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Půdní fauna

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předkládá

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Obsah

Úvod

Pedobiologie je ekologická disciplína, která se zabývá biologií půdních organizmů. První pedobiologické práce vznikly až v osmdesátých letech XIX. století, do té doby převažoval v biologii popisný přístup taxonomický, anatomický, morfologický, fyziologický a embryologický. Mezi nestory pedobiologického výzkumu patří bez pochyb i Charles Robert Darwin (1809-1882), který studoval tvorbu půdy činností žížal (Darwin 1881). Vedl jej k tomu postřeh, že stráň, kterou si z dětství pamatoval jako hodně kamenitou, je o několik desetiletí později relativně zazemněná. Neboli že kameny, které o sebe při došlapu skřípaly, nyní neskřípou, jelikož jsou nyní více zanořené v zemině. Studoval proto proto podrobněji toto zazemňování a popsal, jak si (anektické) žížaly vtahují listový opad do chodbiček a jak vylučují na povrch půdy své exkrementy.

V Německu působící rakouskouherský přírodovědec Raoul Heinrich Francé (Rudolf Heinrich Franze, 1874-1943) ve své studii o ekologii půdních mikroorganizmů zavedl pro ně termín *edaphon* (Francé 1911, 1913). Toto označení vztáhl ve druhém vydání své monografie na všechny organizmy vázané svým způsobem života na půdu (Francé 1921). Termín edafon (odvozený z řeckého *ἔδαφος*) je tudíž z historických důvodů dobře etablovaný v němčině, ale také je používaný v maďarštině, češtině, slovenštině, francouzštině, portugalštině, či rumunštině. V angličtině však příliš nezdomácněl (ale viz např. Gallagher a Daiber 1973), proto se dnes v literatuře (převážně anglicky psané) setkáváme nejčastěji s označením *soil fauna*. Půdní fauna je obvyklé označení pro živočichy, kteří obývají půdní prostředí. Za typické představitele proto považujeme druhy, které se vyskytují v půdě, ať již v jejích svrchních vrstvách včetně nadložního humusu, či v hlubších minerálních vrstvách. Do půdní fauny se však nezařazují obyvatelé jeskyní. Přestože širší definice jeskyně zahrnuje všechy přirozeně vzniklé podzemní prostory, obvykle za jeskyně považujeme prostory, které jsou schopné pojmout člověka.

Jiný přístup ke klasifikaci půdní fauny sleduje taxonomickou příslušnost druhů k vyšším taxonům, které buď jsou, či nejsou považovány za typické představitele obyvatele půdy. Běžnými příklady jsou například žížalovití (Lumbricidae) či chvostoskoci (Collembola). V půdě je však možné najít zástupce velkého množství povrchových a dokonce i vodních taxonů (např. Schlaghamerský a Kobetičová 2005) a je proto na výzkumníkovi, jak se s takovýmito druhy vypořádá.

První problém si můžeme představit na broucích (Coleoptera). Většina takzvaných edafických druhů brouků obývá půdní prostředí pouze během svého larválního vývoje. Larvy se však od dospělců hodně liší (přinejmenším u hmyzu s dokonalou proměnou, taxonu Holometabola), což jejich determinaci komplikuje, jelikož většina entomologů se zaměřuje na určování dospělců a determinační literatura pro larvální stádia je omezena na několik málo skupin. Proto larvy brouků, ale i dvoukřídlého hmyzu, obvykle vystupují v ekologických studiích zaměřených na půdu pouze jako vyšší taxon, jehož význam bývá podceňován a za echtovní půdní faunu se často nepovažují. Půdní biologové je většinou (alibisticky) opomíjejí s tím, že dospělci vlastně žijí mimo půdu a proto význam těchto druhů v půdních procesech je relativně omezený.

Druhým důvodem k vyloučení řady obyvatel půd je jejich poměrně pasivní role v půdním subsystému. Příkladem mohou být květolib včelí či břehule říční. Tito živočichové v půdě hloubí nory z důvodu ochrany svého potomstva. Žádné jejich vývojové stádium se neživí detritem, neloví ostatní obyvatele půdy, ani jejich exkrementy nijak zásadně nepozměňují půdní chemizmus. Nory a chodby takovýchto druhů jsou obvykle relativně krátké a nepřispívají tak významně k aeraci půdy, zasakování srážkové vody, ani k bioturbanci. Jejich význam pro pedogenezi je tudíž minimální. Do této kategorie obvykle spadají také (nejen drobní) savci a plazi, kteří si hloubí nory, řada druhů hmyzu, které v půdě zimují apod.

Další způsob, jak lze definovat půdní faunu a vyhnout se „nežádoucím“ zástupcům, je metodologický přístup. Za půdní bezobratlé jsou považováni ti, kteří se nejlépe chytají pomocí klasických pedozoologických metod a nikoliv pomocí metod jiných. Některé mnohonožky lze sice najít na větvích stromů nejen v tropech, ale i v mírném pásu (Short 2015), ale obvykle se mnohonožky chytají do zemních pastí, proto je považujeme za půdní faunu. Tento přístup je nejrozšířenější, protože poskytuje výzkumníkovi „důvod“ neobtěžovat se determinací čmeláků, žab, sarančí či sýkorek (Anděra, pers. com.), které se (spíše omylem či nešťastnou náhodou) chytí do jeho padacích zemních pastí. Na druhou stranu takovýto přístup obecně zamlžuje naše poznání a informace o způsobu života některých druhů jsou hodně kusé, či zkreslené. Příkladem mohou být chlupule, které se mohou hojně vyskytovat na větvích keřů a stromů. Klasický entomolog, který sklepává z větví hmyz, tyto mnohonožky ignoruje a informaci o záchytu neuvádí. Půdní zoolog, věnující se mnohonožkám, však tyto chlupule potká jen relativně zřídka – občas v opadu, obvykle pod kůrou na úpatí stromu či padlého kmene, kde může najít i jiné druhy mnohonožek. Každopádně to vede k situaci, kdy specialista na danou skupinu vzorkuje pouze obvyklá mikrostanoviště a nezaznamená v terénu přítomnost dalších druhů a jiný specialista, který nějaký konkrétní druh potkává v atypickém (dle jeho názoru) prostředí pravidelně, mu nevěnuje žádoucí pozornost. To je důvodem, proč by se i půdní zoolog měl snažit využívat méně obvyklé metody sběru a vzorkovat různými způsoby. Metodologií vzorkování půdních bezobratlých, zhodnocením efektivity jednotlivých metod a představení některých méně obvyklých metod se věnuje další kapitola této práce. Po ní následující kapitola se věnuje ekologickým souvislostem edafického způsobu života a behaviorálním adaptacím, které živočichům takový život umožňují.

Metody vzorkování půdních bezobratlých

Když odhlédneme od předchozí kapitoly, můžeme přijmout za své obvyklé obecné tvrzení, že půdní fauna obývá půdní profil a vrstvy nadložního humusu. Tomu odpovídají běžné metody vzorkování půdní fauny.

Nejjednodušší metodou je tzv. individuální sběr, na který byli odkázáni přírodovědci až do konce XIX. století. Spočívá v chytání jednotlivých živočichů pomocí pinzety, exhaustoru, případně štětečku. Jedná se o metodu časově náročnou a špatně kvantifikovatelnou. Výzkumníkova pozornost je zaměřena hlavně na větší a nápadnější druhy, menší a/nebo hlouběji žijící jedinci jsou obvykle přehlédnuti. Na druhou stranu lze tímto jednoduchým způsobem nasbírat potřebný počet živočichů například na experimenty. Individuální sběr je v kombinaci s dalšími metodami a pomůckami vhodnou metodou pro provádění faunistické inventarizace. Pro potřeby ekologické inventarizace či biomonitoringu se však nehodí, protože se jedná o metodu špatně opakovatelnou. Individuální sběr je ale neocenitelnou metodou při vzorkování fauny v atypických prostředích, kde si nemůžeme dovolit aplikovat jiné meotdy. Dobrým příkladem jsou například skleníky či botanické zahrady, obecně prostory, kde se dbá na estetickou stránku prostředí, které je veřejnosti hodně na očích. V těchto prostorách jsou jiné metody obvykle považovány za velmi nežádoucí. Naproti tomu individuální sběr například ve sklenících může přinést zajímavá překvapení (Decker a kol. 2014, Dányi a Tuf 2016).

