 36: 18 – 34.
- **Melancon**, M.J., 2003: Bioindicators of Contaminant Exposure and Effect in Aquatic and Terrestrial Monitoring. In: Hoffman D.J., et al. (eds.), Handbook of Ecotoxicology. 2nd edition. Lewis Publishers, USA, pp. 257 – 278.
- **Mickleburgh**, S.P., Hutson, A.M., Racey, P.A., 2002: A review of the global conservation status of bats. Oryx, 36: 18 – 34.

- **Nowak**, R.W., (ed.), 1994: Walker's bats of the world. Johns Hopkins University Press, Baltimore, MD, 287 pp.
- **O'Shea**, T.J., Johnson, J.J., 2009: Environmental contaminants and bats: investigating exposure and effects. In: Kunz, T.H., Parsons, S., (eds.), Ecological and behavioral methods for the study of bats, 2nd edn. Johns Hopkins University Press, Baltimore, pp. 500 – 528.
- **Pikula**, J., Zukal, J., Adam, V., Bandouchova, H., Beklova, M., Hajkova, P., Horakova, J., Kizek, R., Valentikova, L., 2010: Heavy metals and metallothionein in vespertilionid bats foraging over aquatic habitats in the Czech Republic. Environmental Toxicology and Chemistry, 29: 501 – 506.
- **Pybus**, M.J., Hobson, D.P., Onderka, D.K., 1986: Mass mortality of bats due to probable blue-green algal toxicity. J Wildl Dis, 22: 449 – 450.
- **Rachwald**, A., Wodecka, K., Malzahn, E., Kluzinski, L., 2004: Bat Activity in Coniferous Forest Areas Land the Impact of Air Pollution. Mammalia, 68: 445 – 453.
- **Racey**, P.A., Entwistle, A.C., 2000: Life history and reproductive strategies of bats. In: Crighton, E.G., Krutzsch, P.H. (eds.), Reproductive biology of bats. Academic Press, New York, pp. 363 – 414.
- **Rattner**, B.A., 2009: History of wildlife toxicology. Ecotoxicology, 18: 773 – 783.
- **Relyea**, R., Hoverman, J., 2006: Assessing the ecology in ecotoxicology: a review and synthesis in freshwater systems. Ecol Lett, 9: 1157 – 1171.
- **Sánchez**, F., Melcón, M., Korine, C., Pinshow, B. (2010): Ethanol ingestion affects flight performance and echolocation in Egyptian fruit bats. Behavioural Processes, 84: 555 –558.
- **Sanderson**, H., Solomon, K., 2009: Contaminants of emerging concern challenge ecotoxicology. Environ Toxicol Chem, 28: 1359 – 1360.
- **Stahlschmidt**, P., Bruhl, C.A., 2012: Bats at risk? Bat activity and insecticide residue analysis of food items in an apple orchard. Environmental Toxicology and Chemistry, 31: 1556 – 1563.
- **Stephens**, R., Spurgeon, A., Calvert, I.A., Beach, J., Levy, L.S., Berry, H., Harrington, J.M., 1995: Neuropsychological effects of long-term exposure to organophosphates in sheep dip. Lancet, 345: 1135 – 1139.
- **Thomas**, D.W., Dorais, M., Bergeron, J.M., 1990: Winter energy budgets and cost of arousals for hibernating little brown bats, Myotis lucifugus. J Mammal, 71: 475 – 479.
- **UNEP**, 1999: Inventory of information sources on chemicals Persistent Organic Pollutants. 141 pp.
- **Vaughan**, N., Jones, G., Harris, S., 1996: Effects of sewage effluent on the activity of bats (Chiroptera: Vespertilionidae) foraging along rivers. Biological Conservation, 78: 337 – 343.
- **Walker**, L.A., Simpson, V.R., Rockett, L., Wienburg, C.L., Shore, R.F., 2007: Heavy Metal Contamination in Bats in Britain. Environmental Pollution, 148: 483 – 490.
- **Zocche**, J.J., Leffa, D.D., Damiani, A.P., Carvalho, F., Mendonça, R.A, dos Santos, C.E.I., Boufleur, L.A, Dias, J.F., de Andrade, V.M., 2010: Heavy metals and DNA damage in blood cells of insectivore bats in coal mining areas of Catarinense coal basin, Brazil. Environmental Research Volume, 110: 684 – 691.

Table. 1. *Criteria that make bats excellent indicators (reproduced from Jones et al. 2009).*

• Relatively stable taxonomy
• Can be sampled at several levels (e.g. population, feeding rates of individuals)
• Wide geographic range
• Graded responses to habitat degradation correlated with responses of other taxa (e.g. insects)
• Rich trophic diversity
• Slow reproductive rate, meaning that population declines can be rapid
• Perform key ecosystem services (e.g. pollination, fruit dispersal, arthropod control)
• Reservoirs of a wide range of emerging infectious diseases whose epidemiology may reflect environmental stress

Table 2. *List of characteristics that make insectivorous bats important as environmental indicators.*

Parameter		Importance for bio-indication
Long lifespan	=	*Long-term accumulation*
Obligate insectivores	=	*High in the trophic chain*
Variety of feeding habitats	=	*Diversity of contaminants sources*
Active flight	=	*Long-range coverage*
Synanthropy	=	*Human and industrial impact*
High metabolic rate	=	*Accumulation of contaminants*
Hibernation	=	*Mobilisation of residues within a short period*

Těžké kovy a syndrom bílého nosu - potenciální hrozby pro populace letounů

Banďouchová, H., Bartonička, T., Berková, H., Brichta, J., Černý, J., Kováčová, V., Kolařík, M., Köllner, B., Kulich, P., Martínková, N., Řehák, Z., Turner, G. G., Zukal, J., Pikula, J. (2014) *Pseudogymnoascus destructans*: Evidence of Virulent Skin Invasion for Bats Under Natural Conditions, Europe. Transboundary and Emerging Diseases 62, 1-5.

Transboundary and Emerging Diseases

RAPID COMMUNICATION

Pseudogymnoascus destructans: Evidence of Virulent Skin Invasion for Bats Under Natural Conditions, Europe

H. Bandouchova[1,*], T. Bartonicka[2], H. Berkova[3], J. Brichta[1], J. Cerny[4], V. Kovacova[1], M. Kolarik[5], B. Köllner[6], P. Kulich[7], N. Martínková[3,8,*], Z. Rehak[2], G. G. Turner[9], J. Zukal[2,3,*] and J. Pikula[1,*]

[1] Department of Ecology and Diseases of Game, Fish and Bees, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
[2] Department of Botany and Zoology, Masaryk University, Brno, Czech Republic
[3] Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic
[4] Department of Cell Biology, Faculty of Science, Charles University in Prague, Prague, Czech Republic
[5] Laboratory of Fungal Genetics and Metabolism, Institute of Microbiology ASCR, Prague, Czech Republic
[6] Institute of Immunology, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Greifswald-Insel Riems, Germany
[7] Veterinary Research Institute, Brno, Czech Republic
[8] Institute of Biostatistics and Analysis, Masaryk University, Brno, Czech Republic
[9] Pennsylvania Game Commission, Harrisburg, PA, USA

Keywords:
white-nose syndrome; chiroptera; transmission electron microscopy; ultraviolet light diagnostics; morbidity; mortality

Correspondence:
J. Pikula. Department of Ecology and Diseases of Game, Fish and Bees, University of Veterinary and Pharmaceutical Sciences Brno, Palackeho 1–3, 612 42 Brno, Czech Republic. Tel.: +420 5 4156 2655; Fax: +420 5 4156 2657; E-mail: pikulaj@vfu.cz

*These authors contributed equally to this work.

Received for publication March 30, 2014

doi:10.1111/tbed.12282

Summary

While *Pseudogymnoascus destructans* has been responsible for mass bat mortalities from white-nose syndrome (WNS) in North America, its virulence in Europe has been questioned. To shed the light on the issue of host–pathogen interaction between European bats and *P. destructans*, we examined seventeen bats emerging from the fungus-positive underground hibernacula in the Czech Republic during early spring 2013. Dual wing-membrane biopsies were taken from *Barbastella barbastellus* (1), *Myotis daubentonii* (1), *Myotis emarginatus* (1), *Myotis myotis* (11), *Myotis nattereri* (1) and *Plecotus auritus* (2) for standard histopathology and transmission electron microscopy. Non-lethal collection of suspected WNS lesions was guided by trans-illumination of the wing membranes with ultraviolet light. All bats selected for the present study were PCR-positive for *P. destructans* and showed microscopic findings consistent with the histopathological criteria for WNS diagnosis. Ultramicroscopy revealed oedema of the connective tissue and derangement of the fibroblasts and elastic fibres associated with skin invasion by *P. destructans*. Extensive fungal infection induced a marked inflammatory infiltration by neutrophils at the interface between the damaged part of the wing membrane replaced by the fungus and membrane tissue not yet invaded by the pathogen. There was no sign of keratinolytic activity in the *stratum corneum*. Here, we show that lesions pathognomonic for WNS are common in European bats and may also include overwhelming full-thickness fungal growth through the wing membrane equal in severity to reports from North America. Inter-continental differences in the outcome of WNS in bats in terms of morbidity/mortality may therefore not be due to differences in the pathogen itself.

Introduction

White-nose syndrome (WNS) has rapidly become a major threat to hibernating bats in the United States and Canada since its emergence in 2006 (Blehert et al., 2009). The disease has attracted intensive research owing to mass mortalities in six North American bat species. However, many gaps still remain in our understanding of WNS (Blehert, 2012; Cryan et al., 2013). Among the most important discoveries in recent years have been the establishment of a causal relationship between the fungus *Pseudogymnoascus* [formerly *Geomyces*] *destructans* (Minnis and Lindner,

2013) and WNS in *Myotis lucifugus* (Lorch et al., 2011) and induction of WNS, with subsequent mortality, in *M. lucifugus* through inoculation with both North American or European isolates of the fungus (Warnecke et al., 2012). Interestingly, the latter study revealed that European *P. destructans* strains can also be pathogenic for bats. Mass mortalities have not been reported in European bats (Martinkova et al., 2010); however, despite the presence of *P. destructans* in many European countries (Martinkova et al., 2010; Puechmaille et al., 2011). These findings tend to support the 'novel pathogen hypothesis', suggesting that WNS may have originated in Europe (Warnecke et al., 2012). On the other hand, little is known of host–pathogen interactions between European bats and *P. destructans*, previous results having been based on histopathological studies on few individuals (Pikula et al., 2012; Wibbelt et al., 2013). In addition, little is known of inter-continental differences in infection with WNS fungus, an area that deserves much greater attention (Blehert, 2012; Cryan et al., 2013). Therefore, the objectives of the present study were to evaluate pathological findings associated with the WNS fungus infection using non-lethal collection of suspected lesions from several European bat species for standard histopathology and transmission electron microscopy (TEM).

Materials and Methods

In the spring of 2013, we examined bats from caves situated in the Moravian Karst, mines in the Jeseniky Mountains, and pseudo-karst caves in the Podyji National Park (all in the Czech Republic) for WNS. The bats were netted while emerging from hibernacula during early spring (from 15 April to 3 May) and handled so as to minimize stress and duration of sampling procedures. Seventeen bats were collected from six different species, that is *Barbastella barbastellus* ($N = 1$ female, body mass 7.5 g, forearm length 39.6 mm), *Myotis daubentonii* ($N = 1$ male, body mass 6.0 g, forearm length 38.1 mm), *Myotis emarginatus* ($N = 1$ female, body mass 7.0 g, forearm length 37.9 mm), *Myotis myotis* ($N = 6$ males and five females, body mass 23.81 ± 1.64 and 25.66 ± 3.54, forearm length 59.38 ± 1.80 and 60.43 ± 1.45), *Myotis nattereri* ($N = 1$ male, body mass 5.6 g, forearm length 40.4 mm) and *Plecotus auritus* ($N = 2$ males, body mass 7.0 and 7.1 g, forearm length 40.6 and 40.2 mm). Two wing-membrane biopsies were taken from each bat for standard histopathology and TEM, the samples being fixed in 10% neutral buffered formalin and 2% glutaraldehyde, respectively. Biopsy of suspected WNS lesions (i.e. areas of orange–yellow fluorescence; Fig. 1a) was guided by trans-illumination of the wing membranes with ultraviolet light [wavelength 368 nm] (Turner et al., 2014), the biopsy being collected using a sterile disposable 4-mm skin biopsy punch (Kruuse, Denmark) from the left thoracic wing membrane. Formalin-fixed samples were embedded in paraffin, cut into 40 serial tissue sections of 5 μm and stained for fungi with periodic acid–Schiff stain. Glutaraldehyde-preserved biopsies for ultrathin sections were post-fixed in 1% OsO_4, dehydrated in acetone and embedded in Epon–Durcupan mixture (Epon 812, Serva, Germany; Durcupan, ACM Fluka, Switzerland). The sections were then stained with 2% uranyl acetate and 2% lead citrate and observed at 80 kV under a Philips EM 208 TEM (FEI, Czech Republic). Classification of skin lesions as WNS-positive was based on published diagnostic criteria (Meteyer et al., 2009). Skin swabs were also collected from WNS-suspected wing membranes using the FLOQ Swabs system (CopanFlock Technologies, Brescia, Italy) to identify *P. destructans* using real-time polymerase chain reaction [RT-PCR] (Muller et al., 2013). The Czech Academy of Sciences' Ethics Committee has reviewed and approved Animal Use Protocol No. 169/2011 in compliance with Law No. 312/2008 on Protection of Animals against Cruelty, as adopted by the Parliament of the Czech Republic. Permits for non-lethal bat sampling were issued by the Czech Nature Conservation Agency (01662/MK/2012S/00775/MK/2012, 866/JS/2012 and 00356/KK/2008/AOPK).

Results and Discussion

All bats selected for ultramicroscopic analysis exhibited pinpoint orange–yellow fluorescent foci (range = 14–455 loci per left wing; Fig. 1a) and were PCR-positive for *P. destructans*. Microscopic findings were consistent with the histopathological criteria for WNS diagnosis, with fungal infection stages from superficial colonization of skin (Fig. 1b) to tissue-invasive growth observed in sections from all bats examined. The most frequent lesions in WNS-positive sections were cup-like erosions packed with *P. destructans* hyphae locally invading the dermis (Fig. 1 c, d). Severe invasive infection spanning the full thickness of the wing membrane was found in two specimens (*M. daubentonii* and *P. auritus*; Fig. 1e,f). TEM images (Fig. 1b,d,f) provide higher resolution and finer detail than light microscopy, enabling comparison of successive *P. destructans* infection stages on the wing membrane. As shown in Fig. 1b, fungal hyphae penetrate and separate *stratum corneum* layers on both sides of the wing membrane. Other layers of skin had an orderly structure with no sign of inflammation at sites of superficial skin colonization by the fungus. Figure 1 (d) documents the presence of WNS-diagnostic cupping erosions, which were also identified using light microscopy (Fig. 1c). Here, flattened layers of *stratum corneum* with some fungal hyphae become detached from the non-keratinized epidermis. Packed fungal hyphae erode

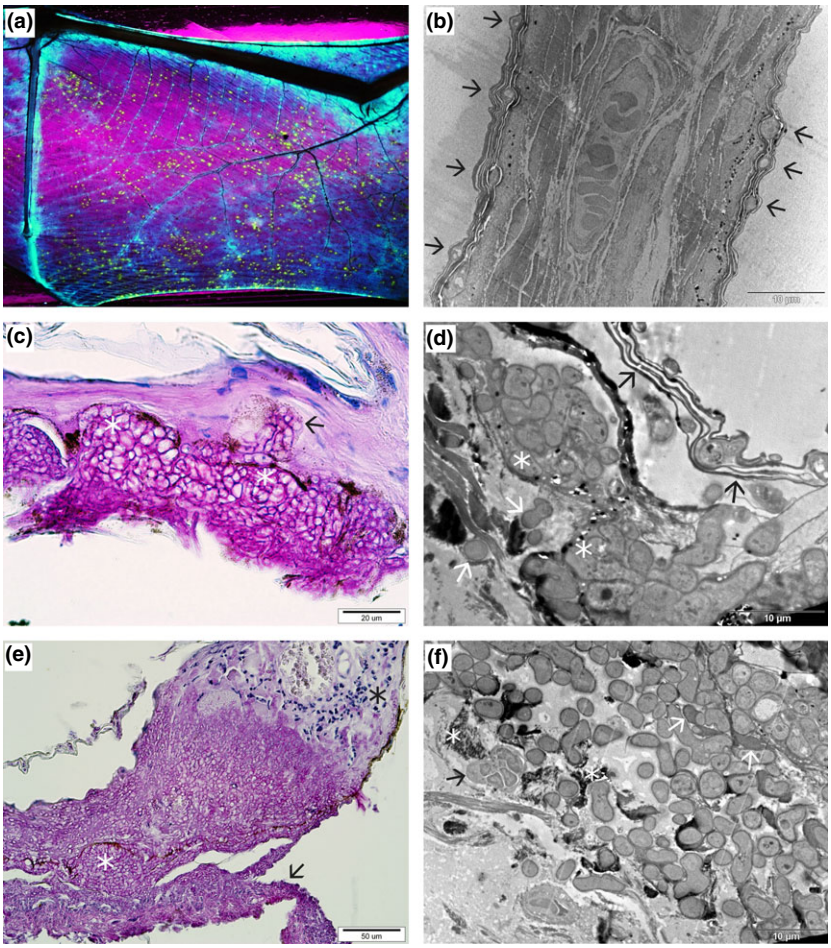


Fig. 1. Wing membrane skin lesions associated with white-nose syndrome (WNS) in European bats, illustrated using trans-illumination by ultraviolet light (a), optical microscopy (c,e) and transmission electron microscopy (b,d,f) in *Myotis myotis* (a,c,d), *Barbastella barbastellus* (b), *Plecotus auritus* (e,f). (a) A section of left wing membrane showing hundreds of pinpoint orange–yellow fluorescent foci. (b) Superficial skin colonization showing *Pseudogymnoascus destructans* hyphae penetrating between separate layers of *stratum corneum* (black arrows). The deeper skin layers remain intact. (c) Cupping erosions packed with WNS fungi (white asterisks). *P. destructans* hyphae obscure the basement membrane and invade the dermis (black arrow). (d) Layers of *stratum corneum* become detached from the epidermis (black arrows) and packed fungal hyphae erode to the epidermal/dermal interface (white asterisks). White arrows indicate dermis-invading fungal hyphae close to elastic fibres. (e) Extensive fungal infection spanning the full thickness of the wing membrane. Indicated are signs of inflammation (black asterisk), cup-like epidermal erosion (white asterisk) and cellular debris on the skin's surface (black arrow). (f) Extensive deep dermal invasion by *P. destructans* hyphae showing derangement of connective tissue structures, including fibroblasts (white arrows) and elastic fibres (white asterisks). Also illustrated is a polymorphonuclear neutrophil (black arrow).

close to the epidermal/dermal interface while individual hyphae penetrate between elastic fibres within the o*edematous dermis*. Figure 1 (f) provides a closer look at skin pathology following extensive fungal infection spanning the full thickness of the wing membrane. This induced a marked inflammatory infiltration by neutrophils at the interface between the damaged part of the wing membrane replaced by the fungus and membrane tissue not yet invaded by the pathogen (Fig. 1e). Ultramicroscopy (Fig. 1f) revealed oedema of the connective tissue and derangement of the fibroblasts and elastic fibres associated with an overwhelming invasion by *P. destructans*. Interestingly, while pathogenic action was clear in living skin layers (Fig. 1d,f), there was no sign of keratinolytic activity in the *stratum corneum* (Fig. 1b,d). This confirms a previous study that found no evidence of keratinase activity within 8 weeks of *in vitro* testing of nutritional capability and substrate suitability for *P. destructans* (Raudabaugh and Miller, 2013).

Using methods considered as the 'gold standard' for diagnosing WNS (Meteyer et al., 2009), we demonstrate that *P. destructans* is virulent for European bats under natural infection conditions. Because we do not also observe the morbidity and mortality associated with WNS in North

America, it is likely that these outcomes of the disease are the result of yet unidentified multifactorial determinants associated with the host, the agent and the environment (Blehert, 2012; Cryan et al., 2013). Our findings further support the idea that inter-continental differences in bat mortality may not be due to differences in the pathogen itself (Warnecke et al., 2012; Cryan et al., 2013). Furthermore, there is no support for the hypothesis that bat species in Europe are completely resistant to *P. destructans* infection (Warnecke et al., 2012; Wibbelt et al., 2013). Indeed, it is clear that natural fungal infection of European bats can result in severe lesions and not just a mild form restricted to the epidermis, as suggested in the latest pathological study (Wibbelt et al., 2013). The *M. myotis* we examined showed extensive skin infection (Fig. 1a), yet survived the hibernation season. Unfortunately, while there can be little doubt that such extensive WNS infection must contribute to bat morbidity, we have no data on winter torpor pattern, hibernation behaviour or alteration in homoeostasis for the specimens sampled; hence, our data cannot be compared directly with the factors underlying mortality in North America (Lorch et al., 2011; Warnecke et al., 2012, 2013).