Kvantitativní vzorkování povrchově aktivních bezobratlých

Mnohem sofistikovanější metodou je využití tzv. padacích zemních pastí. Do ekologie je uvedl Herbert Spencer Barber, který v roce 1931 publikoval práci o jeskynních bezobratlých, nachytaných pomocí padacích pastí s návnadou. Zdůraznil v této práci, že pouze systematický dlouhodobý odchyt (ideálně v průběhu celého roku) může poskytnout představu o velikosti a dynamice populací jednotlivých druhů, a vyzýval čtenáře k využívání pastí poukazováním na jejich vysokou efektivitu lovu ve srovnání s individuálním sběrem (Barber 1931). Jím uvedené pasti, zakopané skleněné tuby jež ústily na povrch substrátu, obsahovaly fixační tekutinu různých druhů (jejich vhodnost v práci posuzoval) a návnadu z hnijícího masa pro lákání brouků. Dnešní pasti jsou obvykle bez návnady, aby nezkreslovaly reprezentativnost odchyceného vzorku celkového společenstva a nenadhodnocovaly početnosti přilákaných druhů. Pasti s návnadou jsou vhodné pro faunistickou inventarizaci, pro výzkum v jeskyních, kde jsou obecně nízké abundance bezobratlých, či je lze opakovaně používat při biomonitoringu konkrétních druhů.

Zemní pasti mají řadu výhod. Hlavní výhodou je jejich dobrá kvantifikovatelnost, kdy lze srovnávat úlovky z jednotlivých odchytových období a také z různých lokalit. Je však nutno si uvědomit, že se nejedná o přímá data o abundanci jednotlivých druhů, jelikož tyto druhy se liší nejen svými početnostmi, ale také svou epigeickou aktivitou (Tuf a kol. 2012), ochotou padat do zemních pastí (Halsall a Wratten 1988, Topping 1993, Gerlach a kol. 2009a, 2009b) i schopností z těchto pastí opět uniknout (Petruška 1969). Pokusy vytvořit vztah mezi trapabilitou druhu a jeho abundancí jsou sporé a diskutabilní (Clark a kol. 1995, Zhao a kol. 2013, Shi a kol. 2014).

Velkou výhodou je ale jejich snadná instalace, časově nenáročná obslužnost a finanční dostupnost. Zemní pasti chytají epigeon v terénu i bez naší přítomnosti, proto se příležitostně využívají i pro dlouhodobé vzorkování chudých společenstev. Tímto způsobem lze získat velmi zajímavý materiál například z vrcholových partií vysokých hor, ze sutí a podobně. Naproti tomu významnou nevýhodou zemních pastí je jejich neselektivnost. Před instalací je vhodné si rozmyslet, jakým způsobem zúročit materiál necílových skupin půdní fauny (New 1999). Také je vhodné pokusit se zabezpečit pasti proti vniknutí drobných obratlovců, jejichž usmrcování je v rozporu se zákonem 114/1992 Sb. Použití poklopu z drátěného pletiva se jeví jako vhodné opatření, nicméně často zabrání vniknutí nejen drobných savců, ale také velkých střevlíků. Podobné poklopy doporučoval již Barber (1931) proti drobným savcům i velkým jeskynním cvrčkům. Druhou možností je použití nálevek, které významně snižují počet odchycených drobných obratlovců (Lange a kol. 2011).

Velmi významným, leč zhusta přehlíženým problémem zemních pastí je nespočet jejich modifikací, které ovlivňují velikost a reprezentativnost jejich úlovku. Vhodné je před instalací zohlednit řadu parametrů zemních pastí. Pokud z jakéhokoliv důvodu nemůžeme či nechceme využít doporučenou metodiku (např. Janáčková a Štorkánová 2004), je vhodné alespoň prostudovat metodiky předchozích studií, se kterými budeme vlastní data srovnávat. Problematice posouzení kvality této metody bylo věnováno nespočet publikací (Adis 1979, Spence a Niemelä 1994, Knapp a Růžička 2012, Siewers a kol. 2014, Baini a kol. 2016, Brown a Matthews 2016, Knapp a kol. 2016).

Na kvalitu i kvantitu úlovku totiž má vliv už samotná instalace zemních pastí. Pasti je samozřejmě třeba zakopat a k tomu lze využít buď lopatky či rýče, nebo vrtáku. Dané způsoby se liší množstvím zeminy, která je po instalaci kolem pasti obnažena. Vrtákem lze udělat perfektní otvor na vsazení sklenice, takže její okolí zůstane nedotčené. Lopatkou je obvykle nutno vykopat větší jámu, ve které je past poté zafixována vyjmutou zeminou. Tato čerstvě vykopaná zemina, která je obvykle vlhká a obsahuje minerály, je atraktivní pro řadu druhů bezobratlých, včeně epigeonu a způsobuje tzv. *digging-in effect*. Ten se projevuje tak, že do čerstvě zakopaných pastí padá více živočichů než do pastí zakopaných dříve, což nadhodnocuje údaje o početnosti populací a pozměňuje strukturu společenstev. Tento fenomén je dosud poměrně neznámý a málo prostudovaný (Digweed a kol. 1995), minimalizovat *digging-in effect* lze pravděpodobně instalací pastí delší dobu před začátkem vegetačního období a nechat je zpočátku uzavřené.

Dalším problémem je konstrukce pasti. Past se může skládat ze skla (oblíbené jsou zavařovací sklenice Omnia o objemu 0,7 l), kovu (plechovky od konzerv) či plastu (PE láhev). Fixační tekutina může být přímo v pasti, nebo se jí naplní vložený kelímek. Kelímek svým průměrem buď přesně koresponduje s hrdlem pasti a tím pádem v pasti visí, nebo je menší a stojí na dně. Mezi hrdlo pasti a stojící kelímek se přitom umísťuje trychtýř. Obě konstrukční řešení mají své nevýhody, obecně však do pastí s trychtýřem padá více živočichů. Příčinou je pravděpodobně „důvěryhodnost“ šikmé plochy trychtýře oproti svislé stěně vloženého kelímku či samotné pasti pro epigeon. Je doloženo, že řada půdních bezobratlých dokáže zabránit vpadnutí do pasti s kolmými stěnami, přestože už v ní většinou těla byla (Gerlach a kol. 2009a, 2009b, Fryčka 2012). Typické je podobné „vycouvání visícího zvířete“ pro mnohonožky, stejnonožce i stonožky, jelikož mají hodně končetin.

Zajímavým aspektem je barva zemní pasti. Přestože se u epigeonu nepřikládá barevnému vidění příliš velký význam (na rozdíl od opylovačů či herbivorů, kteří za letu vybírají vhodnou potravu), je doloženo, že i barva padacích zemních pastí ovlivní velikost úlovku. Pavouci preferují bílou či žlutou barvu, suchozemští stejnonožci naopak zelenou či hnědou, respektive se nápadnějším barvám spíše úspěšněji vyhýbají (Buchholz a kol. 2010).