Host–pathogen interaction can result in evolution of two types of defence mechanisms against infection (Schneider and Ayres, 2008). Apart from resistance mediated by the immune system, hosts can tolerate a pathogen′s presence by reducing its adverse effect on their fitness (Medzhitov et al., 2012). While European bats seem tolerant of wing membrane infection, skin damage has been linked to pathophysiology of WNS and mortality in North American bats (Warnecke et al., 2013). Moreover, restoration of immune responses in North American bats infected with *P. destructans* may result in post-emergent immunopathology (Meteyer et al., 2012). Host fitness parameters plotted against pathogen burden distinguish between resistance and tolerance (Medzhitov et al., 2012). Bats sampled in the present study were not severely emaciated. However, the sample size of seventeen bats from six different species is too low to allow this kind of analysis considering the fact that the body mass index of individual specimens may show great variability in dependence on foraging activities during the early post-hibernation period.

Importantly, our data supporting a lack of resistance to the infection coupled with continent-wide presence of the pathogen (Martinkova et al., 2010; Puechmaille et al., 2011), and identification of multiple European bat species positive for WNS (Zukal et al., 2014) may provide another evidence for a long history of exposure to the pathogen in Europe. To draw a parallel, chytridiomycosis has been implicated in decline of amphibian populations in geographically disparate parts of the world (Ouellet et al., 2005). Although it gained attention in the 1990s as a new emerging pathogen, there is historical evidence of the chytrid fungus infection among North American amphibians since at least 1960s. Outbreaks of lethal chytridiomycosis in amphibians have probably complex causes and are associated with predisposing factors. Likewise, museum specimens of bats collected prior to recognition of WNS may provide answer to the hypothesis that European chiropterans have long been exposed to the WNS fungus.

While we cannot reach conclusions as to which attributes allow European bat species to survive *P. destructans* infection, our findings highlight the importance of inter-continental comparative studies on susceptibility, fungal load and transmission, total affected wing/body surface area, behavioural and physiological traits, hibernation environment and interplay between fungal pathogenic mechanisms and host defences in bat species. Aside from North America, severe WNS lesions have only been reported from the Czech Republic to date. If effective risk assessments and control measures are to be put in place against *P. destructans* infection on both continents, it is imperative that we also gain a greater understanding of these issues in other European countries.

Acknowledgements

This study was supported by the Grant Agency of the Czech Republic (Project No. P506/12/1064), the Internal Grant Agency of the University of Veterinary and Pharmaceutical Sciences in Brno (Grant No. 32/2013/FVHE) and the National Speleological Society of the USA.

References

Blehert, D. S., 2012: Fungal disease and the developing story of bat white-nose syndrome. *PLoS Pathog.* 8, e1002779.

Blehert, D. S., A. C. Hicks, M. Behr, C. U. Meteyer, B. M. Berlowski-Zier, E. L. Buckles, J. T. H. Coleman, S. R. Darling, A. Gargas, R. Niver, J. C. Okoniewski, R. J. Rudd, and W. B. Stone, 2009: Bat white-nose syndrome: an emerging fungal pathogen? *Science* 323, 227.

Cryan, P., C. U. Meteyer, J. Boyles, and D. S. Blehert, 2013: White-nose syndrome in bats: illuminating the darkness. *BMC Biol.* 11, 47.

Lorch, J. M., C. U. Meteyer, M. J. Behr, J. G. Boyles, P. M. Cryan, A. C. Hicks, A. E. Ballmann, J. T. H. Coleman, D. N. Redell, D. M. Reeder, and D. S. Blehert, 2011: Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature* 480, 376–378.

Martinkova, N., P. Backor, T. Bartonicka, P. Blazkova, J. Cerveny, L. Falteisek, J. Gaisler, V. Hanzal, D. Horacek, Z. Hubalek, H. Jahelkova, M. Kolarik, L. Korytar, A. Kubatova, B. Lehotska, R. Lehotsky, R. K. Lucan, O. Majek, J. Mateju, Z. Rehak, J. Safar, P. Tajek, E. Tkadlec, M. Uhrin, J. Wagner, D. Weinfurtova, J. Zima, J. Zukal, and I. Horacek, 2010: Increasing incidence of *Geomyces destructans* fungus in

bats from the Czech Republic and Slovakia. *PLoS ONE* 5, e13853.

Medzhitov, R., D. S. Schneider, and M. P. Soares, 2012: Disease tolerance as a defense strategy. *Science* 335, 936–941.

Meteyer, C. U., E. L. Buckles, D. S. Blehert, A. C. Hicks, D. E. Green, V. Shearn-Bochsler, N. J. Thomas, A. Gargas, and M. J. Behr, 2009: Histopathologic criteria to confirm white-nose syndrome in bats. *J. Vet. Diagn. Invest.* 21, 411–414.

Meteyer, C. U., D. Barber, and J. N. Mandl, 2012: Pathology in euthermic bats with white nose syndrome suggests a natural manifestation of immune reconstitution inflammatory syndrome. *Virulence.* 3, 583–588.

Minnis, A. M., and D. L. Lindner, 2013: Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. *Fungal Biol-UK.* 117, 638–649.

Muller, L. K., J. M. Lorch, D. L. Lindner, M. O'Connor, A. Gargas, and D. S. Blehert, 2013: Bat white-nose syndrome: a real-time TaqMan polymerase chain reaction test targeting the intergenic spacer region of *Geomyces destructans*. *Mycologia* 105, 253–259.

Ouellet, M., I. Mikaelian, B. D. Pauli, J. Rodrigue, and D. M. Green, 2005: Historical evidence of widespread Chytrid infection in North American amphibian populations. *Conserv. Biol.* 19, 1431–1440.

Pikula, J., H. Bandouchova, L. Novotny, C. U. Meteyer, J. Zukal, N. R. Irwin, J. Zima, and N. Martinkova, 2012: Histopathology confirms white-nose syndrome in bats in Europe. *J. Wildl. Dis.* 48, 207–211.

Puechmaille, S. J., G. Wibbelt, V. Korn, H. Fuller, F. Forget, K. Muhldorfer, A. Kurth, W. Bogdanowicz, C. Borel, T. Bosch, T. Cherezy, M. Drebet, T. Gorfol, A. J. Haarsma, F. Herhaus, G. Hallart, M. Hammer, C. Jungmann, Y. Le Bris, L. Lutsar, M. Masing, B. Mulkens, K. Passior, M. Starrach, A. Wojtaszewski, U. Zophel, and E. C. Teeling, 2011: Pan-European distribution of white-nose syndrome fungus (*Geomyces destructans*) not associated with mass mortality. *PLoS ONE* 6, e19167.

Raudabaugh, D. B., and A. N. Miller, 2013: Nutritional capability of and substrate suitability for (*Pseudogymnoascus destructans*), the causal agent of bat white-nose syndrome. *PLoS ONE* 8, e78300.

Schneider, D. S., and J. S. Ayres, 2008: Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. *Nat. Rev. Immunol.* 8, 889–895.

Turner, G. G., C. U. Meteyer, H. Barton, J. F. Gumbs, D. M. Reeder, B. Overton, H. Bandouchova, T. Bartonička, N. Martínková, J. Pikula, J. Zukal, and D. S. Blehert, 2014: Nonlethal screening of bat wing skin using UV fluorescence to detect lesions indicative of white-nose syndrome. *J. Wildl. Dis.* 50, 566–573.

Warnecke, L., J. M. Turner, T. K. Bollinger, J. M. Lorch, V. Misra, P. M. Cryan, G. Wibbelt, D. S. Blehert, and C. K. R. Willis, 2012: Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *P. Natl. Acad. Sci. USA* 109, 6999–7003.

Warnecke, L., J. M. Turner, T. K. Bollinger, V. Misra, P. M. Cryan, D. S. Blehert, G. Wibbelt, and C. K. Willis, 2013: Pathophysiology of white-nose syndrome in bats: a mechanistic model linking wing damage to mortality. *Biol. Letters* 9, 20130177.

Wibbelt, G., S. J. Puechmaille, B. Ohlendorf, K. Mühldorfer, T. Bosch, T. Görföl, K. Passior, A. Kurth, D. Lacremans, and F. Forget, 2013: Skin lesions in European hibernating bats associated with *Geomyces destructans*, the etiologic agent of white-nose syndrome. *PLoS ONE* 8, e74105.

Zukal, J., H. Bandouchova, T. Bartonicka, H. Berkova, V. Brack, J. Brichta, M. Dolinay, K. S. Jaron, V. Kovacova, M. Kovarik, N. Martínková, K. Ondracek, Z. Rehak, G. G. Turner, and J. Pikula, 2014: White-nose syndrome fungus: a generalist pathogen of hibernating bats. *PLoS ONE* 9, e97224.

Těžké kovy a syndrom bílého nosu - potenciální hrozby pro populace letounů

Turner, G. G., Meteyer, C. U., Barton, H., Gumbs, J. F., Reeder, D. A., Overton, B., Banďouchova, H., Bartonička, T., Martínková, N., Pikula, J., Zukal, J., & Blehert, D. S. (2014). Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. Journal of Wildlife Diseases 50, 566-573.

DOI: 10.7589/2014-03-058 *Journal of Wildlife Diseases*, 50(3), 2014, pp. 566–573

NONLETHAL SCREENING OF BAT-WING SKIN WITH THE USE OF ULTRAVIOLET FLUORESCENCE TO DETECT LESIONS INDICATIVE OF WHITE-NOSE SYNDROME

Gregory G. Turner,[1] Carol Uphoff Meteyer,[2,11,12] Hazel Barton,[3] John F. Gumbs,[4] DeeAnn M. Reeder,[5] Barrie Overton,[6] Hana Bandouchova,[7] Tomáš Bartonička,[8] Natália Martínková,[9,10] Jiri Pikula,[7] Jan Zukal,[8,9] and David S. Blehert[2]

[1] Pennsylvania Game Commission, 2001 Elmerton Avenue, Harrisburg, Pennsylvania 17710, USA
[2] US Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, USA
[3] Department of Biology, University of Akron, 185 East Mill Street, Akron, Ohio 44325, USA
[4] BATS Research Center, 107 Meadow View Court, Shohola, Pennsylvania 18458, USA
[5] Department of Biology, Bucknell University, 1 Dent Drive, Lewisburg, Pennsylvania 17756, USA
[6] Department of Biology, Lock Haven University, 301 West Church Street, Lock Haven, Pennsylvania 17745, USA
[7] Department of Ecology and Diseases of Game, Fish and Bees, University of Veterinary and Pharmaceutical Sciences Brno, Palackeho 1/3, 612 42 Brno, Czech Republic
[8] Department of Botany and Zoology, Masaryk University, Kotlarska 2, 611 37 Brno, Czech Republic
[9] Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Květná 8, 603 65 Brno, Czech Republic
[10] Institute of Biostatistics and Analyses, Masaryk University, Kamenice 3, 625 00 Brno, Czech Republic
[11] Current address: US Geological Survey National Center, Environmental Health, 12201 Sunrise Valley Drive, Reston, Virginia 20192, USA
[12] Corresponding author (email: cmeteyer@usgs.gov)

ABSTRACT: Definitive diagnosis of the bat disease white-nose syndrome (WNS) requires histologic analysis to identify the cutaneous erosions caused by the fungal pathogen *Pseudogymnoascus* [formerly *Geomyces*] *destructans* (*Pd*). Gross visual inspection does not distinguish bats with or without WNS, and no nonlethal, on-site, preliminary screening methods are available for WNS in bats. We demonstrate that long-wave ultraviolet (UV) light (wavelength 366–385 nm) elicits a distinct orange–yellow fluorescence in bat-wing membranes (skin) that corresponds directly with the fungal cupping erosions in histologic sections of skin that are the current gold standard for diagnosis of WNS. Between March 2009 and April 2012, wing membranes from 168 North American bat carcasses submitted to the US Geological Survey National Wildlife Health Center were examined with the use of both UV light and histology. Comparison of these techniques showed that 98.8% of the bats with foci of orange–yellow wing fluorescence ($n=80$) were WNS-positive based on histologic diagnosis; bat wings that did not fluoresce under UV light ($n=88$) were all histologically negative for WNS lesions. Punch biopsy samples as small as 3 mm taken from areas of wing with UV fluorescence were effective for identifying lesions diagnostic for WNS by histopathology. In a nonlethal biopsy-based study of 62 bats sampled (4-mm diameter) in hibernacula of the Czech Republic during 2012, 95.5% of fluorescent ($n=22$) and 100% of nonfluorescent ($n=40$) wing samples were confirmed by histopathology to be WNS positive and negative, respectively. This evidence supports use of long-wave UV light as a nonlethal and field-applicable method to screen bats for lesions indicative of WNS. Further, UV fluorescence can be used to guide targeted, nonlethal biopsy sampling for follow-up molecular testing, fungal culture analysis, and histologic confirmation of WNS.

Key words: Bat, Chiroptera, dermatomycosis, fungal infection, *Pseudogymnoascus* (*Geomyces*) *destructans*, ultraviolet (UV) fluorescence, white-nose syndrome.

INTRODUCTION

White-nose syndrome (WNS) is caused by the psychrophilic fungus *Pseudogymnoascus* [formerly *Geomyces*] *destructans* (*Pd*) (Lorch et al. 2011; Minnis and Lindner 2013). Mortality from *Pd* infection has been confirmed for six species of North American bats, including little brown myotis (*Myotis lucifugus*), northern myotis (*Myotis septentrionalis*), Indiana myotis (*Myotis sodalis*), Eastern small-footed myotis (*Myotis leibii*), tricolored bat (*Perimyotis subflavus*), and big brown bat (*Eptesicus fuscus*) (Turner et al. 2011). *Pd* has also been isolated from bats in Europe (Puechmaille et al. 2011a), with documentation of characteristic invasive

lesions diagnostic for WNS (Pikula et al. 2012); unusual mortality has not been reported among European bats infected by *Pd* (Martínková et al. 2010; Puechmaille et al. 2011b; Sachanowicz et al. 2014).

White-nose syndrome is the first invasive cutaneous ascomycosis reported in mammals. Currently, histopathology is required to diagnose WNS (Meteyer et al. 2009). To collect an adequate sample of wing membrane (skin) to conduct a thorough histopathologic analysis, euthanasia is typically required. A rapid, field-applicable, and nonlethal technique to identify presumptive WNS would reduce the need to euthanize bats to obtain a diagnosis. Such a technique would additionally serve to enhance ability to expand diagnostic activities to assess the presence of disease in new species and additional regions of the world, and to screen bats rapidly to determine efficacy of potential mitigation strategies.

Since the historic observation in 1925 that typical fungal dermatophyte infections fluoresce under long-wave ultraviolet (UV) light, this technique has been used as aid for diagnosing keratinaceous fungal infections, including ringworm in domestic animals (Koeing and Schneckenburger 1994) and tinea capitis in humans (Margarot and Deveze 1925). Applying this technique to wing membranes of bats with suspect WNS, long-wave (366–385 nm) UV light was shown to be a rapid, reliable, and field-applicable diagnostic tool for preliminary identification of WNS in bat-wing membranes and an accurate guide for targeted, nonlethal biopsy sampling for subsequent histologic confirmation.

MATERIALS AND METHODS

Paired assessments with the use of UV illumination and histology in the laboratory

The fluorescence of bat wings in response to long-wave UV light was compared to the histologic gold standard for diagnosing WNS. Three different UV light sources were used in these studies described below; a hand-held flashlight for quick detection of fluorescence in the laboratory, a stationary Wood's lamp for photography in the laboratory, and a stationary 9-watt UV light for transillumination in the field. These light sources are described in detail below and all had wavelengths of 366–385 nm.

The wings of 168 bats of 11 species submitted to the US Geological Survey National Wildlife Health Center Madison, Wisconsin, USA (USGS NWHC) from 21 states between March 2009 and April 2012 were evaluated for fluorescence with the use of a hand-held 51-LED 385-nm UV flashlight (model 7202 UV-385 nm, LED Wholesalers, Hayward, California, USA) in a darkened room. Laboratory personnel wore UV-protective eyewear when illuminating bat wings and the same individual performed all visual assessments for fluorescence to ensure consistency. Photography was performed in a darkened room with the use of a Nikon (Tokyo, Japan) D80 digital SLR camera (F-stop 3.3, ISO 200, shutter speed 8 sec) with an AF 60 mm lens with no filter and a Wood's lamp (366 nm; BLAK-RAY Model UVL-56, San Gabriel, California, USA) mounted approximately 13 cm above the bat at a 35–40-degree angle as the sole light source to illuminate the outstretched wing from above.

After external examination, the entire membrane was removed from a wing for histologic evaluation with the use of periodic acid–Schiff stain as described by Meteyer et al. (2009). All samples were coded for impartial histologic assessment for WNS and later compared with the UV-fluorescence status. Fisher's exact test (SigmaPlot 11.0, Systat Software, Inc., San Jose, California, USA) was used to determine whether there was a relationship between fluorescence and WNS lesions.

UV fluorescence for targeted sample collection for WNS confirmation

A field study was conducted to determine if UV fluorescence could provide a preliminary diagnosis of WNS and guide nonlethal collection of wing tissue to determine WNS status by histopathology. Torpid bats were removed from roosts during surveys, captured in flight while exiting hibernacula, or found dead at hibernacula entrances. Methods and equipment used in the field for UV illumination of bat wings were the same in the US and the Czech Republic. White or UV light was used to illuminate wing membrane of bats either from above (light on the same side as the person viewing) or below (transilluminating the wing with the light source on the opposite side of viewing). A GloBox (Artograph, Delano,

Minnesota, USA) was used for white light transillumination, and a field-portable 9-watt 368-nm fluorescent light (WTC 9L-110, Way Too Cool, from Fluorescents.com [www.fluorescents.com]) was used for UV transillumination. The use of white light illumination was discontinued after the effectiveness of UV fluorescence was established. During transillumination of live bats in the field, bats were kept in the dark, placed on the working surface of the light unit with wings extended. Photographs were then taken of wings with the use of a Canon (Melville, New York, USA) EOS 350D digital SLR camera (F-stop 5–10, ISO 200, and shutter speeds 0.5–30 sec) equipped with an EFS 18–55 mm or EF 100-mm lens with 58-mm ultraviolet filter (in Pennsylvania); or a Nikon D300 digital SLR camera (F-stop 5.3–5.8, ISO 1000, and shutter speeds 0.15–0.4 sec) with AF NIKKOR 28–80-mm lens (in the Czech Republic). Cameras were mounted on a tripod (Fig. 1A). Bats were rapidly processed to reduce handling time and minimize stress. To prevent cross-contamination, field equipment was either sanitized between bats or covered with a disposable plastic sheet (Shelley et al. 2013). Dedicated "clean" equipment was used in uninfected sites to decrease risk for inadvertent introduction of a pathogen.

To characterize ability of field biologists to assess WNS-related fluorescence accurately, wings of *M. lucifugus* ($n=6$) from two Pennsylvania sites known to harbor bats with WNS were collected in 2010 and 2011, transilluminated with UV light, and multiple 1-cm^2 regions of wing membrane were outlined on each bat with permanent marker and labeled as either fluorescent ($n=14$) or nonfluorescent ($n=13$). Marked wings were then photographed during UV transillumination, and bats were euthanized by isoflurane overdose. Carcasses were shipped overnight (chilled) to the NWHC for histologic evaluation as described above.

To evaluate the effectiveness of UV transillumination-guided biopsy sampling for WNS testing, four sizes of sterile biopsy punches (McKesson, Richmond, Virginia, USA) were used. One biopsy punch of each size (3, 4, 5, and 6 mm) was used to collect areas of wing fluorescence from each of five bats providing 20 skin biopsy samples of different sizes for histopathology evaluation.

Single biopsy samples (4-mm diameter) guided by UV transillumination were collected from each of 62 live bats of six different species in the Czech Republic as they exited their hibernacula in spring 2012. Following collection, all biopsy samples were placed into individually labeled vials containing 10% neutral buffered formalin for histopathology processing.

RESULTS

The effectiveness of long-wave UV light for detection of lesions consistent with WNS was tested with the use of a combination of field and laboratory studies. Roosting bats with distinct foci of orange–yellow fluorescence could be identified when bats were illuminated from above with UV light (Fig. 1B), but this was infrequent. Wings of bats extended and illuminated from above with white light occasionally showed indistinct white fungal growth (Fig. 1C), but evidence of fungal growth or wing damage was not apparent when the wings of the bats were transilluminated with white light (Fig. 1D). However, when long-wave UV light was used to illuminate outstretched bat wings from above (Fig. 1E) or transilluminate wings from below (Fig. 1F), distinct areas of orange–yellow fluorescence were seen. Photography in the laboratory was most successful with a Wood's lamp illuminating the wing from above (Fig. 1E). When photographing live bats under field conditions, UV transillumination (as opposed to UV illumination from above) provided the most expedient and reliable approach for detecting the orange–yellow fluorescence (Fig. 1F). When white fungal growth was seen on the wings of bats illuminated from above with white light, it corresponded to the pattern of orange–yellow fluorescence seen during UV transillumination (Fig. 1C, F). Computer magnification of digital images enhanced the ability to detect isolated pinpoint areas of fluorescence.