Význam pro efektivitu má i průměr pasti. Velké pasti sice mají teoreticky větší pravděpodobnost, že je živočich nemine a spadne do nich, naproti tomu však díky větší hladině fixační tekutiny v pasti může být tato past snáz detekovatelná a živočich se jí vyhne, nebo je do ní nalákán. Je doloženo, že například pro odchyt sekáčů je vhodné používat pasti s menším průměrem (Stašiov nepubl.). Etylenglykolové pasti s velkým průměrem přilákají absolutně více střevlíků, drabčíků i pavouků, byť při přepočtu na plochu pasti jsou malé pasti relativně účinnější (Work a kol. 2002). Se zmíněnou detekovatelností pastí přitom souvisí typ použité fixační tekutiny. Vhodná tekutina by měla být schopna nejen zafixovat živočicha v pasti (čili jej poměrně rychle usmrtit), ale také zafixovat (konzervovat) jeho tělo proti rozkladu. Zároveň by měla být pokud možno levná a také šetrná vůči životnímu prostředí. Taková látka však zatím nebyla objevena, proto se využívá řada jiných, které mají své jednotlivé nevýhody. Nejběžněji se zřejmě využívá vodný roztok formaldehydu, přičemž však formaldehyd je karcinogenní. Náhražkou tak může být ethylenglykol (který je drahý), ethanol (který se rychle vypařuje), roztok kyseliny octové (který účinně rozpouští vápenité soli v kutikule stonožkovců a stejnonožců), voda (ve které materiál rychle maceruje) a podobně. Přitom například výhodou etanolu je, že má nízké povrchové napětí a bezobratlí se v něm rychle utopí (Szinwelski a kol. 2013). Nejen, že ani jedna látka není z těchto úhlů pohledu ideální, ještě navíc se liší svou atraktivitou či odpudivostí pro jednotlivé skupiny bezobratlých. Je známo, že formaldehyd je atraktivní pro některé druhy střevlíků, ale odpuzuje jiné střevlíky a také sekáče (Pekár 2002). Voda může být atraktantem hlavně v letním období, nebo například v suchém prostředí Středomoří (Simaiakis a Mylonas 2006). V současné době navíc čím dál palčivěji zesiluje poptávka po fixační tekutině, která zachovává DNA pro následné genetické analýzy uloveného meteriálu (Szinwelski a kol. 2012).

Při plánování výzkumu je také nutné uvážit vhodný interval výběru úlovku z pastí. Příliš časté výběry se projeví destrukcí nejbližšího okolí pastí, příliš dlouhé intervaly mezi výběry však sníží celkový úlovek, protože z větší části zaplněná past většinu živočichů olfaktoricky odpuzuje. Ideální intervaly jsou obvykle jeden až tři týdny (Schirmel a kol. 2010). Zároveň je vhodné si naplánovat celkové trvání výzkumu. Entomologové obvykle pasti využívají během vegetační sezóny (ca duben až říjen), nicméně řada druhů mnohonožek, stonožek, sekáčů či dalších je aktivní i v průběhu zimy. Některé hrbule (mnohonožky řádu Chordeumatida) s jednoletým životním cyklem dosahují pohlavní zralosti v únoru a během několika málo dní se páří a hynou (Meyer 1990). Determinace jejich juvenilních stádií na druhovou úroveň je přitom nemožná a proto je nezbytné chytat například mnohonožky po celý rok.

V neposlední řadě je nutné zvážit počet instalovaných zemních pastí a jejich rozmístění v terénu. Počet pastí by měl být kompromisem mezi fyzickými schopnostmi výzkumníka pasti vybírat a třídit, mezi únosným zatížením populací místních druhů odlovem a mezi schopností pastí zachytit reprezentativní vzorek společenstev. Instalace velkého počtu pastí představuje zbytečně usmrcený materiál a zbytečnou práci výzkumníka, malý počet pastí znamená malý počet zaznamenaných druhů. Například je doloženo, že pětinásobné navýšení pastí (z pěti na 25) zvýší počet zaznamenaných druhů střevlíků dvojnásobně (Obrtel 1971). Za ideální počet na jedné lokalitě se proto považuje deset až patnáct zemních pastí (Soviš 2010). Rozmístění pastí je přitom také nutno pečlivě zvážit. Z praktických důvodů je obvyklé rozmístit pasti do čtvercové sítě nebo do linie. Pasti přitom nesmějí být příliš blízko u sebe, aby si „nekonkurovaly“. Je doloženo, že pasti ležící blízko u sebe mají menší úlovky než pasti vzdálenější. Přespříliš vzdálené pasti však přinášejí problémy s jejich dohledáním a zbytečně prodlužují čas potřebný k jejich výběru. Za ideální spon pastí v linii se považuje 10–20 m (Ward a kol. 2001).

Zvýšit efektivitu pastí, neboli eliminovat problém průměru pasti, jejich počtu a vzdálenosti mezi nimi, lze pomocí bariér, které usměrní pohyb živočichů směrem k pasti. Takovéto bariérové pasti se sice používají hlavně při studiu migrací obojživelníků, nicméně neobvyklé nejsou ani při studiu epigeonu (Winder a kol. 2001, Grgič a Kos 2009, Hora 2010).

Zemní pasti jsou také opatřeny stříškou, která chrání past před jejím zanesením listovým opadem. Stříšky však výrazně pozměňují mikroklima v těsném okolí pasti. Často se používají praktické stříšky plechové, jejichž zahnuté rohy poslouží jako podpěra stříšky. Nicméně svítí-li na tyto stříšky během dne byť krátkodobě slunce, je pod nimi příliš sucho a teplo. Takové prostředí může významnou část potenciálního úlovku odradit, pod stříšku nevleze a pasti se tak vyhne. Naproti tomu přírodní materiály, jako jsou větve, kůra, kameny, se nad past umísťují dosti komplikovaně, nerovnoměrně a leží-li přímo na povrchu země, mohou vytvářet bariéru bránící v přístupu k vlastní pasti. V ekologických studiích, kde výzkumník instaluje velké množství pastí, se velmi často využívají papírové, polystyrenové či plastové stříšky, jež jsou obvykle tvořeny táckem z daného materiálu připíchnutého drátem či hřebíky nad past. Barva stříšky velikost úlovku neovlivňuje (Buchholz a Hannig 2009).

Kvantitativní vzorkování půdu obývajících bezobratlých

Hlavní konstrukční nevýhodou padacích zemních pastí je skutečnost, že odchytávají pouze tzv. epigeicky aktivní část půdní fauny. Aby se do pasti živočich ulovil, musí přelézt její okraj, který je na povrchu půdy. Zemní pasti proto silně podhodnocují abundance edafických druhů, které obývají půdní profil, či dokonce tzv. *Milieu Souterrain Superficiel*, neboli systém podzemních povrchů. Jedná se o prostory, jež jsou pod povrchem půdy. Příkladem může být suťový svah, na jehož povrchu se z opadu vytvořila půdní vrstva. Ta pod sebou ukrývá soubor skulin a štěrbin mezi podloženými kameny. Tento fenomén se s ohledem na světový primát francouzské speleologie tradičně označuje uvedeným francouzským termínem a zkratkou MSS, jíž se přizpůsobil i později zaváděný anglický termín *Mesovoid Shallow Substratum* (složenina *mesovoid* se skládá z předpony *meso-* značící v dané souvislosti „středně velký“ a kmene slova *void* znamenající v tomto kontextu „dutina, pór“). Vzorkování společenstev edafonu obývajících MSS je extrémně obtížné. Individuální sběr možný není, jelikož v porézní suti živočichové snadno prchají hlouběji. Alternativou je zakopat padací past pod povrch půdy, inspekce pasti je však příliš destruktivní a tudíž tato past není vhodná na detailnější a dlouhodobé průzkumy (Růžička 1992, Yamaguchi a Hasegawa 1996, Tuf a kol. 2008, Pacheco a Vasconcelos 2012). Podobně málo efektivní jsou tzv. *shingle traps* jež tvoří uzavřený kelímek s návnadou a vlhkým hadříkem, z nějž vede plastová hadička zastrčená do prostor MSS, pukliny ve skále a podobných nedostupných štěrbin (Barber 1997). Kelímek je nutno často kontrolovat, aby se případný úlovek navzájem nezkonzumoval.

Vhodnější a relativně nejsnazší je instalace a kontrola tzv. *drillings*, neboli subterránních pastí. Jedná se o dlouhou plastovou trubku, která je zakopána ve vertikální poloze tak, aby jeden její konec ústil na povrch půdy. Druhý její konec může být tak hluboko, jak to situace na lokalitě dovolí, obvykle se hloubka pohybuje mezi 60 a 150 cm. Stěny trubky jsou perforované v různých hloubkách či po celém profilu. Dovnitř tohoto pláště se spouštějí kelímky s fixační tekutinou. Takový kelímek může být jen jeden, přičemž fixuje materiál vstupující do pasti otvory po celé délce pasti, či jen otvory v konkrétní hloubce. S ohledem na extrémní náročnost instalace těchto pastí například v živých suťových svazích je efektivnější využít soustavu kelímků na středové rozšroubovatelné závitové tyči. Tato soustava umožňuje v jedné pasti srovnávat aktivitu bezobratlých v různých hloubkách. Klasické drillings s jedním kelímkem jsou používány hlavně rumunskými výzkumníky v krasových oblastech (Ilie 2003a, 2003b), soustavy s více kelímky vyvinuli Rakušani pro studium bezobratlých v alpských suťovištích a jsou využívány v Česku i na Slovensku (Laška a kol. 2011, Rendoš a kol. 2012, 2016). Výhodou subterránních pastí je možnost získat zcela unikátní vzorky společenstev, které jsou jinými metodami nedostupné. Pomocí těchto pastí se například podařilo zdokumentovat larvální vývoj strašníků (Ilie 2003a), sezónní vertikální migrace plachetnatek (Kopecký a Tuf 2013), probíhající evoluci pavouků (Růžička a kol. 2011), náhradní nížinné biotopy pro horské a jeskynní stonožky (Tuf a kol. 2017) či brouky (Gilgado a kol. 2014), unikátní druhy pyrenejských mnohonožek (Gilgado a kol. 2015), cesty kolonizace jeskynních systémů (Tuf a kol. 2008, Tajovský a kol. 2013) atp.

Subterránní pasti jsou však velmi náročné na instalaci a přestože poskytují unikátní data o distribuci a způsobu života půdní fauny, mají relativně nízkou účinnost – například celoroční úlovek stonožek do tří pastí na jedné lokalitě se pohybuje pouze v řádech jedinců až desítek jedinců (Rendoš a kol. 2016, Tuf a kol. 2017). Pro vzorkování půdních bezobratlých ve svrchních vrstvách půdy je proto vhodnější využívat odběr půdních vzorků. Principem této metody je vyjmout neporušený vzorek o definované ploše a hloubce a transportovat jej do laboratoře k dalšímu zpracování. Díky tomu jsou půdní vzorky ideální metodou pro kvantitativní ekologii, protože jako jediné dávají přímé informace o abundanci jednotlivých druhů bez ohledu na jejich aktivitu.

Volba vhodné velikosti a hloubky půdního vzorku je zásadní pro správné využití této metody. Velikost neboli plocha vzorku souvisí s abundancemi modelové skupiny, kterou se snažíme studovat. Z pochopitelných důvodů nejsou v žádné původní studii prezentována současně data o abundancích například krytenek a mnohonožek. Krytenky (Arcellinida) patří mezi měňavkovce (Amoebozoa) a dosahují v půdách abundancí řádově milionů až stovek milionů jedinců na metr čtvereční, zatímco mnohonožky dosahují abundancí desítek až stovek jedinců. Proto je velikost adekvátního půdního vzorku pro krytenky nesrovnatelně menší, než vhodná velikost půdního vzorku pro mnohonožky. Z tohoto důvodu se půdní fauna klasifikuje do skupin podle své velikosti (mikro-, meso-, makro- a megafauna), což odráží také ekologické souvislosti jak jejího vzorkování, tak i případných mezidruhových interakcí. Přestože kategorizace živočichů podle jejich velikosti není příliš obvyklá, nelze tento přístup považovat za zcela ojedinělý. Dokladem může být skupina nazývaná „drobní savci“, která také zahrnuje pouze některé druhy vybraných řádů, obvykle hlodavců a hmyzožravců vázaných svou biologií na povrch půdy. Ospravedlněním pro tuto umělou kategorii je, podobně jako u edafonu, shodná metodologie jejich odchytu (živolovné pasti, padací pasti, sklapovací pasti). Jelikož je tato klasifikace víceméně subjektivní, existují regionální odlišnosti projevující se případným zahrnováním letounů, zajícovců, drobných šelem atp. Podobně se projevuje odlišné pojetí této skupiny u ekologů a například veterinářů (Banks a kol. 2010).

Nevýhodou metody půdních vzorků je jejich objem, respektive hmotnost. Vzorek o hloubce 10 cm a povrchu $^1/_{16}$ čtverečního metru, což je vhodná velikost pro studium půdní makrofauny, váží dle půdních vlastností přibližně šest až osm kilogramů. Pomineme-li fyzickou náročnost vlastního odběru půdních vzorků, jejich hmotnost limituje celkový počet odebraných vzorků na množství, které jsme schopni fyzicky transportovat z terénu do laboratoře. Logistika tohoto transportu je tudíž dost zásadním aspektem pedozoologického výzkumu pomocí půdních vzorků.

Další nevýhodou metody půdních vzorků je jejich destruktivita. V lese je půda dosti kyprá a „jámy“ o průměru více než čtvrt metru a hloubce 10 cm jsou poměrně rychle vyplněny materiálem z jejich zborcených stěn, listovým opadem a podobně. Na loukách a pastvinách, kde je povrch půdy protkán hustým kořenovým systémem travin, jsou však místa po odběru vzorků dlouho viditelná a nápadná. Podobně na polích je dopad vzorkování nápadný. Majitelé těchto pastvin a polí proto nepovažují půdní vzorky za příliš vhodnou metodu. Na pastvinách dle nich údajně hrozí také nebezpečí zranění pasoucím se zvířatům, odnos ornice z polí je trnem oku zvláště majitelům polí ovlivněných erozí. Zároveň je nutné si uvědomit, že abundance půdní fauny na polích a pastvinách je výrazně nižší, než abundance v lesních půdách. Je proto vhodné zvážit přínos této metody a všechna případná negativa a zamyslet se nad případnými alternativami.

Vhodnou náhražkou půdních vzorků mohou být návnadové podzemní pasti. Vzorem pro návnadové podzemní pasti je ekologická metoda pro studium dekompozice. Ekologové využívají pro kvantifikaci dekompozice jednak proužky celulózy, jednak tzv. *litter bags*. Zatímco pomocí proužků celulózy se studuje hlavně celulolytická aktivita půd (čili mikrobiální rozklad), lze pomocí *litter bags* posoudit i význam členovců. Tyto sáčky s opadem jsou vytvořeny ze síťoviny, která umožňuje instalovat definované množství opadu na lokalitě a postupným odebíráním jednotlivých sáčků stanovovat rychlost a průběh dekompozice. Velikost ok v síťovině může také umožnit či vyloučit přístup k opadu jednotlivým velikostním skupinám edafonu, čili můžeme např. posoudit význam makrodekompozitorů pro rozklad opadu. Tyto sáčky s opadem jsou občas využívány i jako návnady pro dekompozitory – jsou transportovány do laboratoře, kde jsou z nich extrahováni chvostoskoci či roztoči (Pflug a Wolters 2001).