Paired assessments with the use of UV illumination and histology in the laboratory

Of the 168 bats submitted to the NWHC for diagnostic investigation, 80 had areas of characteristic orange–yellow fluorescence when the wings were illuminated from above with a hand-held 51-LED 385-nm UV flashlight; 79 of these were histologically positive and one histologically negative for

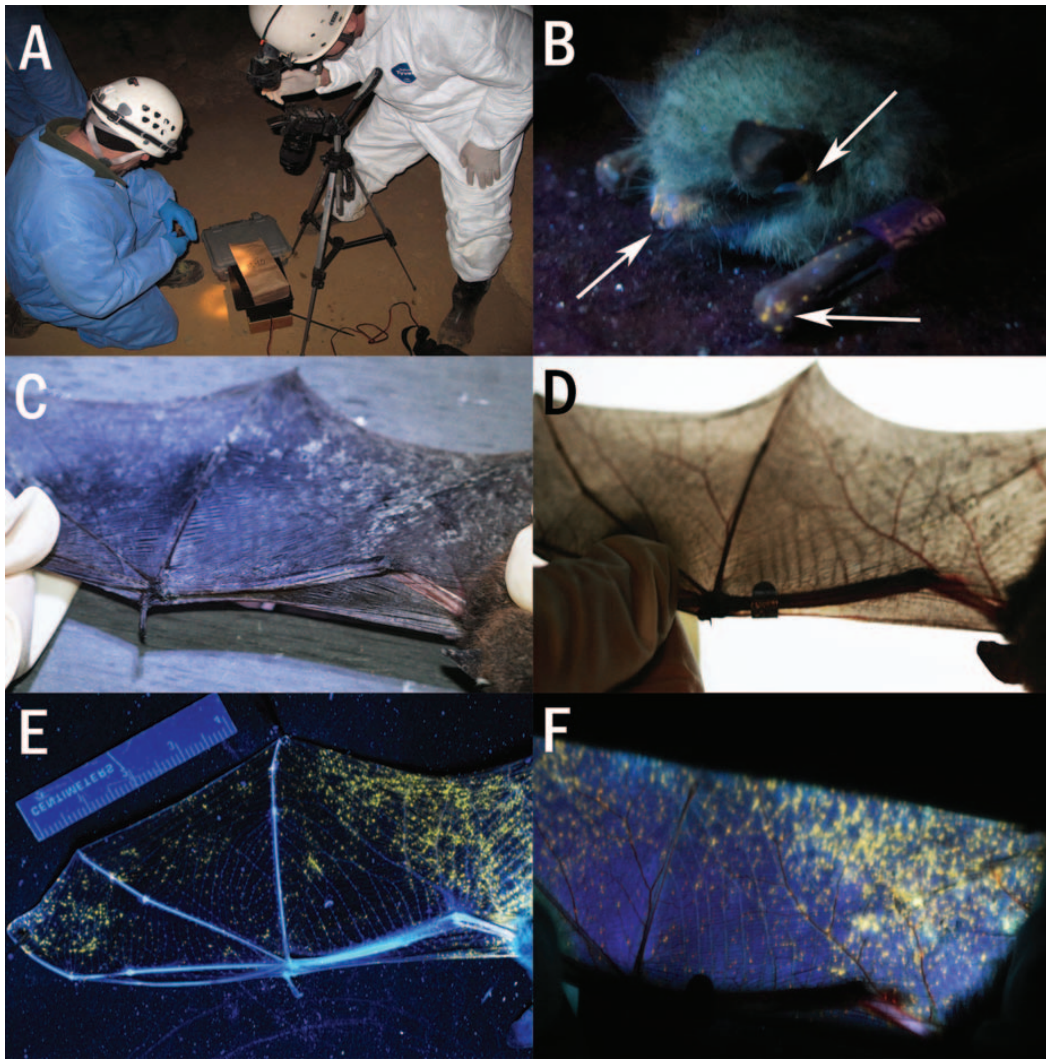


FIGURE 1. Long-wave ultraviolet (UV) and white-light illumination of lesions associated with white-nose syndrome. All photographs are from bats of the US; blurring in photos of live bats in C, D, and F is due to animal movement during long exposure. (A) Camera in cave, mounted on tripod directed at platform constructed to transilluminate bat wings with UV light (photo by Craig Stihler with permission). (B) Points of orange–yellow fluorescence (arrows) detected on a roosting Indiana myotis (*Myotis sodalis*) following surface illumination with a field-portable 9-watt 368-nm fluorescent UV light (photo by Tina Cheng with permission). (C) Wing from live little brown myotis (*Myotis lucifugus*) lit from above in cave with white light shows dispersed pattern of fungal growth. (D) White-light transillumination of wing from the live bat in C shows no obvious pattern of fungal infection or wing damage. (E) Wing from dead tricolored bat (*Perimyotis subflavus*) lit from above with hand-held 51 LED 385-nm UV flashlight shows points of orange–yellow fluorescence. (F) Transillumination of wing from live bat in C with the use of a field-portable 9-watt 368-nm fluorescent UV light. The pattern of orange–yellow fluorescence follows the distribution of surface fungal growth seen in C.

WNS (98.8% agreement between UV and histopathology assessments; Table 1). The 88 bats that were UV-fluorescence negative were all histologically negative for WNS (Table 1). There was a strong Fisher's exact test association between UV fluorescence and WNS lesions ($P<0.001$) in these 168 bats.

TABLE 1. Summary of paired ultraviolet (UV) fluorescence and histologic analyses for bats from North America and UV-targeted biopsy-based study for bats from Europe.

Bat species	Positive		Negative		Total
	Fluorescence	Histology	Fluorescence	Histology	
US (whole carcasses)					
Myotis lucifugus	59	58	40	41	99
Eptesicus fuscus	1	1	1	1	2
Myotis leibii	1	1	0	0	1
Myotis septentrionalis	5	5	7	7	12
Perimyotis subflavus	11	11	16	16	27
Myotis grisescens	0	0	7	7	7
Myotis velifer	0	0	11	11	11
Myotis sodalis	0	0	1	1	1
Myotis yumanensis	0	0	1	1	1
Myotis austroriparius	0	0	3	3	3
Tadarida brasiliensis	0	0	1	1	1
Unidentified *Myotis* sp.	3	3	0	0	3
Total	80	79	88	89	168
Czech Republic (biopsy samples)					
Myotis myotis	17	16	13	14	30
Myotis daubentonii	2	3	10	9	12
Myotis nattereri	2	2	5	5	7
Myotis bechsteinii	0	0	6	6	6
Myotis alcathoe	0	0	5	5	5
Myotis emarginatus	1	1	1	1	2
Total	22	22	40	40	62

Of the 88 bats that were UV-fluorescence negative and histologically negative, 22 had microscopic evidence of fungal colonization in the superficial keratin layer of wing skin that was morphologically distinct from WNS, and these fungi were considered to be different from *Pd*.

Use of UV fluorescence to target sample collection for WNS confirmation

Histologic examination of all 1-cm^2 targeted samples of fluorescent wing membrane collected from bats in Pennsylvania ($n=14$) were positive for the dense aggregates of fungal hyphae that form cupping erosions, which define WNS (Fig. 2A, B). When these 1-cm^2 skin samples encompassed single, pinpoint dots of fluorescence, microscopic examination identified individual fungal erosions diagnostic for WNS as small as 20–40 μm in diameter (Fig. 2B). Nine of 13 1-cm^2 regions of wing membrane marked as nonfluorescent had no cupping erosions when examined microscopically. The remaining 4 of 13 nonfluorescent samples examined microscopically had a single fungal cupping erosion (20–40-μm diameter) diagnostic for WNS. Retrospective computer magnification of the digital images taken in the field of these four fluorescence-negative bats subsequently detected scattered small pinpoint fluorescent areas that were not initially detected, suggesting that the reliable margin of accuracy in assessing unmagnified digital images may be lesions approximately 20–40 μm in diameter.

The utility of nonlethal UV-targeted biopsy sampling and biopsy size requirements was evaluated with the use of wing skin samples from bats in Pennsylvania. Biopsy samples of four diameters (3, 4, 5, and 6 mm) from each of the five bat carcasses provided adequate tissue for diagnosing cupping erosions characteristic of WNS, confirming the usefulness of this nonlethal sampling technique for biopsies as small as 3 mm in diameter.

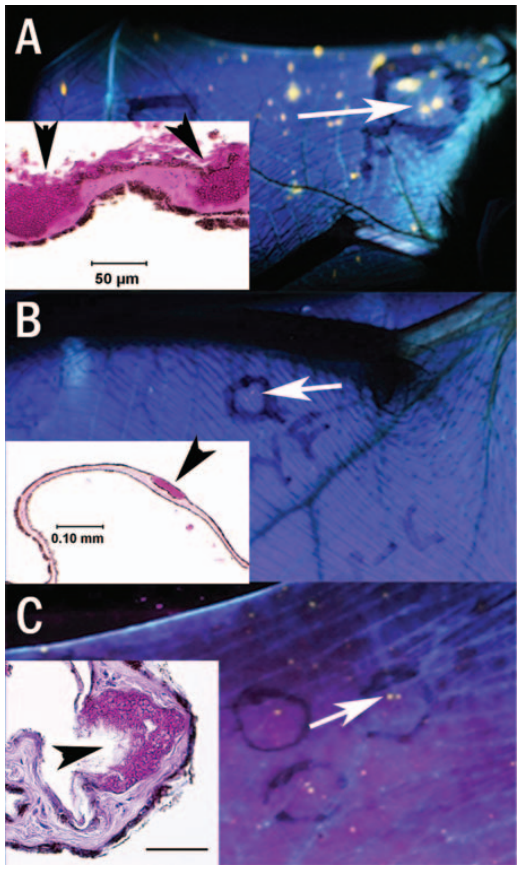


FIGURE 2. Ultraviolet fluorescence in wings of live bats (main images) and periodic acid–Schiff stained histologic sections (insets) of bat-wing skin with lesions diagnostic of white-nose syndrome; blurring in photos is due to animal movement during long exposure. (A) Black circle outlines an approximately 1-cm^2 area of wing from a little brown myotis (*Myotis lucifugus*), Pennsylvania, USA with foci of fluorescence (white arrow). Inset shows the histologic section of this 1-cm^2 area of tissue with densely packed fungal hyphae in cupping erosions (arrowheads). (B) Black circle outlines a 1-cm^2 area of wing from a little brown myotis, Pennsylvania, with a single fluorescent dot (white arrow). Inset shows the only fungal cupping erosion (arrowhead) found in the histologic section from this labeled area of wing membrane. (C) Black circles outline foci of fluorescence on the wing skin of a greater mouse-eared myotis (*M. myotis*) from the Czech Republic (white arrow). Inset (scale bar = 50 µm) shows the histologic section from a 4-mm biopsy sample taken from an area of fluorescence with densely packed fungal hyphae in cupping erosion (arrowhead).

Consistent with samples analyzed from North America, 21 of 22, 4-mm targeted biopsy samples from UV-fluorescent wing skin of bats from the Czech Republic also contained dense aggregates of fungal hyphae filling cupping erosions that are diagnostic for WNS (95.5% agreement between UV and histopathology assessments; Fig. 2C; Table 1). Retrospective review of digital images indicated that, for the histology-negative animal, the circled region of wing skin targeted for biopsy sampling had missed the point of fluorescence. For reporting purposes, however, this animal was classified as fluorescence-positive and histology negative. Additionally, a biopsy sample from 1 of 40 fluorescence-negative bats from the Czech Republic was positive for WNS by histology.

DISCUSSION

The gold standard for diagnosing bat WNS is the histologic identification of aggregates of fungal hyphae that form characteristic cupping erosions and ulceration of wing membrane (Meteyer et al. 2009). The large amount of wing membrane needed to detect these lesions histologically necessitates euthanasia of the bat. Given the detrimental effect that WNS has had on bat populations (Blehert et al. 2009; Frick et al. 2010; Turner et al. 2011), detection protocols that do not require euthanasia would be advantageous.

Illumination/transillumination of wing membranes of bats with WNS with the use of long-wavelength UV light (366–385 nm) elicited a distinct orange–yellow fluorescence that correlated with the presence of fungal cupping erosions used to diagnose WNS by histopathology (Figs. 1, 2). This correlation of fluorescence to WNS histologic lesions was observed in wings from five North American and four European species of bats (Table 1), with 98.8 and 95.5% agreement between UV and histopathology assessments for bats of North America and Europe, respectively. In addition, the 22

of 88 fluorescence negative bats that had fungi along the superficial keratin of wing skin were also histologically negative for the cupping erosions that confirm WNS. This supports our hypothesis that it is the lesion of cupping erosion, characteristic of WNS, that is fluorescing with UV light, and not superficial fungal hyphae. We thus conclude that observation of orange–yellow fluorescence following illumination/transillumination of wing membranes with UV light facilitates identification of bats with WNS. *Pd* is an ascomycete fungus, as are numerous plant pathogens. Ascomycete plant pathogens change morphologically as they penetrate the plant cuticle and the distinct subsurface hyphae release novel products related to virulence at the fungal–tissue interface (Valent and Khang 2010). A similar scenario might explain fluorescence associated with the invasive lesion of WNS and not surface hyphae. Once penetration of the epidermis occurs, *Pd* hyphae may secrete novel proteins, metabolic products, and enzymes that contribute to the erosion of living tissue and fluorescence.

Bats severely affected by WNS had numerous conspicuous large, coalescing regions of fluorescence distributed over much of the wing membrane and were readily identifiable (Fig. 1E, F). In North American bats with mild WNS (Fig. 2B), as in the WNS-positive bats in Europe (Fig. 2C), the random, sparse, and pinpoint pattern of fluorescence was more difficult to see, particularly when environmental white light was not eliminated. In addition, ability to discern sparse, subtle fluorescence often varied by observer, potentially because of factors such as inexperience with the technique, red–green color blindness, or other differences in visual acuity. Because of these difficulties, UV technique may miss individual bats with mild cases of WNS. Laboratory tests including PCR for detection of *Pd* (Muller et al. 2013), culture for *Pd* (Lorch et al. 2010), and histology to diagnose WNS (Meteyer et al. 2009) continue to play a definitive role in confirming WNS. The ability to observe sparse points of fluorescence can be enhanced by using digital photography with extended exposure time and augmentation by computer magnification of the digital images. The smallest points of fluorescence that could be visually detected with the unaided eye correlated to cupping erosions >20 μm in diameter.

In addition to the demonstrated utility of long-wave UV light as a rapid field assessment technique to obtain a preliminary diagnosis for WNS, this technique can also be used to optimize nonlethal collection of small (4-mm) biopsy samples for testing by histology, PCR, or culture. Another benefit of the enhanced accuracy afforded by UV-guided sampling is the ability to identify bats with fluorescent lesions (Fig. 1B) while limiting disturbance to nonfluorescent bats within a hibernaculum. This nonlethal assessment technique can also assist natural resource managers and researchers investigating WNS by facilitating the ability to track progression of disease in individual bats and by providing the potential, in the hands of trained field personnel, to generate accurate preliminary on-site results to inform mitigation strategies more quickly. The ability to perform targeted and nonlethal sampling of bats for WNS offers a needed tool to facilitate enhanced surveillance and research for this disease.

ACKNOWLEDGMENTS

We thank numerous state and federal biologists for submitting diagnostic specimens to NWHC, NWHC technicians for assistance with necropsies, Nathan Ramsay (NWHC) for laboratory photography, Craig Stihler (WV DNR) and Tina Cheng (UCSC) for field photography, Colleen Patterson-Pritcher, Cynthia Hauser, Lee DeWolski, and James Sinclair for assistance in the field, and Jeff Lorch (University of Wisconsin–Madison) for helpful suggestions during preparation of this manuscript. This work was supported by the US Geological Survey, the Czech Science Foundation (P506/12/1064), and the National Speleological Society. Use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US government.

Permits

In Pennsylvania, work with live bats was conducted by personnel of the Pennsylvania Game Commission in compliance with Pennsylvania Statute Title 34, Section 322, and procedures for sampling and euthanasia of bats in the US were conducted in accordance with US Geological Survey National Wildlife Health Center (NWHC) Institutional Animal Care and Use Committee Experimental Protocol 081124-A2. In the Czech Republic, live bats were sampled as they left hibernacula, and work was conducted in accordance with the Czech Academy of Sciences Ethics Committee Animal Use Protocol 169/2011 in compliance with Law 312/2008 on Protection of Animals against Cruelty adopted by the Parliament of the Czech Republic. Nonlethal sampling was in compliance with Law 114/1992 on nature and landscape protection, and was based on permits 01662/MK/2012S/00775/MK/2012, 866/JS/2012, and 00356/KK/2008/AOPK issued by the Nature Conservation Agency of the Czech Republic.

LITERATURE CITED

Blehert DS, Hicks AC, Behr MJ, Meteyer CU, Berlowski-Zier BM, Buckles EL, Coleman JTH, Darling SR, Gargas A, Niver R, et al. 2009. Bat white-nose syndrome: An emerging fungal pathogen? *Science* 323:227.

Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, Turner GG, Butchkoski CM, Kunz TH. 2010. An emerging disease causes regional population collapse of a common North American bat species. *Science* 329:679–682.

Koenig K, Schneckenburger H. 1994. Laser-induced autofluorescence for medical diagnosis. *J Fluoresc* 4:17–40.

Lorch JM, Gargas A, Meteyer CU, Berlowski-Zier BM, Green DE, Shearn-Bochsler V, Thomas NJ, Blehert DS. 2010. Rapid polymerase chain reaction diagnosis of white-nose syndrome in bats. *J Vet Diagn Invest* 22:224–230.

Lorch JM, Meteyer CU, Behr MJ, Boyles JG, Cryan PM, Hicks AC, Ballmann AE, Coleman JTH, Redell DN, Reeder DM, Blehert DS. 2011. Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature* 480:376–378.

Margarot J, Deveze P. 1925. Aspect de quelques dermatoses lumière ultraparaviolette. Note préliminaire. *Bull Soc Sci Méd Biol Montpellier* 6:375–378.

Martínková N, Bačkor P, Bartonička T, Blažková P, Červený J, Falteisek L, Gaisler J, Hanzal V, Horáček D, Hubálek Z, et al. 2010. Increasing incidence of *Geomyces destructans* fungus in bats from the Czech Republic and Slovakia. *PLoS One* 5:e13853.

Meteyer CU, Buckles EL, Blehert DS, Hicks AC, Green E, Shearn-Bochsler V, Thomas NJ, Gargas A, Behr MJ. 2009. Histopathologic criteria to confirm white-nose syndrome in bats. *J Vet Diagn Invest* 21:411–416.

Minnis AM, Lindner DL. 2013. Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. *Fungal Biol* 117:638–649.

Muller LK, Lorch JM, Lindner DL, O'Connor M, Gargas A, Blehert DS. 2013. Bat white-nose syndrome: A real-time TaqMan polymerase chain reaction test targeting the intergenic spacer region of *Geomyces destructans*. *Mycologia* 105:253–259.

Pikula J, Bandouchova H, Novotny L, Meteyer C, Zukal J, Irwin N, Zima J, Martínková N. 2012. Histopathology confirms white-nose syndrome in bats in Europe. *J Wildl Dis* 48:207–211.

Puechmaille SJ, Wibbelt G, Korn V, Fuller H, Forget F, Mühldorfer K, Kurth A, Bogdanowicz W, Borel C, Bosch T, et al. 2011a. Pan-European distribution of white-nose syndrome fungus (*Geomyces destructans*) not associated with mass mortality. *PLoS One* 6:e19167.

Puechmaille S, Frick WF, Kunz TH, Racey PA, Voigt C, Wibbelt G, Teeling EC. 2011b. White nose syndrome: Is this emerging disease a threat to European bats? *Trends Ecol Evol* 26:570–576.

Sachanowicz K, Stępień A, Ciechanowski M. 2014. Prevalence and phenology of white-nose syndrome fungus *Pseudogymnoascus destructans* in bats from Poland. *Cent Eur J Biol* 9:437–443.

Shelley V, Kaiser S, Shelley E, Williams T, Kramer M, Haman K, Keel K, Barton HA. 2013. Evaluation of strategies for the decontamination of equipment for *Geomyces destructans*, the causative agent of white-nose syndrome (WNS). *J Cave Karst Studies* 75:1–10.

Turner GG, Reeder DM, Coleman JTH. 2011. A five-year assessment of mortality and geographic spread of white-nose syndrome in North American bats, with a look at the future. *Bat Res News* 52:13–27.

Valent B, Khang CH. 2010. Recent advances in rice blast effector research. *Curr Opin Plant Biol* 13:343–344.

Submitted for publication 3 March 2014.
Accepted 28 March 2014.

Těžké kovy a syndrom bílého nosu - potenciální hrozby pro populace letounů

Zukal, J., Banďouchová, H., Bartonička, T., Berková, H., Brack, V., Brichta, J., Dolinay, M., Jaroň, K. S., Kováčová, V., Kovařík, M., Martínková, N., Ondráček, K., Řehák, Z., Turner, G. G. & Pikula, J. (2014) White-Nose Syndrome Fungus: A Generalist Pathogen of Hibernating Bats. PLoS One 9, e97224.