Podobně návnadové podzemní pasti představuje organická hmota instalovaná do půdy pod její povrch. Primárně ji tudíž nekolonizují zástupci epigeonu (vzorkovatelní snáze pomocí padacích zemních pastí), ale v půdě se pohybující zástupci edafonu. Organická hmota je instalována v sáčku či kapse z pletiva (kovového či plastového, vhodná velikost ok je kolem 2 cm), do které se dává optimálně seno, vřetena vyloupaných kukuřičných klasů či jiný rostlinný materiál (Prasifka a kol. 2007). Organický materiál je vhodné před instalací navlhčit a ponechat in situ po dobu dvou až tří měsíců. Právě po osmi až deseti týdnech je v materiálu nejvyšší množství zástupců půdních bezobratlých (Tuf a kol. 2015b).

Jinou alternativou půdních vzorků je metoda prosevu. Pomocí prosívadla je možné dosáhnout redukce objemu a hmotnosti vzorku při snaze zachovat v něm pokud možno všechny živočichy. Používané prosívadlo je zařízení vyvinuté entomology, převážně k získávání brouků. Využívá se hlavně v prosevům opadu, nahromaděné organické hmoty, jako je trouch v dutinách stromů, lokální akumulace hrabanky, mechových polštářů ap., principiálně je lze však využít i na vzorky půdy. Hlavní výhodou je velikostní selekce materiálu, kde fragmenty substrátu o velikosti větší než zájmová skupina jsou ze vzorku pomocí síta odděleny a vyhozeny. Proces prosívání však může některé živočichy významně poškodit. Brouci mají převážně pevnou kutikulu a kompaktní tvar, jehož dosáhnou i reflexním přitažením končetin a tykadel k tělu při podráždění. Naprosti tomu řada půdních živočichů má kutikulu měkkou a/nebo končetiny a ostatní tělní přívěsky relativně dlouhé (vidličnatky, chvostatky, stonožky, stejnonožci, hrbule).

Jak oddělit živočichy od substrátu?

Metoda půdních vzorků, prosevů či návnodových pastí má další úskalí – výzkumník získá a do laboratoře transportuje substrát, ve kterém jsou schovaní bezobratlí. Jejich oddělení od substrátu lze dosáhnout pomocí aktivních či pasivních metod. Aktivní metody zahrnují aktivitu výzkumníka a jsou obecně pracnější. Většinou se využívá ruční rozbor vzorků, který je fyzicky i časově náročný a překvapivě neefektivní – dle nepublikovaných výsledků Karla Tajovského se efektivita rozboru půdního vzorku u nezacvičeného pracovníka pohybuje kolem 10 % živočichů. Nicméně i zacvičená osoba s delší praxí dokáže ze vzorku vytřídit pouze okolo třetiny zástupců edafonu. Efektivnější je rozbor prosevu, jelikož ten je velikostně homogennější, tudíž jsou živočichové relativně nápadnější (navíc odpadá potřeba prohlížet změť kořenů ve vzorku). Jinými možnostmi jsou promývání vzorku na soustavě sít, rozplavování v nasyceném cukerném roztoku (anorganický materiál klesne ke dnu, organický materiál plave na hladině) apod. Tyto metody jsou však nejen časově náročné, ale s výjimkou ručního rozboru i relativně drahé.

Pasívní metody naproti tomu přenášejí aktivitu výzkumníka na vlastní živočichy; živočichové se dokáží pohybovat, proto je stimulujeme k pohybu a snažíme se je ze vzorku vypudit. Obvykle se vychází ze skutečnosti, že živočichové reagují na pokles vlhkosti substrátu negativní geotaxí neboli přesunem do hlubších vrstev. Tato strategie je v přírodě vhodnou adaptací na přežívání suchých (letních) měsíců, jelikož v hlubších vrstvách půdy jsou teplotní i vlhkostní podmínky stabilnější a příznivější. Pokud je vzorek (či prosev či návnadová past) umístěn na perforované podložce (například pletivu), živočichové mohou skrze tuto podložku propadávat do podstavené nádoby. Zařízení, která využívají tento princip, se jmenují eklektory či extraktory a byla do zoologické praxe uvedena na počátku minulého století (Berlese 1905, Tullgren 1918). Do dnešní doby prošla tato zařízení mnoha vylepšeními (zdrojem tepla k vysoušení Berleseho vzorků byla horká voda) až k současným typům s udržovaným teplotním gradientem a s regulovaným zesilovaným ohříváním (Tajovský a Pižl 1998).

Volba vhodné metody

Půdní zoologové mají zjevně na výběr řadu metod pro vzorkování společenstev. Pokud se však jednotlivé metody liší svou efektivitou, jak vybrat tu pravou? Existuje několik publikací, které se věnují této problematice. Buď se jedná o patentované pomůcky a nástroje (zvláště v Číně se objevují současné patenty klasických dosud nepatentovaných zařízení), nebo podstatně vzácněji doporučené protokoly pro vzorkování. Tyto protokoly obvykle vycházejí z vlastní zkušenosti a jsou poplatné působišti autora (Kos 1988, Mesibov a kol. 1995, Means a kol. 2015). Mnohem více prací je zaměřeno na srovnání dvou či několika metod s doporučením té kvalitnější (McFadyen 1955, van Straalen 1983, Mesibov a kol. 1995, Mommertz a kol. 1996, Weeks a McIntyre 1997, Simmons a kol. 1998, Holland a Smith 1999, Isaia a kol. 2006, Prasifka a kol. 2007, Sakchoowong a kol. 2007, Sólymos a kol. 2007, Băncilă a Plăiaşu 2009, Véle a kol. 2009, Sabu a kol. 2011, Shaw a Ozanne 2011, Corti a kol. 2013), některé práce obecně upozorňují na nedostatky a omezení široce využívaných metod (Adis 1979, Lemieux a Lindgren 1999, Lang 2000, Gotelli a Colwell 2001, Karaban a kol. 2012), eventuálně navrhují kombinaci různých metod (Querner a Bruckner 2010, Tuf 2015).

Zdá se naprosto zřejmé, že ke kvalitnímu kvantitativnímu popisu taxocenóz stonožek, mnohonožek či suchozemských stejnonožců (tj. skupin obsahující druhy s převažující epigeickou aktivitou i druhy s endogeickým způsobem života) je nezbytné kombinovat vícero metod. Pro dlouhodobější studium je vhodné zkombinovat například metodu padacích zemních pastí a tepelné extrakce půdních vzorků (Tufová a Tuf 2003, Tuf 2015). Pro faunistické potřeby je vhodné vzorkovat i „atypické“ mikrohabitaty, které mohou skrývat některé další druhy s neobvyklým způsobem života. Příkladem může být stonožka *Lithobius borealis* Meinert, 1872, která je odchytávána do zemních pastí relativně vzácně, protože s oblibou žije na stromech (Spitzer a kol. 2010, Božanić a kol. 2013).

Publikace autora k dané tematice, jež jsou součástí této práce

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Evoluční aspekty života v půdě

Suchozemští stejnonožci jako modelová skupina pro evoluční biology

Symplesiomorfním neboli fylogeneticky původním znakem všech korýšů je volně plovoucí vodní larvální stádium. Přestože někteří krabi (ca 2 % druhů řádu Decapoda) a část různonožců čeledi Talitridae (ca 3 % druhů řádu Amphipoda) jsou také schopni žít v dospělosti mimo vodu (a vzácně se mimo vodu i rozmnožovat), pouze suchozemští stejnonožci jsou vyšším taxonem (podřád Oniscidea), kde je terestrický způsob života včetně rozmnožování převažující strategií. Suchozemští stejnonožci představují jeden z jedenácti podřádů stejnonožců, jejich druhová diverzita však představuje více než třetinu (36 %) celkového počtu druhů stejnonožců a dokonce přes 5 % všech popsaných druhů korýšů (Zhang 2013). To samo o sobě dokládá rychlou evoluční radiaci stejnonožců, kteří objevili na souši volnou ekologickou niku.

Vznik suchozemských stejnonožců

Suchozemští stejnonožci nejsou příliš častí ve fosilních nálezech. Jejich vápenitá kutikula není dostatečně chráněna voskovou epikutikulou (srovnatelnou s hmyzí či pavoukovčí epikutikulou), takže na souši špatně fosilizuje a navíc není chráněna proti pryskyřici s jejím kyselým pH. Přesto však máme několik nálezů v jantaru ze svrchní křídy, obsahujících dobře adaptované stejnonožce (Broly a kol. 2013). Starší nálezy v jantaru k dispozici nemáme, jelikož jsou obecně dosti vzácné kvůli nízké produkci pryskyřice u tehdejších stromů. Nejstarší svrchnokřídové fosilie suchozemských stejnonožců pocházejí z jihozápadní Francie, severního Španělska a Myanmaru, z podobného období pocházejí také četné ischnofosilie. Ačkoliv z nich není jasné, zda je vytvořili stejnonožci suchozemští či mořští zástupci jiných podřádů, je zjevné, že nějací stejnonožci se pohybovali v jemných nánosech bahna v intertidální zóně a opouštěli tehdy vodu.

Nejstarší fosilizovaní stejnonožci jsou známi již z karbonu, jedná se o zástupce bazální skupiny Phreatocidea. Podle molekulárních i morfologických analýz je zřejmé, že vznik podřádu Phreatocidea následovalo odštěpení podřádu Asellota a po něm i Oniscidea. Zbylé podřády vznikly až později. Přitom fosilní zástupci modernějšího podřádu Sphaeromatoidea jsou doloženi z triasu, pravděpodobně i z permu. Z těchto nepřímých dokladů lze vyvodit, že společný předchůdce všech suchozemských stejnonožců se vyskytoval nejpozději v permu (Broly a kol. 2013).

Nicméně existují další nepřímé důkazy toho, že suchozemští stejnonožci pravděpodobně vznikli již v karbonu. Tím je výskyt všech hlavních (tzn. velkých, druhově početných) čeledí na všech kontinentech. Jelikož suchozemští stejnonožci mají velmi nízký disperzní potenciál (neumějí létat, nejsou dobří běžci, nevyužívají forezi ani balooning, nemají odolná vývojová stádia preadaptovaná k pasivnímu šíření), velmi pravděpodobně osídlili tyto kontinenty v době, kdy byly tyto propojené, tzn. během svrchního karbonu, kdy se vytvořil superkontinent Pangea. Navíc v tomto období vznikly také rozsáhlé lesy, které produkovaly množství opadu. Vznik této nové niky (život v lesní hrabance) suchozemským stejnonožcům umožnil zabydlet Zemi a mimořádně diverzifikovat. Společné vyhodnocení poznatků z paleontologie, paleoekologie, paleobiogeografie i fylogeneze nám tedy umožňuje pohlížet na suchozemské stejnonožce jako taxon vzniklý v karbonu, čili před ca 300 miliony let.

Morfologické a fyziologické adaptace na terestrický způsob života

Opuštění vodního prostředí a přesun na souš byl pro stejnonožce spojen s vyřešením řady problémů. Zcela nejzásadnější jistě bylo ochránit se před vyschnutím. Tomu mohly napomáhat preadaptace spojené s velkým kolísáním hladiny oceánu, které vedly k vysychání mělkých moří. S tím souvisely změny salinity moří a vznik adaptací, které chránily živočichy před osmotickým vysušováním ve slanější vodě. Druhou významnou adaptací spojenou s ochranou před vysycháním byla redukce dýchacích povrchů. Nízké množství kyslíku ve vodě vyžaduje relativně velký povrch žaberních lupínků, naproti tomu obsah kyslíku ve vzduchu dosahující 35 % umožnil invaginaci dýchacího epitelu dovnitř žaberních lupínků a jejich vyústění poměrně malým otvorem (stigmatem).

Současní stejnonožci zahrnují i druhy, které jsou schopny zotavit se z vyschnutí, jež představuje ztrátu až 25 % jejich tělesné hmotnosti (Carefoot 1993). Přestože hemolymfa obsahuje jen 42 % celkového množství vody v těle stínky obecné (*Porcellio scaber* Latreille, 1804), podílí se až 69 % na reverzibilních ztrátách vody této stínky (Edney 1968), což samozřejmě doprovází také změny koncentrace iontů v hemolymfě. Stínka zední (*Oniscus asellus* Linnaeus, 1758) dokáže tolerovat kolísání koncentrace NaCl mezi 1,59 % a 2 % (Bursel 1955). Změny osmolality během vysychání však nejsou tak výrazné, jak by odpovídalo pouze ztrátám vody. Stejnonožci dokáží silně regulovat osmotický tlak v hemolymfě aktivním přesunem iontů Na^+ a Cl^- do zadního střeva a hepatopankreatu pomocí asociovaných makromolekul (Wright a kol. 1997). Proto hemolymfa může fungovat jako zásobník vody během vysychání.

Další významné změny ve způsobu života suchozemských stejnonožců spočívaly v přesunu potravní specializace ze stélek řas na rostlinná pletiva (respektive rostlinný opad) charakteristická přítomností složitých odolných (špatně stravitelných) látek, jako je celulóza či lignin. Velmi užitečnou adaptací pro trávení celulózy se ukázala akvizice endosymbiotických bakterí do trávicího traktu (Zimmer a Topp 1998). Další potravní nároky souvisely s nedostatkem prvků (např. Ca, Mg), které získávají mořští stejnonožci v nadbytku přímo z vody.

Preadaptací mořského předchůdce suchozemských stejnonožců na život na souši bylo jeho dorzoventrální zploštění, které mu umožnilo poměrně energeticky nenáročnou lokomoci (např. ve srovnání s laterálně zploštělými blešivci). Velmi užitečnou preadaptací, kterou sdílejí všichni váčkovci (Peracarida), je přímý vývoj spojený s nošením snůšky ve váčku (marsupiu) samic. Přestože řada korýšů pečuje o snůšku vajíček tak, že ji nosí při sobě, váčkovci se takto starají i o první larvální stádia a životní cyklus váčkovců tudíž nezahrnuje volně plovoucí larvu. Přechod na souš u suchozemských stejnonožců byl touto okolností značně usnadněn. Nejstarší fosilní doklad přítomnosti marsupia a v něm se vyvíjejících larev u suchozemských stejnonožců máme z mexického jantaru starého 23 milionů let (Broly a kol. 2017), péče o potomstvo a marsupium u váčkovců však pochopitelně vznikly podstatně dříve.

Marsupium suchozemských stejnonožců je dvojího typu. Primitivní zástupci (např. rod *Ligia*) mají marsupium otevřené na předním i zadním konci a volně jím vzlíná voda z otevřeného vodovodního systému na povrchu stejnonožce. Naproti tomu uzavřené marsupium pokročilejších forem s vodovodním systémem nesouvisí. Uvnitř marsupia jsou prstovité výběžky (4–28) nazývané kotyledony, které do jisté míy mají stejný význam, jako kotyledony dělohy savců. Vyrůstají ze sternitů prvního až pátého pereonitu, prorůstají mezi nakladená vajíčka a poskytují jim a posléze i larvám výživu, kyslík a vápník (Hoese a Janssen 1989). Dlouhé kotyledony se vyvíjejí u druhů obývajících humidní prostředí, zatímco větší počet krátkých kotyledonů je typický pro pouštní druhy (Lewis 1991). Marsupium teoreticky může bránit volvaci, proto druhy, které se dokáží svinout do kuličky, mají marsupium nevystouplé, ale břišní strana těla samice je prohnutá dovnitř. Tímto způsobem navýší velikost snůšky, i při zachování schopnosti svinout se, o 10–25 % (Appel a kol. 2011).