OPEN ACCESS Freely available online

PLOS ONE

White-Nose Syndrome Fungus: A Generalist Pathogen of Hibernating Bats

Jan Zukal[1,2], Hana Bandouchova[3], Tomas Bartonicka[2], Hana Berkova[1], Virgil Brack[4], Jiri Brichta[3], Matej Dolinay[2], Kamil S. Jaron[5], Veronika Kovacova[3], Miroslav Kovarik[6], Natália Martínková[1,5], Karel Ondracek[3], Zdenek Rehak[2], Gregory G. Turner[7], Jiri Pikula[3]*

1 Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic, **2** Department of Botany and Zoology, Masaryk University, Brno, Czech Republic, **3** Department of Ecology and Diseases of Game, Fish and Bees, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, **4** Environmental Solutions & Innovations Inc., Cincinnati, Ohio, United States of America, **5** Institute of Biostatistics and Analysis, Masaryk University, Brno, Czech Republic, **6** Administration of the Moravian Karst Protected Landscape Area, Nature Conservation Agency of the Czech Republic, Blansko, Czech Republic, **7** Pennsylvania Game Commission, Harrisburg, Pennsylvania, United States of America

Abstract

Host traits and phylogeny can determine infection risk by driving pathogen transmission and its ability to infect new hosts. Predicting such risks is critical when designing disease mitigation strategies, and especially as regards wildlife, where intensive management is often advocated or prevented by economic and/or practical reasons. We investigated *Pseudogymnoascus [Geomyces] destructans* infection, the cause of white-nose syndrome (WNS), in relation to chiropteran ecology, behaviour and phylogenetics. While this fungus has caused devastating declines in North American bat populations, there have been no apparent population changes attributable to the disease in Europe. We screened 276 bats of 15 species from hibernacula in the Czech Republic over 2012 and 2013, and provided histopathological evidence for 11 European species positive for WNS. With the exception of *Myotis myotis*, the other ten species are all new reports for WNS in Europe. Of these, *M. emarginatus, Eptesicus nilssonii, Rhinolophus hipposideros, Barbastella barbastellus* and *Plecotus auritus* are new to the list of *P. destructans*-infected bat species. While the infected species are all statistically phylogenetically related, WNS affects bats from two suborders. These are ecologically diverse and adopt a wide range of hibernating strategies. Occurrence of WNS in distantly related bat species with diverse ecology suggests that the pathogen may be a generalist and that all bats hibernating within the distribution range of *P. destructans* may be at risk of infection.

Citation: Zukal J, Bandouchova H, Bartonicka T, Berkova H, Brack V, et al. (2014) White-Nose Syndrome Fungus: A Generalist Pathogen of Hibernating Bats. PLoS ONE 9(5): e97224. doi:10.1371/journal.pone.0097224

Editor: Justin G. Boyles, Southern Illinois University, United States of America

Received January 5, 2014; **Accepted** April 16, 2014; **Published** May 12, 2014

Funding: This study was supported by the Grant Agency of the Czech Republic (Project No. P506/12/1064) and by the National Speleological Society of the USA. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: V.B. is currently affiliated with a commercial company Environmental Solutions & Innovations Inc. He provided expertise in bat ecology in this paper. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

* E-mail: pikulaj@vfu.cz

Introduction

Host-pathogen dynamics represent a balance between the pathogen's ability to infect and the host's ability to resist, with an intensive arms race between the two reflected in co-evolutionary adaptations. With host switching, pathogens temporarily escape the arms race. New, naive hosts may show lower resistance and other characteristics favourable to the pathogen. Overlapping distribution of a pathogen and its potential host(s) is key to host switching driven by opportunity [1]. The spread of emerging wildlife pathogens may have economic consequences, even in species indirectly linked to humans [2]. Fungal infections in amphibians and bats that result in population declines [3], for example, can lead to increased agricultural costs where humans chemically compensate for ecosystem services provided by these organisms in terms of insect control.

White-nose syndrome (WNS) is an emerging disease of hibernating bats associated with skin infection by *Pseudogymnoascus [Geomyces] destructans*, a recently recognised fungal pathogen [4–7]. Severe skin damage results in disruption of torpor pattern, premature depletion of fat reserves and mortality in affected bats in North America [8]. High mortality rates at affected localities and the rapid spread of the infection since 2006 continues to threaten bat diversity [9–11].

While WNS has characteristics of an epizootic, gradually expanding through North American hibernacula from its original detection site [12,13], *P. destructans* is pan-European in distribution [14,15]. Aside from seasonality in the appearance of white fungal growth [15], detailed spatio-temporal data for *P. destructans* infection in Europe are lacking. Fortunately, mass mortality has not been observed in European bats to date [14].

WNS can be transmitted either directly through bat-to-bat contact or indirectly through contact with pathogen propagules in the environment [6,16] and the infection's spread is assumed to be both density- and frequency-dependent [10]. Multiple factors, such as hibernation in large assemblages or length of hibernation season, play a role in the epidemiology of this fungal disease and ecological and behavioural characteristics of bat species may affect the risk of infection [3]. Traits such as selection of hibernaculum roost sites with differing microclimatic conditions and solitary

versus gregarious hibernation behaviour may also influence the impact of WNS [10]. Other risk factors associated with hibernating bat mortality in North America include distance from the first WNS-affected site, cluster size, species diversity and composition and type of hibernaculum [13].

Prior to 2012, bat species positive for *P. destructans* in North America included *Myotis austroriparius, M. grisescens, M. leibii, M. lucifugus, M. septentrionalis, M. sodalis, M. velifer, Perimyotis subflavus* and *Eptesicus fuscus* [11,16,17]. Dermatohistopathology has revealed fungal infection with cupping erosions and skin invasion diagnostic for WNS in a *M. myotis* specimen hibernating in the Moravian Karst, Czech Republic [18] and eight species (*M. myotis, M. oxygnathus/M. blythii, M. brandtii, M. daubentonii, M. dasycneme, M. mystacinus, M. nattereri* and *M. bechsteinii*) have been reported positive for the WNS fungus in Europe based on direct microscopy of characteristic *P. destructans* conidia, fungal culture and genetic analysis [14,15,19–22]. Photographic evidence of fungal growth suggests that *M. emarginatus, E. nilssonii* and *Rhinolophus hipposideros* may also prove positive for *P. destructans* [14].

In summary, a total of 17 vespertilionid bat species had been reported positive for the WNS fungus in North America and Europe prior to 2012 and, as the epizootic spreads through North America and surveillance continues in Europe, it is expected that the number of infected species will increase. Hereinafter, the term *P. destructans*-infected or -positive relates to those species for which the fungal pathogen has been confirmed by laboratory methods such as fungal culture and genetic analysis. WNS-positive represents those species where the infection has been diagnosed through characteristic histopathological lesions, such as fungal hyphae densely packed in so-called cupping erosions and/or invasion of the dermis [23].

Little is known about *P. destructans* infection in European bat species less abundant or less commonly observed in hibernacula. Knowledge of pathological effects associated with the WNS fungus in European bat species is even poorer. While it is a commonly held view that European bats are more resistant or resilient than those in North America [17], our monitoring revealed three further species positive for *P. destructans* infection in 2012. Differences in their hibernation behaviour and taxonomy inspired us to examine the ecological and behavioural traits and phylogeny of European and North American species reported positive for *P. destructans* in order to identify any similarities in behaviour and habitat use and to identify any other species that may be at risk.

First, we examined the hypothesis that more bat species are positive for WNS in Europe than currently reported via histopathology, considered as the 'gold standard' for diagnosing WNS [23,24]. Second, we hypothesised that ecology, behaviour and phylogenetic relationships of hibernating bat species influence risk of infection by *P. destructans*. Aside from ecological similarities, those species most often found positive for *P. destructans* and WNS belong to the genus *Myotis*, indicating that phylogenetic relatedness of hosts may facilitate invasion by the fungus. To test this, we compared the ecological and behavioural traits of hibernating bats from Europe and North America. We grouped species with similar behaviour and habitat use and used confirmed positive species to propose possible additional species susceptible to infection. We constructed a phylogeny of vespertilionids and rhinolophids from Europe and North America, to test the hypothesis that infected bats are phylogenetically closely related. Finally, we screened species of unknown infection status in Czech hibernacula to test the validity of our models and predictions.

Here, we provide histopathological evidence of multiple European species positive for WNS. We found that infected bat species are ecologically diverse, utilising a range of hibernating and feeding strategies. Although bat species previously described as being *P. destructans*-positive have been phylogenetically related, the pattern begins to break down with the newly diagnosed taxa; the data presented herein demonstrating that the host range for this fungal pathogen is more diverse than previously realized.

Materials and Methods

Ethics statement

The Czech Academy of Sciences' Ethics Committee has reviewed and approved Animal Use Protocol No. 169/2011 in compliance with Law No. 312/2008 on Protection of Animals against Cruelty, as adopted by the Parliament of the Czech Republic. Bats were monitored for WNS and presence of the causative agent *P. destructans* in the spring of 2012 and 2013 in caves of the Moravian Karst, mines near Mala Moravka in the Jeseniky Mountains, and in the Podyji National Park, all in the Czech Republic. Non-lethal sampling was in compliance with Law No. 114/1992 on Nature and Landscape Protection and was based on permits 01662/MK/2012S/00775/MK/2012, 866/JS/2012 and 00356/KK/2008/AOPK issued by the Nature Conservation Agency of the Czech Republic. Bats were handled so as to minimise stress and duration of sampling procedures between capture and release. Numbered aluminium rings were attached around the forearm for long-term identification prior to release at the site.

Screening bat species in Czech hibernacula for *P. destructans* infection

When screening bats for WNS and *P. destructans* we 1. captured bats emerging from hibernacula at the end of the hibernation season using mist nets, 2. swabbed the wing membrane for fungal culture using the Fungi-Quick transport system (Copan Innovation, Italy), 3. briefly illuminated the bats with a flashlight to detect any visible fungal growth, 4. trans-illuminated the wing membrane using ultraviolet light (UV; wavelength 368 nm) to detect any WNS lesions, 5. photographed wing membranes of each bat under both visible and UV light, 6. took a wing punch biopsy from all WNS-suspected skin lesions (i.e. areas of orange-yellow fluorescence) using a sterile and disposable 4 mm skin biopsy punch (Kruuse, Denmark), 7. used polymerase chain reaction (PCR) to confirm *P. destructans* from fungal cultures or skin swabs using the FLOQSwabs system (CopanFlock Technologies, Italy), and 8. undertook complete histopathological examinations of skin samples.

A total of 276 bats were screened for WNS and *P. destructans* and 123 skin biopsies were taken for histological examination from 15 bat species (Table 1).

Formalin-fixed punch biopsy samples were embedded in paraffin and serial 5 μm tissue sections were prepared and stained for fungi with periodic acid–Schiff stain. Histopathological findings were classified as WNS based on previously described diagnostic criteria [23]. Samples collected to cultivate fungi were transferred onto Petri dishes containing Sabouraud agar, sealed with parafilm, inverted and incubated in the dark at 10 °C. Pure fungal cultures were established from fungal growth developing at 14 days or later. *Pseudogymnoascus destructans* was confirmed through characteristic asymmetrically curved conidia via direct microscopy [5]. Fungal isolates or skin swabs were further identified using PCR and follow-up sequencing of amplicons [25] and real-time PCR [24]. A pure culture of *P. destructans* isolate grown at 10 °C on Sabouraud agar and genetically confirmed (EMBL-Bank accession number: HE588133; [18]) served as a PCR control.

Table 1. Bats examined for white-nose syndrome and *Pseudogymnoascus destructans* infection in Czech hibernacula (Europe).

Species	Screened	Biopsied	Histo+	WNS prevalence (%)	St. error
Myotis myotis[a]	67	56	37	55.22	6.08
Myotis daubentonii[b]	25	13	4	16.00	5.76
Myotis bechsteinii[b]	21	7	2	9.52	6.78
Myotis nattereri[b]	20	8	3	15.00	7.10
Myotis brandtii[b]	17	1	1	5.88	8.23
Myotis alcathoe[b]	8	7	0	0	15.94
Myotis emarginatus[b]	39	7	5	12.82	5.35
Rhinolophus hipposideros[b]	28	5	1	3.57	5.18
Eptesicus nilssonii[b]	4	1	1	25.00	26.89
Plecotus auritus[c]	23	11	5	21.73	8.60
Barbastella barbastellus[c]	17	3	3	17.64	8.24
Plecotus austriacus	3	1	0	0	32.22
Eptesicus serotinus	2	1	0	0	39.61
Pipistrellus pipistrellus	1	1	0	0	48.47
Myotis dasycneme[b]	1	1	1	100	48.47
Total	**276**	**123**	**63**	**22.82**	**2.53**

[a] = species reported positive for WNS fungus prior to 2012, [b] = species recognised as positive in 2012, [c] = bat species recognised as positive in 2013.
Screened = numbers of bats captured and examined using UV light trans-illumination to detect WNS lesions, biopsied = numbers of bats biopsied due to WNS-suspected skin lesions viewed under UV light, histo+ = specimens positive for WNS diagnostic features under histopathological examination (i.e. cupping erosions and fungal invasion of dermis), WNS prevalence = percentage of bats positive on histopathology out of the total number screened.
doi:10.1371/journal.pone.0097224.t001

Analysis of European and North American bat ecological traits

The list of European and North American bat species was prepared according to Simmons [26]; those species included in the study being those with complete or partial distribution in continental Europe or North America for which data are available. In total, we reviewed ecological and behavioural variables for 87 species. Of these, 47 were assessed for all variables and were subjected to ecological modelling analysis.

Eleven traits were chosen to describe bat species: 1. Infection status (*P. destructans*-positive/*P. destructans*-negative), 2. Cave or non-cave hibernation, 3. Region (Palearctic/Nearctic distribution), 4. Clustering during hibernation (clustering/non-clustering; i.e. hibernating in groups where multiple individuals touch), 5. Temperature preference (thermophilic/cryophilic; mainly according to Webb *et al.* [27]), 6. Preferred roost type during hibernation (exposed/hidden/both), 7. Size of clusters during hibernation (no clustering/small clusters $<$ 50 bats/medium clusters of 51 to 500 bats/large clusters $>$ 500 bats), 8. Distribution range (very large area/large area of approximately half a continent/moderate size/small area/very small area; according to Horáček *et al.* [28]), 9. Food (dominant insect food group represented by Diptera/Lepidoptera/Coleoptera/generalists), 10. Foraging habitat (open/edge/closed), 11. Body size (small - up to 5 g/medium - 5 to10 g/large - over 10 g).

These traits, which were assessed based on a primary literature review and expert evaluation, were chosen as those most likely to influence susceptibility of bats to WNS, the spread of *P. destructans* infection or survival rate during hibernation [10]. As the disease can inflict long-term damage to affected bats surviving the hibernation season, other medically relevant factors may also influence survival and reproduction in the active season [29]. Categorical variables were coded as $n - 1$ binary, dummy variables, where n is the number of categories. Data for the traits of each bat species are provided in Table S1. Grouping of ecologically similar species was performed via neighbour-joining clustering of squared Euclidean distances using ape in R language [30,31].

Phylogenetic reconstruction of European and North American bats

The phylogeny of bats from Europe and North America was extracted from a maximum likelihood phylogenetic tree using Phylocom version 4.2 [32]. The complete phylogeny of the Vespertilionidae, Miniopteridae and Cistugidae families (from which the tree used here was pruned) was reconstructed from a concatenated DNA sequence matrix of 13 mitochondrial and nuclear genes with 64% of missing data (Table S2). Three *Rhinolophus* species were used as an outgroup. Phylogeny was reconstructed using the partitioning scheme suggested by the greedy algorithm, utilising the Bayesian Information Criterion assessment in PartitionFinder [33]. The tree space was searched using maximum likelihood analysis with automatic majority-rule bootstopping option [34]. By extracting the target species' phylogeny from a comprehensive tree, we were able to obtain a phylogeny that exploits currently available diversity to optimise relationships and branch lengths, and thus mitigate possible analysis artefacts.

Statistical analysis and hypothesis testing of *P. destructans* infection occurrence

We explored the distribution pattern of *P. destructans* infection on a tree based on bat trait variation and molecular phylogeny. Occurrence of *P. destructans* infection represents a presence/absence variable, rather than a continuous trait, meaning that it is suitable for community structure analysis. The phylogenetic signal for explanatory variables and for *P. destructans* infection was calculated in Phylocom using the comstruct function. In order to

assess relatedness of species that share a specific characteristic, mean phylogenetic distance (MPD) and mean nearest phylogenetic taxon distance (MNTD) were compared to the null model, which assumes random dispersal of the trait on the tree. MPD measures the mean branch length between two randomly selected taxa from a sample, and is calculated as the sum of branch lengths to the node representing their most recent common ancestor. MNTD is the mean branch length between a taxon within the sample and its nearest relative. The null model randomised samples across phylogeny in 9,999 replicates. The distribution of the trait on a tree is clustered if values of MPD and MNTD obtained are higher than 95% of values obtained from the null samples standardised by the standard deviation of the null samples. The comparison is expressed as net relatedness index (NRI) and nearest taxon index (NTI) greater than zero [32]. Clustered distribution of a trait or phylogenetic signal means that species that share the trait are more closely related to one another than to a random taxon sampled from the tree.

Species' ecological and behavioural characteristics often show a heritable component such that close relatives have similar traits [35]. Such characteristics might then be adaptive and their evolution further decoupled from the assumption of sample independence needed for general statistical approaches. The evolutionary relationships of traits in our dataset were removed from comparisons by using phylogenetic generalised least squares (PGLS) in the Caper package of R [36]. We used a variance-covariance matrix calculated from the phylogeny with branch lengths transformed according to the Ornstein-Uhlenbeck model in geiger [37]. The PGLS model was developed via a step-down procedure, using the Akaike Information Criterion (AIC) to compare alternative models.

Results

Screening species with unknown infection status in Czech hibernacula

We tested a broad diversity of European hibernating bats for *P. destructans* infection and skin lesions pathognomonic for WNS. Analysis of 123 skin biopsy samples collected in 2012 and 2013 revealed histopathological findings matching criteria used for diagnosis of WNS in 63 bats (22.82% prevalence; Table 1) of 11 species, i.e. *M. myotis*, *M. daubentonii*, *M. bechsteinii*, *M. nattereri*, *M. brandtii*, *M. emarginatus*, *M. dasycneme*, *E. nilssonii*, *R. hipposideros*, *B. barbastellus* and *P. auritus* (Figure 1). With the exception of *M. myotis*, the other ten species are all new reports of WNS in Europe. Of these, *M. emarginatus*, *E. nilssonii*, *R. hipposideros*, *B. barbastellus* and *P. auritus* are new to the list of *P. destructans*-infected bat species. Fungus isolates or skin swabs from histopathologically positive bats were identified as *P. destructans* using PCR.

Risk of *P. destructans* infection in bat species of unknown infection status

Testing the hypothesis of phylogenetic relatedness. *P. destructans*-infected species were clustered together by molecular phylogeny (MPD = 0.212, NRI = 2.913, $p < 0.001$), meaning that pairs of infected species were, on average, more closely related than random species pairs from Europe and North America. When sister species or nearest relatives were considered, however, our results indicated that infection of both, either or neither was random (MNTD = 0.111, NTI = 1.556, $p = 0.06$; Table 2, Figure 2). Nine explanatory variables showed NRI and/or NTI not equal to zero, indicating that phylogenetic comparative methods were needed due to relatedness of taxa with shared traits (Table 2).