Hospodaření s vodou

Suchozemští stejnonožci mají na svém těle tzv. *water conducting system* neboli vodovodní systém (Hoese 1981). Ten se skládá z mělkých kanálků plných jemných kolíkovitých výrůstků. Hlavní kanálky vedou podél těla po břišní straně poblíž bází končetin. Kanálek začíná u vyústění maxilárních žláz a končí u prvních pleopodů (Carefoot 1993). Rozlišujeme dva typy těchto systémů:

- Typ Ligia je otevřený vodovodní systém. Moč z maxilárních žláz je vedena k pleopodům a konečníku, ve kterém je reabsorbována. Během transportu se vypařuje čpavek. Stejným systémem je transportována i voda, která do systému vstupuje pomocí kapilárních sil. *Ligia* přiloží k sobě dvě poslední kráčivé končetiny a vnoří je do kapky vody (Horiguchi a kol. 2007).
- Typ Porcellio je uzavřený vodovodní systém. Kromě dvou podélných kanálků existují ještě příčné spojky na hřbetní straně těla podél zadních okrajů tergitů. Moč cestou ztrácí čpavek a svlažuje dýchací povrchy pleopodů (Hoese 1981).

Pravděpodobně všichni stejnonožci dokáží přijímat vodu pitím a také ponořením uropodů do vody. Voda, která vzlíná po uropodech, svlažuje pleopody, případně je absorbována v konečníku (Spencer a Edney 1954). Příjem vody uropody přitom svým významem převyšuje příjem vody ústy (Drobne a Fajgelj 1993).

Kromě příjmu vody v tekutém skupenství mohou stejnonožci absorbovat také vodní páru, respektive získávat vodu ze vzdušné vlhkosti. Tuto schopnost stínek předpokládal již Eric Berry Edney (1951), experimentálně ji jako zvýšení hmotnosti stínek obecných ve vlhkém vzduchu potvrdil Piet den Boer (1961). Stínky jsou schopny absorbovat vodu ze vzduchu s vlhkostí nad 90 % díky hyperosmotickým roztokům v kutikule (Wright a Machin 1990, 1993). Pleon je obvykle zvlhčen roztokem isoosmotickým s hemolymfou, což je potřeba pro respiraci. V případě potřeby však může být tento roztok nahrazen zmíněným hyperosmotickým roztokem s osmolalitou až desetkrát vyšší než hemolymfa (Wright a O´Donnell 1995), čehož je dosaženo exkrecí Na^+ a Cl^- iontů. Efektivitu absorbce vody zvyšují stejnonožci také rytmickými pohyby ploeopodů, které napomáhají cirkulaci vlhkého vzduchu. Schopnost absorbovat vodu ze vzduchu mají všechny testované druhy ze skupiny Crinocheta a Diplocheta, ale žádný ze skupiny Synocheta (Wright a Machin 1993). Drobní synochetní stejnonožci ale žijí endogeicky ve svrchních vrstvách půdy, kde je stálá vlhkost půdního vzduchu kolem 100 % (Tuf a Jeřábková 2008) a kde tudíž nedochází ke ztrátám vody evapotranspirací.

Dýchání

Respirace, nezbytná podmínka metabolizmu živočichů, souvisí s průchodem molekul O_2 a CO_2 přes dýchací povrch živočicha. Nicméně molekula kyslíku či oxidu uhličitého je podstatně větší než molekula vody – dýchacím povrchem tudíž dochází také k evaporaci. U drobných terestrických forem může být kyslík přijímán difúzí přes pokožku, malí živočichové se totiž mohou schovat v půdě, kde je obvykle vlhkost vzduchu stoprocentní. Větší druhy se brání evaporaci tvorbou silné kutikuly, která omezí ztráty vody, a omezením dýchacího povrchu na nezbytné minimum potřebné k dostatečnému zásobení těla kyslíkem.

Vodní stejnonožci dýchají buď celým povrchem těla, nebo pomocí pleopodů (3. až 5. pár), čili přívěsků zadečkových článků. Každý pleopod se skládá z lístkového exopoditu, který kryje endopodit, jenž funguje jako žábry. Pohyby exopoditů jednak přihánějí k žábrám vodu, jednak napomáhají při plavání. Suchozemští stejnonožci vyřešili příjem kyslíku několika způsoby. Nejdrobnější formy dýchají celým povrchem těla. Hygrofilní formy (např. čeleď Ligiidae) stále využívají endopodity, jež fungují jako žábry, a také vnitřní povrch exopoditů. Žijí v prostředí, kde je dost vody, kterou mohou k pleopodům přivádět pomocí kapilárních sil – přiloží k sobě dvě poslední kráčivé končetiny a kanálkem, který mezi nimi vznikne, voda vzlíná k pleopodům. U větších a více adaptovaných forem dýchací povrchy na exopoditech invadují v různé míře a vytvářejí tzv. preudotracheální žábry, které fungují stejně jako plicní vaky pavoukovců. Tyto dýchací struktury jsou u jednotlivých druhů různě pokročilé, často i uvnitř jedné čeledi. To naznačuje jejich nezávislý opakovaný vznik u různých skupin suchozemských stejnonožců. Příkladem mohou být dýchací orgány u zástupců čeledi Eubelidae, kteří se vyskytují kromě vlhké rovníkové Afriky a jihovýchodní Asie také v (polo)pouštních podmínkách Blízkého východu a severovýchodní Afriky. Paoli a kol. (2002) u zástupců této čeledi rozlišují šest typů dýchacích struktur:

- Typ Atracheodillo: jednoduchý nepřekrytý dýchací epitel na povrchu exopoditu.
- Typ Synarmadilloides: jednoduchý dýchací epitel částečně vchlípený pod povrch exopoditu.

- Typ Eubellum: uzavřený typ plicního vaku, dýchací epitel ve formě rozvětvených kanálků, které ústí na povrch exopoditu několika jednoduchými stigmaty obklopených specificky modifikovaným povrchem.
- Typ Somaloniscus: podobný předchozímu, ale tvar stigmata je modifikovaný a začátek pseudotracheí u stigmat je pokryt kartáčkem krátkých chloupků.
- Typ Aethiopopactes: podobný předchozímu, ale stigma je pouze jediné a pseudotracheje vyplňují celý exopodit.
- Typ Periscyphis: nejpokročilejší typ, kde jediné štěrbinovité stigma, neopatřené modifikovaným okolním povrchem, ústí do širokého atria, ze kterého vychází shluk pseudotrachejí, jež vedou až dovnitř zadečku.

Specificky modifikovaný povrch okolo stigmat, typický pro typ Eubellum, Somaloniscus a Aethiopopactes, tvoří hexagonální struktura připomínající včelí plástev. Funkcí této struktury je rychle odvádět vodu, která by mohla ucpat stigmata. Podobné nebezpečí nehrozí pouštním stejnonožcům rodu *Periscyphis*, u nichž tato struktura vyvinuta není. Podobně štěrbinovité stigma je oproti kruhovému adaptací omezující ztráty vody evaporací. Suchý vzduch, vstupující stigmatem do atria, je zde před vstupem do pseudotrachejí zvlhčován vzduchem vydechovaným, který se zde naopak potřebné vlhkosti zbavuje.

Behaviorální adaptace na terestrický způsob života

Problémy, jimž stejnonožci na souši čelí, je možné řešit buď pomocí zmíněných morfologických či fyziologických adaptací, nebo pomocí změny chování. Behaviorální adaptace jsou přitom obvykle plastičtější. Nejjednodušším způsobem, jak omezit ztráty vody z těla, je omezení aktivity do období, kdy je toto nebezpečí nejnižší. Dalším způsobem, typickým pro suchozemské stejnonožce, je vytváření agregací.