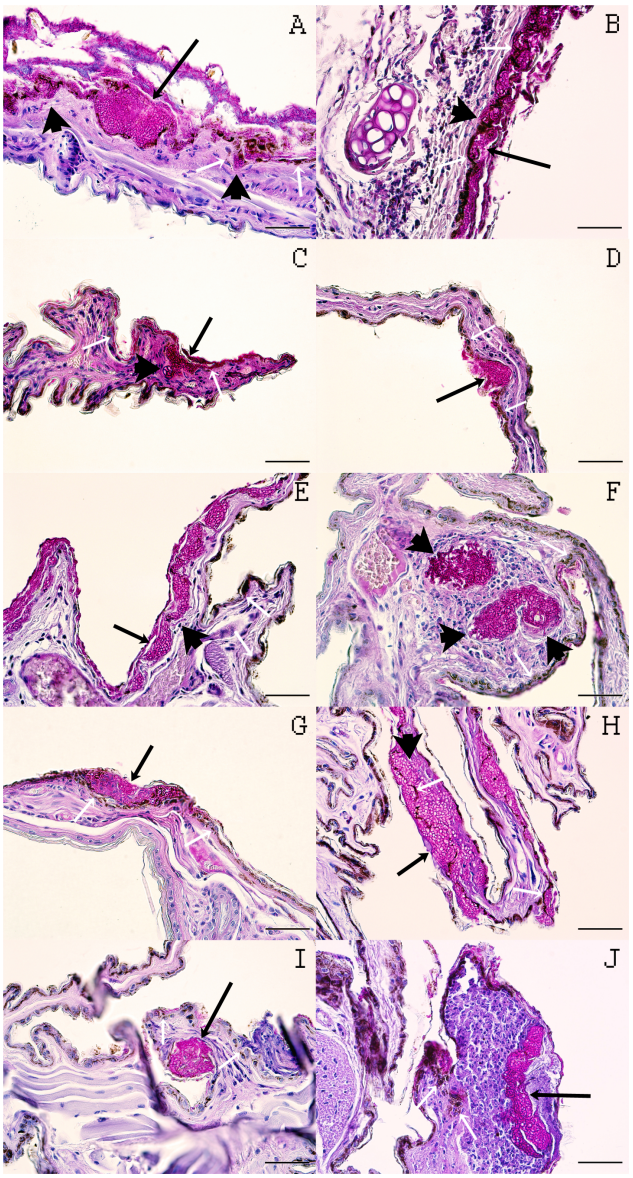


Figure 1. Histopathological skin lesions consistent with white-nose syndrome in ten European bat species. (A) *Myotis emarginatus*, (B) *Eptesicus nilssonii*, (C) *Rhinolophus hipposideros*, (D) *Plecotus auritus*, (E) *Barbastella barbastellus*, (F) *M. dasycneme*, (G) *M. nattereri*, (H) *M. daubentonii*, (I) *M. bechsteinii*, (J) *M. brandtii*. The photographs illustrate i) extensive infection of the wing membrane and cup-shaped epidermal erosions (A, E, H, J; long black arrow); ii) cup-like epidermal erosions in the pinna (B; long black arrow), iii) *Pseudogymnoascus destructans* hyphae obscuring the basement membrane and invading the dermis (A, B, C, E, H; black arrow); iv) a single cupping erosion packed with fungal hyphae in the wing membrane (C, D, G, I; long black arrow); v) colonisation of a hair follicle by *P. destructans*, fungal hyphae present in the associated sebaceous gland and regional connective tissue (F; black arrow); vi) marked signs of inflammation (B, F, J); and vii) a cellular inflammatory crust that sequesters fungal hyphae (A, J). White arrows within each photograph indicate the interface between epidermis and dermis. Periodic acid-Schiff stain; scale bar = 50 μm. *M. myotis* not shown because WNS lesions in this species have already been documented elsewhere [18].
doi:10.1371/journal.pone.0097224.g001

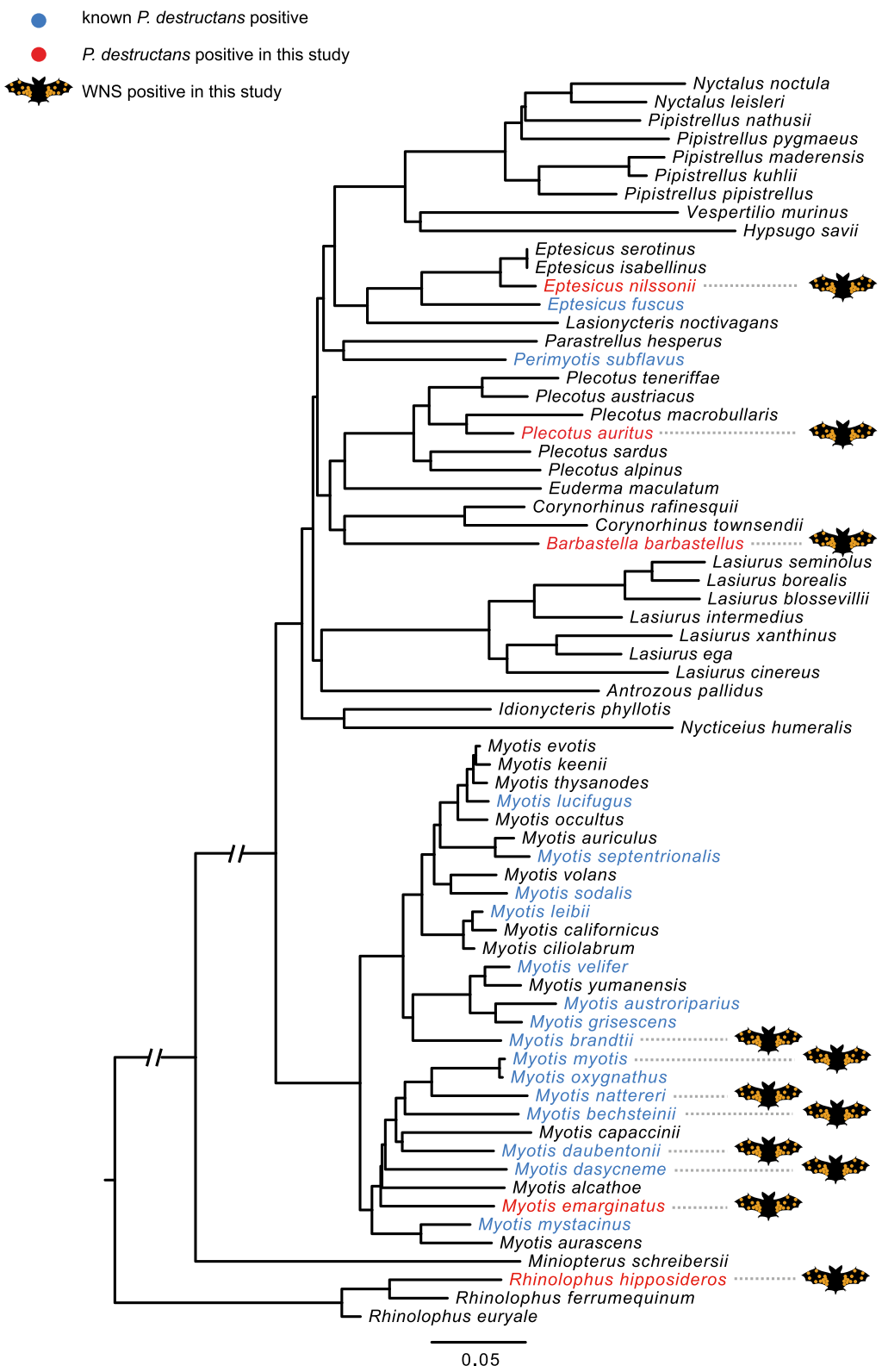


Figure 2. Phylogenetic reconstruction of bats from Europe and North America. The reconstruction was based on a concatenated DNA sequence matrix from 13 loci, purged from a maximum likelihood vespertilionid phylogeny rooted on *Rhinolophus*. Blue = species reported positive for WNS fungus prior to 2012, red = species recognised positive in this study, bat image = bat species diagnosed as WNS positive in this study.
doi:10.1371/journal.pone.0097224.g002

Table 2. *Pseudogymnoascus destructans* infection in relation to chiropteran phylogeny and ecological similarity.

Variable	*N*	MPD	NRI	p_{NRI}	MNTD	NTI	p_{NTI}
Explanatory							
CAVE	42	0.270	1.300	0.105	**0.091**	**1.984**	**0.025**
REGION	23	0.332	−1.606	0.944	0.114	1.125	0.135
CLUSTER	27	**0.241**	**2.151**	**0.012**	0.109	1.180	0.124
TEMPERATURE	21	0.318	−1.020	0.841	0.128	0.569	0.292
SHELTERhidden	31	**0.246**	**2.147**	**0.013**	0.121	0.115	0.450
SHELTERexposed	21	0.316	−0.929	0.814	0.119	1.004	0.165
SHELTERboth[a]	5	0.224	1.051	0.106	0.170	0.754	0.229
CSIZEno	20	0.337	−1.639	0.947	0.125	0.736	0.240
CSIZEsmall	7	**0.126**	**3.025**	**0.001**	**0.082**	**2.474**	**0.004**
CSIZEmedium	9	0.280	0.201	0.474	0.162	0.404	0.358
CSIZElarge	11	0.265	0.592	0.306	0.166	0.064	0.488
RANGEverylarge	15	0.333	−1.217	0.875	0.188	−1.328	0.902
RANGElarge	13	0.311	−0.565	0.708	**0.213**	**−1.872**	**0.970**
RANGEmoderate	14	0.246	1.189	0.112	0.133	0.859	0.203
RANGEsmall	5	**0.098**	**2.865**	**0.001**	**0.059**	**2.675**	**0.001**
FOODcolleoptera	5	0.258	0.482	0.330	0.169	0.706	0.249
FOODdiptera	9	0.237	1.093	0.117	0.144	0.902	0.188
FOODgeneralist	18	0.313	−0.763	0.770	0.128	0.772	0.232
FOODlepidoptera	14	0.286	0.117	0.475	0.147	0.355	0.368
FOODother	1	n/a					
HABITATclosed	11	0.280	0.228	0.458	0.142	0.802	0.220
HABITATopen	13	0.314	−0.623	0.724	0.178	−0.652	0.739
HABITATedge	23	0.267	0.855	0.211	**0.093**	**2.321**	**0.007**
BODYsmall	10	0.292	−0.097	0.576	0.142	0.855	0.204
BODYmedium	21	**0.211**	**2.829**	**0.001**	**0.105**	**1.698**	**0.048**
BODYlarge	16	**0.368**	**−2.259**	**0.989**	0.149	0.070	0.475
Response							
Pd+ on phylogeny (Figure 2)	20	**0.212**	**2.914**	**0.001**	0.111	1.555	0.058
Pd+ on 'eco' tree (Figure 3)	20	7.839	0.862	0.201	4.356	0.277	0.384

[a] = species using both types of shelters are also included in the previous categories.

Phylogenetic signal of explanatory variables on a phylogeny of bats from Europe and North America and of *P. destructans* infection on both phylogeny and a neighbour-joining tree based on squared Euclidean distances of ecological and behavioural traits of hibernating bat species. Values in bold indicate significant clustering or over-dispersion of *P. destructans* infection on the tree. Note that all categories of explanatory variables were tested here, but *n* - 1 dummy variables were included in the PGLS model. *N* = number of species scored positive for the given variable, MPD = mean phylogenetic distance, NRI = net relatedness index, MNTD = mean nearest taxon phylogenetic distance, NTI = nearest taxon index.

doi:10.1371/journal.pone.0097224.t002

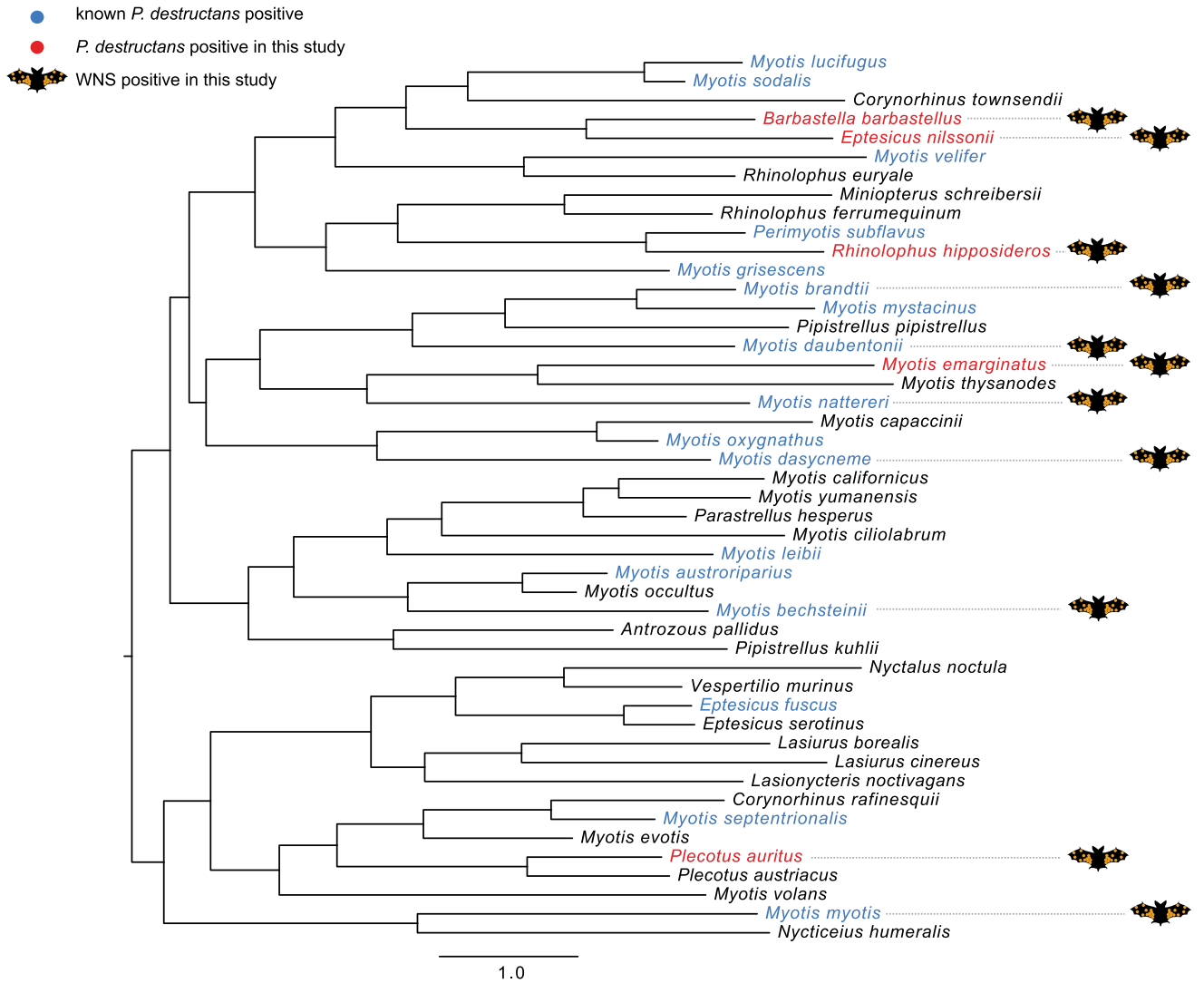


Figure 3. Neighbour-joining tree based on ecological and behavioural traits of bats from Europe and North America (rooted at midpoint). See Figure 2 for a description of the colour scheme.
doi:10.1371/journal.pone.0097224.g003

Testing the hypothesis of ecological similarity. The ecological similarity tree for European and North America bats was constructed using the squared Euclidean distances of the traits dataset (Figure 3). Analysis of *P. destructans*-infected species distribution indicated that infected species were randomly distributed (MPD = 7.839, NRI = 0.862, $p = 0.201$) across the ecological diversity of bats from these two continents. The most ecologically similar species were also infected randomly (MNTD = 4.356, NTI = 0.277, $p = 0.384$; Table 2, Figure 3).

Predicting species at risk from *P. destructans* infection. We explored relationships between ecological traits after removing the effects of bat species relatedness using PGLS. The final model, displaying lowest AIC (AIC = 69.08, *F*-statistic = 8.98, df = 7 and 39, $p < 0.001$, adjusted $R^2 = 0.55$), differed from two more complex models by $\Delta AIC < 3$ (Table S3). The addition of the variables did not markedly alter results of the analysis as reported below, and therefore we used the model with the lowest AIC. It describes the relationship between *P. destructans* infection in bat species and Temperature preference during hibernation ($\beta = -0.207$, SE = 0.085); Roost Shelter during hibernation: Hidden ($\beta = -0.109$, SE = 0.211), Exposed ($\beta = 0.560$, SE = 0.192); Cluster Size during hibernation: Small ($\beta = -0.386$, SE = 0.130), Medium ($\beta = -0.309$, SE = 0.194); Distribution range size: Moderate ($\beta = -0.201$, SE = 0.089); and Feeding habitat: Closed ($\beta = 0.319$, SE = 0.113). Shapiro-Wilks' normality test indicated that model residuals were normally distributed (W = 0.981, $p = 0.63$).

The fitted values of *P. destructans* infection in bats based on the PGLS model showed overlap between the *P. destructans*-positive and -negative bats (Figure 4). Bat species currently recognised as *P. destructans*-negative with highest fitted PGLS values were (in descending order): *Corynorhinus townsendii*, *Lasiurus cinereus*, *Plecotus austriacus*, *Rhinolophus ferrumequinum*, and *Miniopterus schreibersii*.

Discussion

At the end of the hibernation seasons of 2012 and 2013, we screened 276 bats of unknown infection status and biopsied all bats with WNS-suspected skin lesions. Both the number of bats and the number of taxa ($n = 15$) examined make this the most extensive

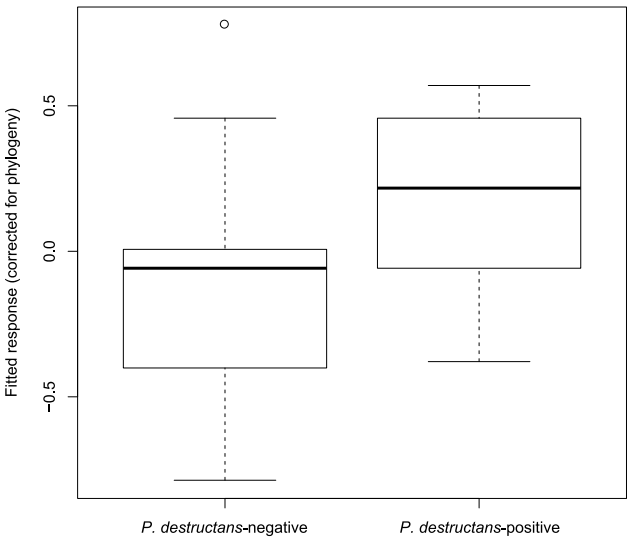


Figure 4. Boxplot of fitted values from the phylogenetic generalised least squares model of *P. destructans* infection. Predictions for infected and non-infected species overlap.
doi:10.1371/journal.pone.0097224.g004

and species-rich study of *P. destructans* infection in Europe to date. Earlier European studies have provided data from bats originally sampled for fungal microscopy, culture and genetic analysis when they exhibited obvious fungal growth during hibernation, numbers of species examined ranging from 1 to 12 and numbers of specimens from 1 to 107 [14,15,19–22].

This study documented five additional bat species as positive for *P. destructans* infection and added ten species from the genera *Myotis*, *Eptesicus*, *Plecotus*, *Barbastella* and *Rhinolophus* to the list of European bat species showing histopathological findings consistent with WNS [23]. Species-specific prevalence of WNS-diagnostic skin lesions ranged from 0 to 100% (4–55% for species with $n > 20$; Table 1). Highest prevalence of WNS lesions was observed in *M. myotis* (after excluding species for which just one specimen was caught, i.e. *M. dasycneme*). Note, however, that with prevalence differing by an order of magnitude, detection of positive specimens may have been biased by small sample size in rarer species and species less frequently visible in hibernacula. With our experimental design (i.e. trapping of winter survivors leaving hibernacula and non-destructive UV fluorescence screening), we were able to confirm that *M. myotis* had highest prevalence of WNS lesions (based on histopathology), with the possible exception of *M. dasycneme*. Our results confirmed WNS skin lesions in 11 bat species, which is in contrast to a previous study that found no invasive growth of *P. destructans* in European bats [38]. The latter study, however, examined a low number of bats and used skin biopsy methods lacking in sensitivity and this may explain the failure to detect lesions diagnostic of WNS.

Bat species with *P. destructans* infection exhibited diverse hibernation behaviours. Among these, *R. hipposideros* was special as it is an exclusively solitary hibernator and hangs free in exposed places with the wing membranes covering the body. It is capable of hibernation at higher temperatures but requires the microclimate stability ensured by using the inner parts of caves [39]. *Rhinolophus hipposideros* is also the most abundant species in winter-monitoring counts, amounting to more than 50% of all bats registered in Czech hibernacula (unpublished data, Czech Bat Conservation Trust). Moreover, as a member of the suborder Pteropodiformes, it is phylogenetically distantly related to all other species infected by *P. destructans* (Figure 2). As documented by the low infection prevalence (3.57%), environmentally mediated indirect and density-dependent transmission probably does not result in higher risk for this rhinolophid species [10]. Similarly, *E. fuscus* and *M. leibii*, both solitary hibernators from North America, were the least impacted species. In comparison, higher declines were observed in large winter colonies of two species that roost solitarily or in small groups, i.e. *P. subflavus* and *M. septentrionalis* [10]. Disease risk, however, was not related to conspecific transmission only. When multiple co-occurring species can host the pathogen, density-dependent transmission can be amplified [40,41].

We confirm here that bat species previously known to be positive for *P. destructans* may later show as WNS-positive based on histopathology. As the lists of bats positive for *P. destructans* or WNS lesions in North American and European species are nearly equal, conclusions drawn from analysis of infection or disease risk should be similar. Traits describing ecological and behavioural characteristics of bats occurring within the known distribution of *P. destructans* indicate that species belonging to additional genera may also be found positive for the infection in the future. Our screening of Czech underground hibernacula, however, demonstrates that the initial, relatively low, number of bat species positive for *P. destructans* infection is more likely the result of sampling bias than a biological phenomenon. Currently, affected species are ecologically diverse, to the point where predictions for infected and non-infected species overlap. We therefore assume that more species will be revealed as WNS-positive with increased sensitivity of detection methods [42].

Phylogenetic representation of *P. destructans* infection indicates that closely related species are most likely to be infected. In terms of field surveys, therefore, some *Myotis* bats are universally likely to be WNS-positive and most effort should be devoted to these species. Interestingly, *M. alcathoe* was free of WNS-positive skin lesions in the present study (but note the low sample size). *Myotis* species typically form clusters during hibernation and such behaviour promotes frequency-dependent transmission of the infection, independent of population size, and may yet drive the species to extinction [9] unless they change their social behaviour, as documented in *M. lucifugus* and *M. sodalis* [10]. In light of our new data, clustering behaviour is not a descriptor common to infected species. Rather, a suite of characteristics, including cluster size, type of shelter during hibernation, temperature at hibernation, as well as size of the distribution range and feeding in closed habitats, play a role in characterising bats with *P. destructans* infection.

Based on the list of species currently known to be affected by WNS or *P. destructans*, it is clear that the fungus is neither species-, genus- nor family-specific. The multi-host occurrence of the pathogen might make the disease less predictable using ecologically- and phylogenetically-based analysis [43]; however, this is likely to change in the future as additional species are revealed as susceptible. Hibernation in contaminated caves and mines under conditions favourable for fungal growth [5,44] appears to be the main risk factor [4,12]. Distribution of *P. destructans* is also correlated with disease in hibernating bats [45]. Importantly, 25 species of insectivorous bats presently hibernate in the United States and Canada, all of which represent possible hosts of the fungal pathogen should the disease spread to their geographic range [46]. This scenario is predicted to happen in most counties with caves in the contiguous United States by the winter of 2105-2106 [47].

The reason bats in North America have been so hard-hit, with millions dying, while bats in Europe apparently cope better with the infection, has not yet been explained. Likewise, the pathogen-

esis of WNS still remains unclear [12]. Behavioural aberrations, physiological disruption and immunosuppression during hibernation are, however, considered key pathomechanisms [4,48,49]. On the other hand, restoration of immune responses in WNS-positive bats early in post-hibernation may result in immune-mediated destruction of infected tissues and death [50].

The fact that our samples were mostly collected from bats emerging from hibernacula at the end of the hibernation season indicates that European bat species can survive *P. destructans* infection and highlights the need for a comparison of European and North American bat population responses to this fungal disease. As all European bat species are strictly protected and any thorough pathological study of *P. destructans* infection would be controversial [18,22], implementation of non-lethal sampling methods is necessary, such as the wing membrane biopsy used in this study [42]. While a detailed comparison of histopathological findings in European and North America bats represents a valid approach to the better understanding of WNS mortality [17,48], it was outside the objectives of this ecological study. We are however, planning a comprehensive study to investigate the extent of WNS wing lesions in hibernating bats from the two continents.

Conclusions

This hypothesis-driven study explored clustering of *P. destructans* infection in relation to chiropteran ecological and behavioural trait variation and phylogeny and supported this with field data. Extension of the surveillance to a broader number of species to test the study's hypotheses identified multiple European species positive for WNS. The increased number of positive bat species resulted in random dispersion of *P. destructans* infection across trait trees and weakened the pattern of phylogenetic clustering of *P. destructans* infection. Distantly related bat species, characterised by diverse life histories, were infected and all hibernating bats may, therefore, be at risk from *P. destructans* infection. Ecological and evolutionary constraints on hibernating bats do not pose a barrier to this generalist fungal pathogen, with WNS occurring in both suborders of bats. Our findings indicate that a wider focus is needed in studying the ecology and epidemiology of this fungal disease of major conservation concern.

Supporting Information

Table S1 Ecological and behavioural traits of European and North American bat species. The dataset includes the most common behavioural or trait value for each bat species.
(XLSX)

Table S2 Accession numbers for phylogeny reconstruction. A total of 13 available mitochondrial and nuclear genes were used for phylogeny reconstruction of bat species from Europe and North America.
(XLSX)

Table S3 Phylogenetic generalized least squares model selection. The model predicting *P. destructans* infection based on ecological and behavioural characteristics of bats was selected with the step-down procedure, where the full model is given on the first line and removed variables are listed subsequently.
(PDF)

Acknowledgments

The authors would like to thank Kevin Roche for his correction of the English text. Comments from Justin Boyles and two anonymous reviewers helped to improve the manuscript.

Author Contributions

Conceived and designed the experiments: JZ H. Bandouchova NM JP. Performed the experiments: H. Bandouchova JP JZ TB H. Berkova JB MD KSJ VK MK KO ZR. Analyzed the data: JZ MD NM KSJ VB GGT. Contributed reagents/materials/analysis tools: JZ H. Bandouchova NM JP GGT. Wrote the paper: JZ H. Bandouchova NM JP. Compiled the ecological and behavioural traits of bats: JZ. Reconstructed the phylogeny of European and North American bats: MD NM. Performed the statistical analysis: JZ KSJ NM. Examined the histopathological findings: H. Bandouchova JP. Collected field data: JZ H. Bandouchova TB H. Berkova JB MD KSJ VK MK KO ZR. Cultured and diagnosed the fungus: JB VK KO. Reviewed compiled data on North American bats: VB GGT. Provided a new method to detect WNS lesions: GGT.

References

1. Irwin NR, Bayerlová M, Missa O, Martínková N (2012) Complex patterns of host switching in New World arenaviruses. Mol Ecol 21: 4137–4150.
2. Boyles JG, Cryan PM, McCracken GF, Kunz TH (2011) Economic importance of bats in agriculture. Science 332: 41–42.
3. Eskew EA, Todd BD (2013) Parallels in amphibian and bat declines from pathogenic fungi. Emerg Infect Dis doi: http://dx.doi.org/10.3201/eid1903.120707.
4. Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-Zier BM, et al. (2009) Bat white-nose syndrome: An emerging fungal pathogen? Science 323: 227–227.
5. Gargas A, Trest MT, Christensen M, Volk TJ, Blehert DS (2009) *Geomyces destructans* sp nov associated with bat white-nose syndrome. Mycotaxon 108: 147–154.
6. Lorch JM, Meteyer CU, Behr MJ, Boyles JG, Cryan PM, et al. (2011) Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. Nature 480: 376–378.
7. Minnis AM, Lindner DL (2013) Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. Fungal Biol 117: 638–649.
8. Warnecke L, Turner JM, Bollinger TK, Lorch JM, Misra V, et al. (2012) Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. Proc Natl Acad Sci USA 109: 6999–7003.
9. Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, et al. (2010) An emerging disease causes regional population collapse of a common North American bat species. Science 329: 679–682.
10. Langwig KE, Frick WF, Bried JT, Hicks AC, Kunz TH (2012) Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. Ecol Lett 15: 1050–1057.
11. Turner GG, Reeder DM (2009) Update of white-nose syndrome in bats, September 2009. Bat Research News 50(3): 47–53.
12. Blehert DS (2012) Fungal disease and the developing story of bat white-nose syndrome. PLoS Pathog 8: e1002779.
13. Wilder AP, Frick WF, Langwig KE, Kunz TH (2011) Risk factors associated with mortality from white-nose syndrome among hibernating bat colonies. Biol Lett 7: 950–953.
14. Martínková N, Bačkor P, Bartonička T, Blažková P, Červený J, et al. (2010) Increasing incidence of *Geomyces destructans* fungus in bats from the Czech Republic and Slovakia. PLoS One 5: e13853.
15. Puechmaille SJ, Wibbelt G, Korn V, Fuller H, Forget F, et al. (2011) Pan-European distribution of white-nose syndrome fungus (*Geomyces destructans*) not associated with mass mortality. PLoS One 6: e19167.
16. Foley J, Clifford D, Castle K, Cryan P, Ostfeld RS (2010) Investigating and managing the rapid emergence of white-nose syndrome, a novel, fatal, infectious disease of hibernating bats. Conserv Biol 25: 223–231.
17. Puechmaille SJ, Frick WF, Kunz TH, Racey PA, Voigt CC, et al. (2011) White-nose syndrome: is this emerging disease a threat to European bats? Trends Ecol Evol 26: 570–576.
18. Pikula J, Bandouchova H, Novotny L, Meteyer CU, Zukal J, et al. (2012) Histopathology confirms white-nose syndrome in bats in Europe. J Wildl Dis 48: 207–211.
19. Kubátová A, Koukol O, Nováková A (2011) *Geomyces destructans*, phenotypic features of some Czech isolates. Czech Mycol 63: 65–75.
20. Puechmaille SJ, Verdeyroux P, Fuller H, Gouilh MA, Bekaert M, et al. (2010) White-nose syndrome fungus (*Geomyces destructans*) in bat, France. Emerg Infect Dis 16: 290–293.

21. Šimonovičová A, Pangallo D, Chovanová K, Lehotská B (2011) *Geomyces destructans* associated with bat disease WNS detected in Slovakia. *Biologia* 66: 562–564.
22. Wibbelt G, Kurth A, Hellmann D, Weishaar M, Barlow A, et al. (2010) White-nose syndrome fungus (*Geomyces destructans*) in bats, Europe. Emerg Infect Dis 16: 1237–1243.
23. Meteyer CU, Buckles EL, Blehert DS, Hicks AC, Green DE, et al. (2009) Histopathologic criteria to confirm white-nose syndrome in bats. J Vet Diagn Invest 21: 411–414.
24. Muller LK, Lorch JM, Lindner DL, O'Connor M, Gargas A, et al. (2013) Bat white-nose syndrome: a real-time TaqMan polymerase chain reaction test targeting the intergenic spacer region of *Geomyces destructans*. Mycologia 105: 253–259.
25. Lorch JM, Gargas A, Meteyer CU, Berlowski-Zier BM, Green DE, et al. (2010) Rapid polymerase chain reaction diagnosis of white-nose syndrome in bats. J Vet Diagn Invest 22: 224–230.
26. Simmons NB (2005) Chiroptera. In: Wilson DE, Reeder DM, editors. Mammal species of the world: A taxonomic and geographic reference. Smithsonian series in comparative evolutionary biology, 3rd edition. Washington DC: Smithsonian Inst. Press. pp. 312–529.
27. Webb PI, Speakman JR, Racey PA (1996) How hot is a hibernaculum? A review of the temperatures at which bats hibernate. Can J Zool 74: 761–765.
28. Horáček I, Hanák V, Gaisler J (2000) Bats of Palearctic region. In: Woloszyn BW, editor. Proceedings of the VIIIth European bat research symposium: 23–27 August 1999. Krakow, Poland: Institute of Systematics and Evolution of Animals PAS. pp. 11–157.
29. Reichard JD, Kunz TH (2009) White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*). Acta Chiropt 11: 457–464.
30. Paradis E, Claude J, Strimmer K (2004) APE: Analyses of phylogenetics and evolution in R language. Bioinformatics 20: 289–290.
31. R Core Team: R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available: http://www.R-project.org. Accessed 2013 Oct 10.
32. Webb CO, Ackerly DD, Kembel SW (2008) Phylocom: software for the analysis of phylogenetic community structure and character evolution. Bioinformatics 24: 2098–2100.
33. Lanfear R, Calcott B, Ho SYW, Guindon S (2012) PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol 29: 1695–1701.
34. Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A (2010) How many bootstrap replicates are necessary? J Comput Biol 17: 337–354.
35. Felsenstein J (1985) Phylogenies and the comparative method. Am Nat 125: 1–15.
36. Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, et al. (2013) Caper: Comparative analyses of phylogenetics and evolution in R. R package version 0.5.2. Available: http://CRAN.R-project.org/package = caper. Accessed 2014 Feb 24.
37. Harmon LJ, Weir JT, Brock CD, Glor RE, Challenger W (2008) GEIGER: investigating evolutionary radiations. Bioinformatics 24: 129–131.
38. Wibbelt G, Puechmaille SJ, Ohlendorf B, Mühldorfer K, Bosch T, et al. (2013) Skin lesions in European hibernating bats associated with *Geomyces destructans*, the etiologic agent of white-nose syndrome. PLoS ONE 8: e74105.
39. Zukal J, Berková H, Řehák Z (2005) Activity and shelter selection by *Myotis myotis* and *Rhinolophus hipposideros* hibernating in the Kateřinská cave (Czech Republic). Mamm Biol 70: 271–281.
40. Keesing F, Holt RD, Ostfeld RS (2006) Effects of species diversity on disease risk. Ecol Lett 9: 485–498.
41. Ostfeld RS, Keesing F (2012) Effects of host diversity on infectious disease. Annu Rev Ecol Evol Syst 43: 157–182.
42. Turner GG, Meteyer CU, Barton H, Gumbs JF, Reeder DM, et al. (2014) Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. J Wildl Dis, doi: 10.7589/2014-03-058.
43. Woolhouse MEJ, Taylor LH, Haydon DT (2001) Population biology of multihost pathogens. Science 292: 1109–1112.
44. Verant ML, Boyles JG, Waldrep W, Wibbelt G, Blehert DS (2012) Temperature-dependent growth of *Geomyces destructans*, the fungus that causes bat white-nose syndrome. PLoS ONE 7: e46280.
45. Lorch JM, Muller LK, Russell RE, O'Connor M, Lindner DL, et al. (2013) Distribution and environmental persistence of the causative agent of white-nose syndrome, *Geomyces destructans*, in bat hibernacula of the Eastern United States. Appl Environ Microbiol 79: 1293–1301.
46. U.S. Geological Survey (2012) White-Nose Syndrome Threatens the Survival of Hibernating Bats in North America. Available: http://www.fort.usgs.gov/WNS/Default.asp. Accessed 2014 Jan 05.
47. Maher SP, Kramer AM, Pulliam JT, Zokan MA, Bowden SE, et al. (2012) Spread of white-nose syndrome on a network regulated by geography and climate. Nat Commun 3: 1306.
48. Cryan PM, Meteyer CU, Boyles JG, Blehert DS (2010) Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. BMC Biology 8: 135.
49. Cryan PM, Meteyer CU, Blehert DS, Lorch JM, Reeder DM, et al. (2013) Electrolyte depletion in white-nose syndrome bats. J Wildl Dis 49: 398–402.
50. Meteyer CU, Barber D, Mandl JN (2012) Pathology in euthermic bats with white nose syndrome suggests a natural manifestation of immune reconstitution inflammatory syndrome. Virulence 3: 583–588.

Těžké kovy a syndrom bílého nosu - potenciální hrozby pro populace letounů

Zukal, J., Banďouchová, H. & Pikula, J. (2015) Bats as bioindicators of heavy metal pollution: history and prospect. Mammalian Biology 80, 220-227.

Mammalian Biology 80 (2015) 220–227

Contents lists available at ScienceDirect

Mammalian Biology

journal homepage: www.elsevier.com/locate/mambio

Review

Bats as bioindicators of heavy metal pollution: history and prospect

Jan Zukal[a,*], Jiri Pikula[b], Hana Bandouchova[b]

[a] Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Brno, Czech Republic
[b] University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

ARTICLE INFO

Article history:
Received 27 June 2014
Accepted 5 January 2015
Handled by Danilo Russo
Available online 15 January 2015

Keywords:
Chiroptera
Bioaccumulation
Heavy metals
Metalloids
Review

ABSTRACT

Bats today face a number of important threats, including that of heavy metal exposure. While the numerous adverse health effects of heavy metals have long been documented, exposure to heavy metal pollution continues, and is even increasing in some parts of the world. The eleven heavy metal elements of highest wildlife protection concern are arsenic, cadmium, cobalt, chromium, copper, mercury, manganese, nickel, lead, tin and thallium. This paper reviews 52 studies reporting on heavy metal concentrations in bats, their organs and guano, and aims to provide an overview of heavy metal research on wild bat populations, and particularly its temporal, geographic, methodological and biological aspects.

The published data are biased both temporally and spatially, with the greatest number of articles published over the last decade. While most studies reporting on heavy metal contamination have come from North America and Europe, these are generally restricted to one or two reports per country/state. General trend analysis of heavy metal content in bats is not possible due to variation in the data and the analysis of stratigraphically dated guano deposits provides inconsistent results. Moreover, variability in heavy metal content observed in bat bodies is influenced by background levels and a direct comparison of results between geographically distant areas is, therefore problematic. Comparison of contaminated and reference localities at a regional scale is useful and is regularly used. From a methodological point of view, the determination of heavy metal concentration in tissues may be limited by the typically small sample sizes available. Heavy metals have been analyzed in a range of matrices, with the four most sampled types (liver, kidney, whole body/carcass and guano) and the actual number of compounds analyzed gradually increasing over time as more sophisticated and precise instrumentation are developed. Non-lethal sampling methods are increasingly used for monitoring as these have minimal impact on threatened and highly protected animals. In total, heavy metal content has been studied in 65 bat species, though the species, sex, age, year of collection and locality varies widely with no clear pattern. Only four species (big brown bat *Eptesicus fuscus*, gray bat *Myotis grisescens*, greater mouse-eared bat *Myotis myotis* and common pipistrelle *Pipistrellus pipistrellus* sensu lato) have been analyzed more than five times, and only five heavy metals (cadmium, chromium, copper, lead, and zinc) have been measured in fructivorous/nectarivorous species. Insectivorous bats have lower mean contaminant values in tissues than both fructivorous/nectarivorous species and guano. While exposure pathway may have influenced differences between the various food guilds, lowered bioavailability of heavy metals from digested food displaying lower bioaccumulation factors may account for differences observed between guano and other types of samples.

While the number of articles confirming direct adverse effects and toxicity of heavy metals on bats is low some impacts and poisoning cases have been documented, including hepatopathy, DNA damage, hemochromatosis, renal inclusion bodies, ascending paralysis, and changes in cholinergic functions. Moreover, results suggest that the effects of chronic sub-lethal exposure to heavy metal contamination may be a more important threat to bat populations as bats under natural environmental conditions are frequently exposed to multiple anthropogenic stressors at the same time. One of the main challenges facing bat ecotoxicology today is the preparation of standardized monitoring programs using modern analytical technologies that offer more precise data on heavy metal contamination.

* Corresponding author. Tel.: +420 777201776.
E-mail address: zukal@ivb.cz (J. Zukal).

http://dx.doi.org/10.1016/j.mambio.2015.01.001
1616-5047/

Introduction

Bats occur on every continent except Antarctica and represent the second largest mammal order, comprising around 20% of mammal species, with the greatest diversity in the tropics (Nowak 1994). Given their wide distribution and high species richness, it is not surprising that bats face an unprecedented array of threats in the early 21st century, from traditional concerns such as habitat disturbance and loss of roost sites (*e.g.* through deforestation or building reconstruction) to pollutants, light pollution, diseases such as white nose syndrome, and collisions with wind turbines. Most of these threats are directly related to an ever increasing human population, with the greatest pressure in tropical countries.

Although the adverse health effects of heavy metals have long been documented, exposure to heavy metal pollution continues, and is even increasing in some parts of the world (Melancon, 2003; Li et al., 2014). Heavy metals occur naturally in the environment and there is always a natural background concentration in soils, rocks, sediments, water, and living organisms, with concentrations varying greatly. However, anthropogenic pollution results in higher concentrations of these metals relative to the normal background values. Emissions of heavy metals into the environment occur *via* a wide range of processes and pathways, including air pollution through combustion, extraction and processing; surface water pollution *via* runoff and releases from storage and transport; into soils and consequently into ground waters, insects and crops (Clark, 1981).

There is no clear definition of what a heavy metal is and, in most cases, elemental density is taken to be the defining factor. Heavy metals are thus commonly defined as "elements having a specific density of more than 5 g/cm^3" (Järup, 2003). Eleven elements are recognized as being of greatest wildlife concern: arsenic, cadmium, cobalt, chromium, copper, mercury, manganese, nickel, lead, tin and thallium (Beyersmann and Hartwig, 2008). The heavier metals, such as lead, mercury, arsenic and cadmium, are amongst the most hazardous; however, even light metals such as aluminum and selenium can be toxic to living organisms at higher concentrations. Some heavy metals play no physiological role in living organisms, and these can be toxic at low concentrations; others are essential elements, yet can be toxic at elevated concentrations. Further, while all heavy metals are potentially hazardous to health, local environmental conditions, such as alkalinity, pH, or water hardness, can affect both the bioavailability and toxicity of the elements (Laskowski et al., 1995). In all living organisms, heavy metal ion levels tend to be strictly controlled at the cellular level (Bremner and Beattie, 1990) as free ions can cause many serious problems, including oxidative stress or permanent signaling within the cell.

Exposure to contaminants, including heavy metals, has been implicated as a major factor contributing to recent decreases in bat populations (Mickleburgh et al., 2002). Bayat et al., (2014), in summarizing actual data on organic contaminants (mainly pesticides) and their effects on bats, was able to show that organic pesticides and PCBs (polychlorinated biphenyls) are still being detected in bat tissue, many years after their use was banned. On the other hand, potential detrimental effects of heavy metals on wild bat populations are poorly documented, despite bats being recognized as a potential bioindicators species (Jones et al., 2009). There are many features of bat life-history and biology that make bats a perfect species for monitoring of environmental contaminants including heavy metals. First, bats are long-lived, with life-spans much longer than those of other similarly-sized mammals. The oldest bats, for example, live up to 40 years, and most have an average age of between five and six years (Gaisler et al., 2003). Such longevity not only makes bats more susceptible to the negative effects of heavy metals through bioaccumulation, it can also result in large concentrated doses of lipophilic contaminants being transferred to offspring in milk. Second, the metabolic processes of insectivorous bats are very rapid and these small animals must consume a great deal of food, with individuals catching prey weighing up to 100% of their body mass in one night (Kurta et al., 1989). Greater food intake increases the amount of contaminant available for concentration in body fat. Moreover, insectivorous bat species occupy a relatively high trophic level, which increases their susceptibility to environmental contaminant accumulation through their diet and ability to show the consequences of toxic pollution. Third, bats often coexist with humans in urban, industrial, and agricultural landscapes (Gaisler et al., 1998; Bartonička and Zukal, 2003; Park, 2015; Russo and Ancillotto, 2015), thereby potentially exposing themselves to increased pollution levels. While synanthropy (living with humans) has allowed bats to spread into regions were the suitable natural shelters are limited (Russo and Ancillotto, 2015), it has also increased the threat of heavy metal contamination through proximity to human activities. In polluted areas, bats accumulate metals through the food chain and long-term exposure to elevated levels can result in a variety of pathological conditions or even death. Fourth, bats also feed on insects emerging from the water surface. Riparian habitats support large numbers of insects and are prime foraging areas for insectivorous bat species (Vaughan et al., 1996; Korine et al., 2015). Inflow of heavy metals and other toxins from industrial waste, however, can not only affect water quality but accumulate in the invertebrate community, which then forms food for bats (Van De Sijpe et al., 2004; Jones et al., 2009). Finally, heavy metals often accumulate in fat, and are more likely to have adverse physiological effects in bats when they are depleting their fat reserves during hibernation, migration, or lactation (Speakman and Thomas, 2003).

Some factors, such as their high mobility, limit the use of bats as bioindicators, with long distances travelled to foraging areas (several kilometers every night) resulting in low geographical accuracy for detection of specific polluting sites. Further, the nocturnal and reclusive nature of these mammals makes recognition of die-offs associated with contaminants more difficult than in other wild animals.

In general, both bioindication and ecological risk assessment in wildlife is limited by a lack of data, including toxicological sensitivity and geographical variability. Furthermore, many bat species are rare or threatened and their protected status means that acquisition of such data may be strictly limited, or be totally unavailable. Strict world-wide protection and conservation of most bat species also prevents their use in standardized monitoring programs for environmental contaminants, such as those undertaken with game animals (Mickleburgh et al., 2002). There is, however, a great need for ecotoxicological support with respect to decision making in wildlife conservation.

As a first step, this review sets out to provide an overview of heavy metals research on wild bat populations to date; to point out major gaps in our present knowledge; and to suggest future directions and approaches for the study of heavy metal contamination and its possible direct adverse effects on bats.

Material and methods

In this review, we summarize all primary literature sources, including original papers, reports and published theses that present original data on heavy metal contamination in bats. Abstracts from conferences and methodological chapters in books were excluded as these tended not to present new data on heavy metal content. Only three theses (Hariono, 1991; Massa, 2000; Land, 2001) were not included as we were unable to obtain copies; however, some results from these studies have already been published as original scientific papers (*e.g.* Hariono et al., 1993).

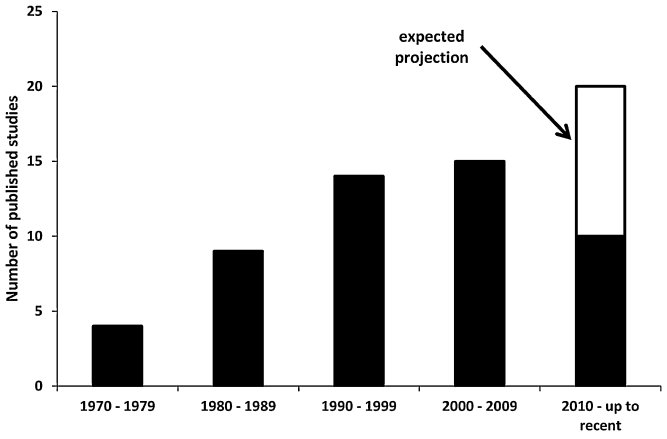


Fig. 1. Number of articles on heavy metal contamination in bats published over the last forty years. The expected projection (calculated as average number of publications per year multiplied by 10) is the number of new publications expected by the end of 2019.

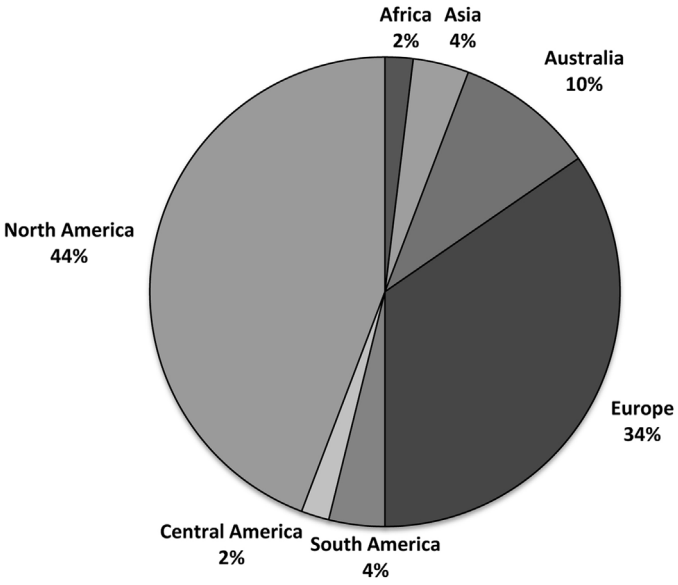


Fig. 2. Geographical distribution of studies undertaken on heavy metals in bats ($n = 52$).

All available information was collected and analyzed, including size of sample, species studied, age and sex structure of samples, matrix used for chemical analysis, locality, and heavy metal content. Thereafter, bat species were categorized by preferred food type (*i.e.* fructivorous/nectarivorous or insectivorous). Unfortunately, extremely high heterogeneity in the published data prevents statistical analysis and we could only compare basic parameters characterizing general variability, such as mean values and ranges.

Results and discussion

Temporal and geographical aspects

We reviewed a total of 52 studies reporting on heavy metal concentrations in bats, their organs or guano. The first article on this subject was published in 1970 (Zook et al., 1970) and describes lead poisoning in three captive Australian fruit bats (*Pteropus poliocephalus*) based on clinical and histological findings. All the bats subsequently died and lead-based paint from the walls of the bats' cage was identified as the source of poisoning. Similarly to Bayat et al., (2014), who reviewed data on organic contaminants in bats, we then faced many difficulties when trying to analyze summarized data in subsequent publications. Such data tend to become increasingly biased, both temporally and spatially, as the number of articles published in the 1990s has increased (Fig. 1). This clearly reflects the increasing interest in heavy metal pollution and its effects on bat populations all over the world (Fig. 2). With the exception of Miura et al., (1978), who published the first report on a survey of heavy metal contamination in wild bat populations, all studies from Asia, Africa, and Central or South America were conducted between 2000 and 2013. Most studies undertaken report on heavy metal contamination in North America or Europe, where more funding tends to be available compared to developing countries (Fig. 2). Even in these regions, however, studies are restricted to one or two reports per country/state, with a limited number of exceptions (*e.g.* Germany and France in Europe, or Virginia and Arizona in North America).

As mentioned above, the application of a general trend analysis for heavy metal contamination levels was not possible due to a scarcity of data or its highly localized availability. As early as the 1970s, Petit and Altenbach (1973) had proposed the analysis of stratigraphically dated guano deposits to address this problem, as this could provide a chronological record of selected heavy metals in the food chain of bats in a given area. Their study on the migratory free-tailed bat *Tadarida brasiliensis* found a correlation between annual fluctuations of mercury in bat guano with annual copper production in a nearby smelter. However, the results of European studies analyzing bat guano strata are less consistent (Uloth et al., 1987; Rackow, 1991; Hartmann, 2000), with lead and cadmium concentrations in different guano layers remaining stable or declining in more recent years, with high local variability. Unfortunately, bat guano deposits maybe contaminated by other heavy metal sources, such as dust or soil (*e.g.* Cuculić et al., 2011). Similar results were obtained by Hartmann (2000) when analyzing heavy metal content in bat forearms, fur and organs. A different approach was used by Miura et al., (1978), when they compared museum specimens with recent bat samples for analysis of mercury content before, during and after the use of organo-mercury fungicides when dusting crops. They found an increased mercury content in bat samples collected during and after the use of mercury pesticides.

Some heavy metals that occur naturally in the environment are essential for a range of normal functions in animals (*e.g.* trace doses of manganese, nickel, cobalt, copper, iron, and zinc). Variability in the levels found in bat bodies is certainly influenced by background environmental levels, which will be mirrored in the amounts accumulated. Direct comparison of results from geographically distant areas is impossible, therefore, though comparison of contaminated and reference localities on a regional scale remains useful (Gerell and Gerell Lundberg, 1993; Zocche et al., 2010; Naidoo et al., 2013). O'Shea et al., 2001 have shown that bats roosting at some distance from confined, concentrated sources of persistent food-chain contaminants can bioaccumulate these substances through exposure during foraging, despite their high mobility and relatively large ranges compared with terrestrial small mammals. Concentrations of arsenic and mercury in guano collected at roosts of big brown bats (*Eptesicus fuscus*) closest to locality contaminated by military and industrial use were significantly higher than those from roosts in the reference area, thereby implicating the study site as the contaminant source (O'Shea et al., 2001).

Heavy metal pollution can also exert an influence on other ecological, genetic, physiological and behavioral parameters, such as bat diversity, relative abundance, population structure, flight activity (Vaughan et al., 1996; Andrews and Allen, 2004; Rachwald et al., 2004; Van De Sijpe et al., 2004; Naidoo et al., 2013), or DNA damage in blood cells, blood parameters, plasma glucocorticoids, mortality,

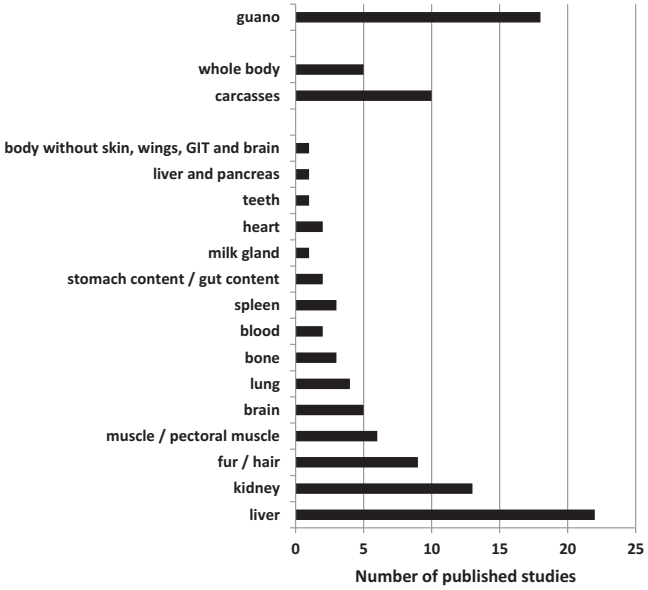


Fig. 3. Number of published heavy metal studies separated based on matrix analyzed. Note that some articles published data from more than one matrix.

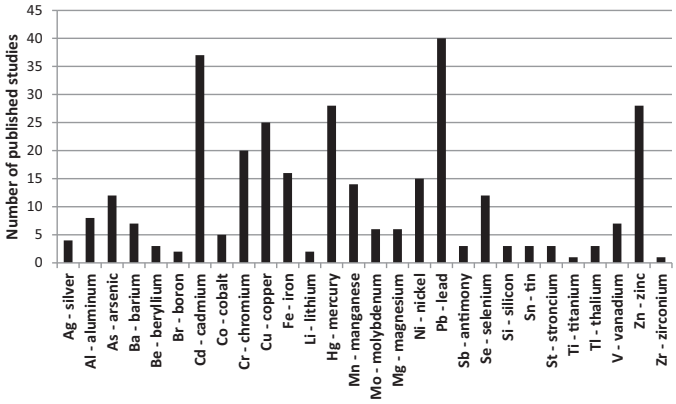


Fig. 4. Frequency of occurrence of particular elements in published articles on heavy metal contamination in bats. In each case, articles presented data on one or more elements.

activity of ligands, and neurochemical alterations (Farina et al., 2005; Courtin et al., 2010; Pikula et al., 2010; Wada et al., 2010; Zocche et al., 2010; Nam et al., 2012; Pilosof et al., 2014). The differences in the levels of such parameters should be compared between nearby localities with differing heavy metal contamination levels.

Methodological aspects

The approaches used in the literature reviewed tend to be inconsistent, with nostandardized methodology used by the different research teams. The only parameter that does allow direct comparison is that of measurement unit, expressed as ppm, mcg/g or mg/kg. Data tend to be presented as either geometric median or arithmetic mean of wet/fresh or dry weight. Note that dry weight concentrations can be estimated by multiplying the wet weight result by a factor of 4, a widely used conversion factor (Mochizuki et al., 2008), but its use increases the inaccuracy of the results obtained. Hartmann (2000) proposed a range of conversion factors for normalization of wet weight assay results from organ samples to dry weight (*i.e.* liver or heart 3.7, kidney 3.9 and lung 4.3), the factors being calculated on the basis of moisture content in the bat's target organs. Such limitations were also mentioned by O'Shea and Johnson (2009), who clearly summarized and described all aspects of contaminant studies, from the planning and design of research studies up to practical aspects of sample dissection, preparation, and storage; chemical analysis techniques; and statistical analysis.

Determination of heavy metal concentrations in tissues can also face limitations due to the small size of the matrix samples used. Heavy metals have been analyzed in various matrices (Fig. 3), with liver, kidney, whole body/carcasses and guano the four most numerous sample types used. Whole body/carcasses or guano are frequently used as they offer sample sizes large enough for chemical analysis. At the same time, these matrices are easily available and sampling is not limited due to conservation-related legislation. Nevertheless, comparing whole body or carcass concentrations between studies is difficult as dissection methods often differ. What one author refers to as a whole body or carcass may not match the definition of other authors (King et al., 2003). Frequent sampling of livers and kidneys for contaminant determination has highlighted a further limitation, *i.e.* that heavy metals accumulate differently in different target organs. Lead, for example, accumulates in bones, liver, and kidneys; arsenic in liver, kidneys, and brain; cadmium in kidneys and liver; and mercury in fur and liver. While this means that it is impossible to compare levels between different organs, heavy metal concentrations within particular tissue types are highly correlated (Hariono et al., 1993).

As more sophisticated and precise instrumentation has been developed, the number of compounds analyzed for in the various matrices has gradually increased (Fig. 4). These new methods allow for analysis of a wider range of elements in much smaller matrix samples, opening the way for non-lethal sampling methods and wider monitoring programs (*i.e.* more samples can be analyzed and more heavy metals assessed). Yates et al., (2014), for example, was recently able to report mercury content in fur and blood of 1447 specimens from northeastern North America (Maine, Maryland, Massachusetts, Virginia, New Hampshire, Pennsylvania, New York, and West Virginia).

Importantly, bat toxicology is a multidisciplinary field with investigations based on analytical chemistry, biochemistry, statistical and mathematical modelling, and biological and ecological studies of the various species, including their pathology and behavior (Rattner, 2009). A new methodological approach is represented by the paper of Hernout et al., (2013), who describe the development, parameterization, and application of a spatially explicit modelling framework predicting the risks of soil-associated metals (lead, copper, zinc and cadmium) to bat health in the United Kingdom.

Biological aspects

Sixty-five bat species (*i.e.* approximately 5% of all known bat diversity) have been included in at least one heavy metal contamination study. However, only two North American (*E. fuscus* and *Myotis grisescens*) and two European insectivorous bat species (*Myotis myotis* and *Pipistrellus pipistrellus* sensu lato) have been analyzed more than five times (Table 1).

Heavy metal concentrations vary greatly between species, sex, age, year of collection, and locality, with no clear pattern observed (see Supplementary data). The fact that heavy metals are naturally present in the environment makes any conclusions concerning risk and adverse effects to bats difficult (Hernout et al., 2013). It is also known that many species in contaminated environments are able to adapt to high concentrations of some metals (Ma and Talmage, 2001).

Only five heavy metals (cadmium, chromium, copper, palladium, and zinc) have been measured in fructivorous/nectarivorous species (Table 2). In every case, mean values were higher than those for insectivorous bats, while nectar and pollen feeders showed

Table 1
Summary of bat species included in studies on heavy metal contamination in bats.

Undetermined species	2	*Myotis austroriparius*	2
		Myotis brandtii	1
Pteropodiformes		*Myotis californicus*	2
Pteropodidea		*Myotis dasycneme*	1
Pteropus alecto	4	*Myotis daubentoni*	2
Pteropus conspicillatus	1	*Myotis emarginatus*	1
Pteropus hypomelanus	1	*Myotis grisescens*	7
Pteropus poliocephalus	4	*Myotis leibii*	2
Pteropus scapulatus	2	*Myotis lucifugus*	5
Rousettus aegyptiacus	2	*Myotis myotis*	9
Dobsonia moluscense	1	*Myotis mystacinus*	4
Sphaerias blanfordi	1	*Myotis nattereri*	2
Rhinolophidae		*Myotis septentrionalis*	3
Rhinolophus cornutus	1	*Myotis sodalis*	2
Rhinolophus ferrumequinum	3	*Myotis vivesi*	1
Rhinolophus hipposideros	3	*Myotis yumanensis*	1
		Molossidae	
Vespertilioniformes		*Tadarida brasiliensis*	5
Vespertilionidae		*Molossus molossus*	1
Eptesicus diminutus	1	Phyllostomidae	
Eptesicus fuscus	9	*Macrotus californicus*	2
Eptesicus serotinus	3	*Carollia brevicauda*	1
Lasionycteris noctivagans	1	*Carollia manu*	1
Lasiurus borealis	1	*Carollia pespicilata*	1
Lasiurus cinereus	1	*Desmodus rotundus*	1
Nyctalus noctula	3	*Anoura caudifer*	1
Pipistrellus abramus	1	*Anoura cultrata*	1
Pipistrellus kuhlii	1	*Anoura geoffroyi*	1
Pipistrellus nathusii	1	*Artibeus cinereus*	1
Pipistrellus pipistrellus	7	*Artibeus jamaicensis*	1
Pipistrellus pygmaeus	1	*Plathyrrhinus masu*	1
Parastrellus hesperus	1	*Sturnira erythromos*	1
Plecotus auritus	2	*Sturnira lilium*	1
Plecotus townsendii (incl. P. t. ingens)	2	*Sturnira oporaphyllum*	1
Vespertiliomurinus	1	*Sturnira tildae*	1
Vespertilio superans	1	*Phylloderma stenops*	1
Neoromicia nana	1	Noctilionidea	
Perimyotis subflavus	2	*Noctilio leporinus*	1
Miniopterus fuliginosus	1		
Miniopterus schreibersii (incl. M. s. bassanii)	3		

Table 2
Summarized values for contamination of bats with heavy metals. Values marked by an asterix are expressed in wet weight. Values in brackets show values for bats poisoned by copper and lead, and values for zinc in dry weight only. Abbreviations: INS—insectivorous species, FRU nectarivorous/fructivorous species.

Element	INS all types of tissue samples			INS guano			FRU all types of tissue samples		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Ag—silver	0.004	0.01	0.019	<1		<6.5			
Al—aluminum	1.92	126.6	1099	470.59	3557.74	6810.34			
As—arsenic	0.13	1.27	36.6	<0.4	6.54	66			
Ba—barium	1.1	14.8	238	13	54.07	139.59			
Be—beryllium				<0.18	0.35	0.45			
Br—boron	0.38	7.94	45.2						
Cd—cadmium	0.001	1.32	180*	0.03	4.13	8.5	<0.03	2.76	19.5
Co—cobalt	0.062	0.081	0.103	2					
Cr—chromium	0.007	3.237	120	<0.5	7.1	57			
Cu—copper	2.29	20.97	762	40	205.66	2869	4.38	23.92	69.67 (4540)
Fe—iron	72	794.6	3071	320	4295.12	10,068.26	174*	1921.5*	3669*
Li—lithium	<0.1	0.6	0.6						
Hg—mercury	0.02	4.3	707.64	0.07	0.54	4.2			
Mn—manganese	1.4	22.5	544	66.3	372.71	840.52			
Mo—molybdenum	0.12	2.41	4	<1.77	5.59	7.82			
Mg—magnesium	99	1093	6340	927.42	6809.11	25,669.02			
Ni—nickel	0.015	1.94	19.656	<1	4.46	<16			
Pb—lead	0.14	8.21	90	0.52	12.75	65	0.04	11.92	370.03 (>500)*
Sb—antimony				<2.16		<8.06			
Se—selenium	0.27	6.76	69	<0.35	5.12	15.6			
Si—silicon	4.8	194.64	710						
Sn—tin	0.45	7.95	28.8	<2.10	8.86	17.88			
St—stroncium	0.2	20.92	58.8	9.26	37.84	90.8			
Ti—titanium	0.4	5.8	14						
Tl—thalium				<0.1		<16.13			
V—vanadium	0.011	0.97	6.29	3*	6.22	10.43			
Zn—zinc	0.25* (25.4)	98.37* (115.9)	2500* (331)	64	544.33	1079.83	9.49	108.96	188.78
Zr—zirconium	0.3	1.17	2.8						

lower organic residue concentrations than insectivorous species. Note, however, that other factors, such as roosting location, foraging habitat, and bat metabolism are also known to significantly influence accumulation (Bayat et al., 2014). Interestingly, differences in organic pesticide accumulation between different bat species are more evident than in heavy metals. It seems likely, therefore, that heavy metal exposure pathways differ between fructivorous and insectivorous bat species. The primary means of heavy metal contamination in fruit bats is likely to be through atmospheric pollution, with secondary contamination from contact with contaminated foliage whilst searching for and eating food (Sutton and Hariono, 1986; Hariono et al., 1993). As the contaminants are later ingested directly when grooming, this represents a very rapid ingestion pathway. Insectivorous bat species, on the other hand, become contaminated mainly through bioaccumulation through the food-chain, *i.e.* heavy metals are transferred from sediments/water/soil/plants or other sources to insect larvae and adults, and finally to the bats themselves (Reinhold et al., 1999; Hsu et al., 2006; Hernout et al., 2013). Not only will pathways differ within the food-chain but they may also differ depending upon the type of toxicant.

The elemental composition of bat guano probably reflects heavy metal residues present in the undigested portion of ingested prey species, and as such may provide some clues to the location of contaminants in the environment (Martin, 1992). However, mean heavy metal concentrations for insectivorous bats (Table 2) show higher levels of all elements in guano than in tissues, with the exception of selenium. This difference may result from lower bioavailability of heavy metals from digested food and lower bioaccumulation or bioconcentration factors, expressed as the ratio between soil heavy metal concentration and the respective tissue concentration (Hsu et al., 2006). Bats also display different food ingestion rates and weights depending on their life-stage (*i.e.* juvenile, male in spermatogenesis, pregnant female, or lactating female) and cycle (winter torpor or summer active); hence, sensitivity to chemical exposure may vary depending which stage the bat is in (Hernout et al., 2013).

Only limited data are available on potential heavy metal toxicity levels in bats as current risk assessment studies tend to focus on the sensitivity of laboratory animals such as mice (*e.g.* lab derived LC50, EC50 and NOEC values for each contaminant). Similar data are totally lacking for bats as no study has yet been performed to assess toxic thresholds of different heavy metals. Studies on other insectivorous mammals indicate that such species can be more tolerant to heavy metals than rodents (Clark, 1979; Ma and Talmage, 2001). In the only laboratory study involving bats and heavy metals, a single subcutaneous injection of cadmium chloride administered to males of rat-tailed bats (*Rhinopoma kinneari*) caused testicular necrosis, with shrinkage of the seminiferous tubules but no loss of testicular weight (Dixit and Lohiya, 1974). Laboratory mice given similar injections showed greater destruction of the seminiferous epithelium and significant loss of testicular weight. Other impacts and poisoning cases have also been documented, including hepatopathy, DNA damage, hemochromatosis, renal inclusion bodies, ascending paralysis, and changes in cholinergic functions (Skerratt et al., 1998; Hoenerhoff and Williams, 2004; Farina et al., 2005; Zocche et al., 2010; Nam et al., 2012). Despite these cases, the number of articles confirming direct adverse effects of heavy metals on bats remains low. Wada et al., (2010), for example, observed no site-specific differences in adrenocortical response, despite the high mercury concentrations observed in bat tissues. Their results suggest that bats at the contaminated site were exposed to mercury concentrations but that these were still below the critical threshold for any adverse effect on the adrenal axis.

There remain many unanswered questions in relation to the metabolism of heavy metals in bats. These include the efficiency of absorption, the level required to show clinical effects, and whether or not excretion occurs. For temperate-zone bats in particular, the influence of hibernation on heavy metal toxicity was yet to be studied. Hypothermic bats substantially reduce their body temperature and metabolic rate during torpor and this period appears to be crucial for the survival of many species (Speakman and Thomas, 2003). The effects of chronic sub-lethal exposure to environmental contaminants are also poorly understood (Bayat et al., 2014). Additionally, wild bats are frequently exposed to multiple anthropogenic stressors at the same time, which may show both antagonistic or, more frequently, combined or synergic effects. Such stressors may include natural toxins, anthropogenic pollutants such as heavy metals, and infectious agents. While the combined effects of such stressors remain practically unexplored, Skerratt et al., (1998) has co-diagnosed lyssaviral infection in wild black flying foxes (*Pteropus alecto*) poisoned by lead, while Courtin et al., (2010) have reported potentially toxic levels of lead (13% of bats) and arsenic (4% of bats) in liver tissue of bats affected by white-nose syndrome. Furthermore, a number of epizootic infectious diseases have been noted as more severe in areas contaminated by environmental pollutants (Grasman, 2002), demonstrating the possibility of population-level effects associated with contaminant-induced immunosuppression.

Conclusions

While the present review documents both the worldwide exposure of bats to toxic elements due to contamination of their foraging habitats and the suitability of this trophic guild as a bioindicator of general environmental condition, direct adverse effects and/or mortality have only been confirmed infrequently. Despite a largely anecdotal and presumed higher tolerance of bats to heavy metals, there is evidence for a similar range of adverse effects as in other mammal species, including toxicity to the liver, kidney, immune, nervous and reproductive systems.

Data show that bats can be exposed to different toxic elements at the same time. While a multiple-stressor scenario (whether natural or anthropogenic in origin) is realistic, exposure to low-level toxicant mixtures and sub-lethal effects are less recognized and the causality of bat population declines hard to document. On a long-term basis, chronic and indirect effects are known to be more hazardous for wildlife. Apart from their primary modes of action, toxic elements can also modulate physiological responses in bats and may contribute to morbidity and mortality elicited by other stressors, such as infectious agents.

Critical toxic threshold levels are mostly lacking for both bat species and particular toxic elements. Given the conservation and protection status of bats in many countries, the preparation of experimental in-vivo bat models to obtain standard toxicological data is not feasible. Ecotoxicological data are essential for risk assessment and decision-making in bat conservation, however, and the question remains as to what can be done to address this important issue?

Apart from making use of bat carcasses to monitor exposure of populations to toxic substances, we would like to encourage other non-lethal means of research. Systematic sampling of guano deposits from various areas, coupled with the known foraging habits of individual bat species and prey analysis, may prove a relevant means of monitoring trends in exposure of bat populations to toxic substances and a good indicator of both aquatic and terrestrial environmental quality.

The relationship between levels of heavy metals in bat guano, insect prey, and the various components of the environment in which the insects develop, should prove a fruitful area for future research. Further, the collection of fur samples may also be

employed as a non-lethal method of analysis as lead levels in fur and kidney have been shown to be correlated, as have levels of mercury in both the liver and brain.

Cutting edge *in vitro* methods that allow the study of direct cellular effects of toxins on specific organ- and tissue-derived cell types under controlled conditions (such as those simulating hibernation temperatures) also appear to be a highly promising method for obtaining relevant toxicological data for bats. Cell lines for individual bat species should be established and immortalized in order to study molecular mechanisms of detoxification following exposure to heavy metals. Importantly, it is now possible for cell lines to be obtained *via* non-lethal collection methods from bats captured and handled so as to minimize stress. Examples include wing membrane puncture biopsies to establish fibroblasts and blood collection for white blood cell lines.

The present situation of non-systematic and haphazard collection of data regarding bat heavy metal pollution requires urgent standardization, both of research approaches and sample collection methodology, through an international monitoring program. On a continental scale, the exchange of information and coordination of international research and monitoring initiatives have to be organized within scientific community. The main purpose of similar initiatives must be to assess the potential risk of heavy metals for bats in order to enhance their future protection as their results could be used by local or international conservation bodies such as EUROBATS (the Agreement on the Conservation of Populations of European Bats) or Bat Conservation International.

Acknowledgements

The authors received support from the Grant Agency of the Czech Republic (Grant no. 506/12/1064), the Institute of Vertebrate Biology of the Academy of Sciences of the Czech Republic (RVO: 68081766), and the Internal Grant Agency of the University of Veterinary and Pharmaceutical Sciences in Brno (Grant no. 23/2014/FVHE). The authors wish to thank Kevin Roche for correction of the English text.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mambio.2015.01.001

References

Andrews, M.M., Allen, J., 2004. Aerial deposition of cadmium before and after the closure of an oil-fired power station in the vicinity of two nursery roosts of *Rhinolophus Ferrumequinum* (Chiroptera, Rhinolophidae). Mammalia 68, 437–444.
Bartonička, T., Zukal, J., 2003. Flight activity and habitat use of four bat species in a small town revealed by bat detectors. Folia Zool. 52, 155–166.
Bayat, S., Geiser, F., Kristiansen, P., Wilson, S.C., 2014. Organic contaminants in bats: trends and new issues. Environ. Int. 63, 40–52.
Beyersmann, D., Hartwig, A., 2008. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch. Toxicol. 82, 493–512.
Bremner, I., Beattie, J.H., 1990. Metallothionein and the trace minerals. Annu. Rev. Nutr. 10, 63–83.
Clark Jr., D.R., 1979. Lead concentrations: bats vs. terrestrial small mammals collected near a major highway. Environ. Sci. Technol. 13, 338–341.
Clark Jr., D.R., 1981. Bats and environmental contaminants: a review. In: United States Fish and Wildlife Service Special Scientific Report-Wildlife, Number 235.
Courtin, F., Stone, W.B., Risatti, G., Gilbert, K., Van Kruiningen, H.J., 2010. Pathologic findings and liver elements in hibernating bats with white-nose syndrome. Vet. Pathol. 47, 214–219.
Cuculić, V., Cukrov, N., Kwokal, Ž., Mlakar, M., 2011. Distribution of trace metals in anchialine caves of Adriatic Sea, Croatia. Estuar. Coast. Shelf Sci. 95, 253–263.
Dixit, V.P., Lohiya, N.K., 1974. Histological changes in testis of the non-scrotal mammal, *Rhinopoma kinneari* (Wroughton) following the administration of cadmium chloride. Indian J. Exp. Biol. 12, 200–202.
Farina, L.L., Heard, D.J., Leblanc, D.M., Hall, J.O., Stevens, G., Wellehan, J.F.X., Detrisac, C.J., 2005. Iron storage disease in captive Egyptian fruit bats (*Rousettus Aegyptiacus*): relationship of blood iron parameters to hepatic iron concentrations and hepatic histopathology. J. Zool. Wildl. Med. 36, 212–221.
Gaisler, J., Hanák, V., Hanzal, V., Jarský, V., 2003. Results of bat banding in the Czech and Slovak Republics, 1948–2000. Vespertilio 7, 3–61.
Gaisler, J., Zukal, J., Řehák, Z., Homolka, M., 1998. Habitat preference and flight activity of bats in a city. J. Zool. 244, 439–445.
Gerell, R., Gerell Lundberg, K., 1993. Decline of a bat *Pipistrellus pipistrellus* population in an industrialized area in south Sweden. Biol. Conserv. 65, 153–157.
Grasman, K.A., 2002. Assessing immunological function in toxicological studies of avian wildlife. Integr. Comp. Biol. 42, 34–42.
Hariono, B., 1991. A Study of Lead (Pb) Levels in Animals and the Environment with Particular Reference to the Fruit Bat (*Pteropus* sp.). University of Queensland, Brisbane, Australia, pp. 242 (Ph. D. Thesis).
Hariono, B., Ng, J., Sutton, R.H., 1993. Lead concentrations in tissues of fruit bats (*Pteropus* sp.) in urban and non-urban locations. Wildl. Res. 20, 315–320.
Hartmann, R., 2000. Deskription der Schwermetallgehalte in Knochen, Organen und Haaren von Fledermäusen (Chiroptera) im Zeitraum 1987 bis 1999. Dissertation Georg-August-Universität, Göttingen, 120 pp.
Hernout, B.V., Somerwill, K.E., Arnold, K.E., McClean, C.J., Boxall, A.B.A., 2013. A spatially-based modelling framework for assessing the risks of soil-associated metals to bats. Environ. Pollut. 173, 110–116.
Hoenerhoff, M., Williams, K., 2004. Copper-associated hepatopathy in a Mexican fruit bat (*Artibeus jamaicensis*) and establishment of a reference range for hepatic copper in bats. J. Vet. Diagn. Invest. 16, 590–593.
Hsu, M.J., Selvaraj, K., Agoramoorthy, G., 2006. Taiwan's industrial heavy metal pollution threatens terrestrial biota. Environ. Pollut. 143, 327–334.
Järup, L., 2003. Hazards of heavy metal contamination. Br. Med. Bull. 68, 167–182.
Jones, G., Jacobs, D.S., Kunz, T.H., Willig, M.R., Racey, P.A., 2009. Carpe noctem: the importance of bats as bioindicators. Endang. Species Res. 8, 93–115.
King K.A., Velasco A.L., Marr C.L.H., Kearns R.L., 2003. Trace elements in bats roosting in mines at Kofa National Wildlife Refuge, Arizona, 2001–2002. In: U.S. Fish and Wildlife Service, Phoenix, Arizona. Project Report, Project 22410-1261-2N41, 28 pp.
Korine, C., Adams, A., Shamir, U., Gross, A., 2015. Effect of water quality on species richness and activity of desert-dwelling bats. Mamm. Biol. 80, 185–190.
Kurta, A., Bell, G.P., Nagy, K.A., Kunz, T.H., 1989. Energetics of pregnancy and lactation in free-ranging little brown bats (*Myotis lucifugus*). Physiol. Zool. 62, 804–818.
Land, T.A., 2001. Population Size and Contaminant Exposure of Bats Using Caves on Fort Hood Military Base, Texas. Texas A&M University, College Station, TX, pp. 102 pp (M.S. Thesis).
Laskowski, R., Niklinska, M., Maryanski, M., 1995. The dynamics of chemical elements in forest litter. Ecology 76, 1393–1406.
Li, Z., Ma, Z., van der Kuijp, T.J., Zuan, Z., Huang, L., 2014. A review of soil heavy metal pollution from mines in China: pollution and health risk assessment. Sci. Total Environ. 468–469, 843–853.
Ma, W.C., Talmage, S., 2001. Insectivora. In: Shore, R.F., Rattner, B.A. (Eds.), Ecotoxicology of Wild Mammals. John Wiley & Sons, New York, NY, pp. 159–215.
Martin, D.B., 1992. Contaminant Studies on Endangered Bats in northeastern Oklahoma. U.S. Fish and Wildlife Service, Ecological Services, Tulsa, Oklahoma, 16 pp.
Massa, S.A., 2000. Mercury Levels in Arkansas Bats from Counties under Advisories for Fish Consumption. Arkansas State University, Arkansas, pp. 98 (Thesis).
Melancon, M.J., 2003. Bioindicators of contaminant exposure and effect in aquatic and terrestrial monitoring. In: Hoffman, D.J., et al. (Eds.), Handbook of Ecotoxicology. , second ed. Lewis Publishers, USA, pp. 257–278.
Mickleburgh, S.P., Hutson, A.M., Racey, P.A., 2002. A review of the global conservation status of bats. Oryx 36, 18–34.
Miura, T., Koyama, T., Nakamura, I., 1978. Mercury content in museum and recent specimens of Chiroptera in Japan. Bull. Environ. Contam. Toxicol. 20, 696–701.
Mochizuki, M., Mori, M., Hondo, R., Ueda, F., 2008. A new index for evaluation of cadmium pollution in birds and mammals. Environ. Monit. Assess. 137, 35–49.
Nam, D.H., Yates, D., Ardapple, P., Evers, D.C., Schmerfeld, J., Basu, N., 2012. Elevated mercury exposure and neurochemical alterations in little brown bats (*Myotis lucifugus*) from a site with historical mercury contamination. Ecotoxicology 21, 1094–1101.
Naidoo, S., Vosloo, D., Schoeman, M.C., 2013. Foraging at wastewater treatment works increases the potential for metal accumulation in an urban adapter, the banana bat (*Neoromicia nana*). Afr. Zool. 48, 39–55.
Nowak, R.W., 1994. Walker's Bats of the World. Johns Hopkins University Press, Baltimore, MD, pp. 287.
O'Shea, T.J., Everette, A.L., Ellison, L.E., 2001. Cyclodiene insecticide, DDE, DDT, arsenic, and mercury contamination of big brown bats (*Eptesicus fuscus*) foraging at a Colorado Superfund site. Arch. Environ. Contam. Toxicol. 40, 112–120.
O'Shea, T.J., Johnson, J.J., 2009. Environmental contaminants and bats: investigating exposure and effects. In: Kunz, T.H., Parsons, S. (Eds.), Ecological and Behavioral Methods for the Study of Bats. Johns Hopkins University Press, Baltimore, MD, pp. 500–528.
Park, K., 2015. Mitigating the impacts of agriculture on biodiversity: bats and their potential role as bioindicators. Mamm. Biol. 80, 191–204.
Petit, M.G., Altenbach, J.S., 1973. A chronological record of environmental chemicals from analysis of stratified vertebrate excretion deposited in a sheltered environment. Environ. Res. 6, 339–343.
Pikula, J., Zukal, J., Adam, V., Banďouchová, H., Beklová, M., Hájková, P., Horáková, J., Kížek, R., Valentíková, L., 2010. Heavy metals and metallothionein in vespertilionid bats foraging over aquatic habitats in the Czech Republic. Environ. Toxicol. Chem. 29, 501–506.
Pilosof, S., Korine, C., Moore, M.S., Krasnov, B.R., 2014. Effects of sewage-water contamination on the immune response of a desert bat. Mammal Biol. 79, 183–188.

Rachwald, A., Wodecka, K., Malzahn, E., Kluzinski, L., 2004. Bat activity in coniferous forest areas land the impact of air pollution. Mammalia 68, 445–453.
Rackow, W., 1991. Nachweise von Blei und Cadmium im Kot des Mausohrs (*Myotis myotis* Borkhausen 1797). Nyctalus (N.F.) 4, 140–144.
Rattner, B.A., 2009. History of wildlife toxicology. Ecotoxicology 18, 773–783.
Reinhold, J.O., Hendriks, A.J., Slager, L.K., Ohm, M., 1999. Transfer of microcontaminants from sediment to chironomids and the risk for the pond bat *Myotis dasycneme* (Chiroptera) preying on them. Aquat. Ecol. 33, 363–376.
Russo, D., Ancillotto, L., 2015. Sensitivity of bats to urbanization: a review. Mamm. Biol. 80, 205–212.
Skerratt, L.F., Speare, R., Berger, L., Winsor, H., 1998. Lyssaviral infection and lead poisoning in black flying foxes from Queensland. J. Wildlife Dis. 34, 355–361.
Speakman, J.R., Thomas, D.W., 2003. Physiological ecology and energetics of bats. In: Kunz, T.H., Fenton, M.B. (Eds.), Bat Ecology. The University of Chicago Press, Chicago, IL, pp. 430–490.
Sutton, R.H., Hariono, B., 1986. Lead poisoning in flying-foxes (Chiroptera: Pteropodidae). Aust. Mammal 10, 125–126.
Uloth, W., Tress, C., Körner, R., Majohr, D., 1987. Das Verhalten von Cadmium und Blei im Fledermausguano. Mengen und Spurenelemente. Arbeitstagung, Leipzig, pp. 107–109.
Van De Sijpe, M., Vandendriessche, B., Voet, P., Vandenberghe, J., Duyck, J., Naeyaert, E., Manhaeve, M., Martens, E., 2004. Summer distribution of the pond bat *Myotis Dasycneme* (Chiroptera, Vespertilionidae) in the west of Flanders (Belgium) with regard to water quality. Mammalia 68, 377–386.
Vaughan, N., Jones, G., Harris, S., 1996. Effects of sewage effluent on the activity of bats (Chiroptera: Vespertilionidae) foraging along rivers. Biol. Conserv. 78, 337–343.
Wada, H., Yates, D.E., Evers, D.C., Taylor, R.J., Hopkins, W.A., 2010. Tissue mercury concentrations and adrenocortical responses of female big brown bats (*Eptesicus Fuscus*) near a contaminated river. Ecotoxicology 19, 1277–1284.
Yates, D.E., Adams, E.M., Angelo, S.E., Evers, D.C., Schmerfeld, J., Moore, M.S., Kunz, T.H., Divoll, T., Edmonds, S.T., Perkins Ch Taylor, R., O'Driscoll, N.J., 2014. Mercury in bats from the northeastern United States. Ecotoxicology 23, 45–55.
Zocche, J.J., Leffa, D.D., Damiani, A.P., Carvalho, F., Mendonca, R.A., dos Santos, C.E.I., Boufleur, L.A., Dias, J.F., de Andrade, V.M., 2010. Heavy metals and DNA damage in blood cells of insectivore bats in coal mining areas of Catarinense coal basin. Braz. Environ. Res. 110, 684–691.
Zook, B.C., Sauer, R.M., Garner, F.M., 1970. Lead poisoning in Australian fruit bats (*Pteropus poliocephalus*). J. Am. Vet. Med. Assoc. 157, 691–694.