Anekdotická pozorování (nepatřičné) aktivity suchozemských stejnonožců se sporadicky objevují v tisku již od konce XIX. století. Za průkopníka systematického studia aktivity suchozemských stejnonožců však je možno považovat britského zoologa Johna Leonarda Cloudsley-Thompsona, který se studiu diurnální aktivity věnoval v padesátých letech minulého století a příležitostně se k tomuto tématu vracel i později (viz např. Cloudsley-Thompson 1977). Zjistil jako první, že spouštěčem epigeické aktivity mimo úkryty je intenzita světla, že však na početnost aktivních stejnonožců má vliv teplota i vlhkost. Zevrubně však v tomto období prostudovali aktivitu stínky obecné v terénních podmínkách nezávisle na sobě australský zoolog John Le Gay Brereton a holandský ekolog Pieter Johannes den Boer. Oba zjistili, že stínky se přes letní období často zdržují také na stromech, kde se schovávají ve štěrbinách kůry, zimují však hlavně v půdě a opadu při úpatí stromů (Brereton 1957, den Boer 1961). Vhodný úkryt jim pomáhají najít senzory, které registrují vlhkostní (např. Waloff 1941), teplotní (např. Dietrich 1931) a světelný (např. Warburg 1964) gradient a které jsou zodpovědné za jejich thigmotaxi (Friedlander 1964). Je tudíž dobře známo, že stínky tráví většinu dne v úkrytech kvůli vysokému odparu vody z těla přes kutikulu (Edney 1968). Pokud je venkovní teplota vysoká a teplota v úkrytu dostatečně nízká, toto chování se umocňuje (Hassall a Tuck 2007). Pokud je však teplota v úkrytu příliš nízká, mohou stejnonožci reagovat i pozitivní fototaxí, zřejmě ve snaze ohřát se na slunci (Refineti 1984).

Obecně lze shrnout, že stejnonožci upouštějí úkryty v noci a je možné je najít i na místech, kde se přes den prakticky nevyskytují, např. lezoucí po zdech (Cloudsley-Thompson 1951). Délka aktivity mimo úkryt je přibližně jedna hodina (den Boer 1961), po té se množství vody v těle sníží na kritickou hladinu (Edney 1951) a musí se schovat do úkrytu s vyšší vzdušnou vlhkostí. V těchto úkrytech potom tráví potravu a aktivně absorbují vodu ze vzduchu (Wright a Machin 1990). Z tohoto důvodu jsou vhodné úkryty pro stejnonožce esenciální – umožňují jim připravit se na další prozkoumávání habitatu (Hassall a Tuck 2007).

Schopnost stejnonožců odolávat vyschnutí se přitom liší mezi jednotlivými druhy, často udávané pořadí rodů dle jejich odolnosti je (od nejcitlivějšího po nejodolnější): *Ligia, Philoscia, Oniscus, Porcellio, Cylisticus, Armadillidium* (Edney 1951). Z toho důvodu je denní aktivita relativně často pozorovatelná u svinek rodu *Armadillidium* (druh *Armadillidium versicolor* Stein, 1859, je velmi početný i v Olomouci a lze se s ním potkat na chodnících během dne poměrně běžně). V našem výzkumu jsme zjistili, že denní aktivita je obvyklá i u stínek *Porcellium conspersum* (C.L. Koch, 1841), které také mají relativně silnější kutikulu (Tuf a Jeřábková 2008). Separace aktivity různých druhů do různých částí dne, neboli koncept časové niky, je znám také u jiných skupin živočichů (např. Tuf a kol. 2006, 2012, Krumpálová a Tuf 2013).

S ohledem na typický pattern aktivity suchozemských stejnonožců je zajímavá skutečnost, že stínky rodu *Ligia* je obvykle možné spatřit pobíhat po kamenech během dne. Přestože jejich odolnost vůči vyschnutí je nízká, jejich denní aktivita má adaptivní význam. Jedná se totiž o suchozemské stejnonožce, kteří obývají intertidální zónu. Vlhkost v tomto prostředí není limitujícím faktorem, ale problém, kterému často čelí, je vysoká teplota rozpálených kamenů, jež aktuálně nejsou ochlazovány vlnami. Odpar vody z těla stínky významně snižuje její teplotu, přičemž vlhkost může aktivně doplnit přímo z mořské vody, do které se aktivně potápí (Davenport 1994), či ve vlhkých štěrbinách mezi kameny.

Vedle specifického patternu cirkadiánní aktivity pomáhá stejnonožcům v hospodaření s vodou také agregační chování. Již před 90 lety Warder Clyde Allee experimentálně prokázal, že pokud jsou stínky osamoceny a ponechány v suchém prostředí, je pro ně ztráta 30 % hmotnosti v důsledku vyschnutí fatální a že k této ztrátě dojde již během dvou až sedmi hodin (v závislosti na vlhkosti vzduchu a hmotnosti, respektive velikosti jedince). Pokud však jsou stínky ve skupině deseti jedinců, ztratí po osmi hodinách průměrně pouze 15 % hmotnosti (Allee 1926).

Význam agregačního chování

Suchozemští stejnonožci agregují spontánně a objasnění tohoto fenoménu bylo věnováno velké úsilí řady výzkumníků. První studie se zaměřovaly na profit jednotlivců z tohoto chování ve snaze najít evoluční význam neboli adaptivnost tohoto chování. Hypotéz bylo navrženo i testováno poměrně mnoho. Jednou z těch základních je vyšší pravděpodobnost nalezení partnerky, kde významným atraktorem je signalizace ochoty k páření samicemi. Tato schopnost je adaptivní nejen pro samce, ale i pro samice, které se tímto způsobem dokáží chránit před sexuálním obtěžováním ze strany samců v dobu, kdy vnímavé nejsou. Kromě doložené atraktivity samic pro samce je doložena i atraktivita samců pro samice (Beauché a Richard 2013). Zároveň však vůně jedince stejného pohlaví jako atraktant nepůsobí, proto lze poměrně spolehlivě předpokládat, že dostupnost sexuálních partnerů je jedním z důvodů ochoty stínek agregovat.

Pokud by stimulem pro vznik agregací bylo jen sexuální chování, těžko by bylo uvěřit, že agregují i samice s plným marsupiem, které se pářit nemohou, či dokonce juvenilní jedinci. Pro juvenilní jedince je však možnost připojit se k agregaci velmi významná. Důvodem je poněkud překvapivě kanibalizmus. Nejedná se o lov a následnou konzumaci příslušníků stejného druhu, ale o konzumaci mrtvých jedinců. Důvodem může být příjem nedostatkového vápníku, jak je tomu zřejmě i v případě kanibalizmu larev vně (Hatchett 1947) i uvnitř marsupia (Warburg 1994). Dalším důvodem však je snaha získat endosymbiotické bakterie žijící výlučně v hepatopankreatu stejnonožců, které významně zefektivňují jejich schopnost trávit celulózu (Zimmer a Topp 1998), ale také se zřejmě podílejí na absorbci mastných kyselin a vitamínů. Kromě toho významně zlepšují přežívání, jak zjistila Terézia Horváthová se svými kolegy (2015) pomocí důmyslných experimentů se sterilními stejnonožci. Tyto bakterie (dva druhy, *Candidatus* Hepatoplasma a *Candidatus* Hepatincola, se nikdy nevyskytují společně v jednom jedinci) přitom nejsou vylučovány v exkrementech (Brandstädter a Zimmer 2008). Možnost vertikálního přenosu byla experimentálně zavržena (Horváthová a kol. 2015), tudíž konzumace hepatopakreatu mrtvých jedinců stejného druhu mláďaty je jediný předpokládaný způsob akvizice těchto bakterií. Přebývání v agregacích tudíž zvyšuje pravděpodobnost, že mladá stínka nalezne uhynulého jedince, jehož hepatopankreat může zkonzumovat. Nutno podotknout, že kanibalizmus je sice adaptivním řešením potřeby získání endosymbiontů, zároveň je to však způsob, který mohou posléze využít paraziti pro efektivní horizontální přenos mezi jednotlivými hostiteli (Le Clec'h a kol. 2013).

Další výhodou zdržování se v agregaci je snížení ztrát vody v těle odparem, jak doložil Allee (1926). Tento aspekt je nespecifický, mikroklima kolem kteréhokoliv jedince je ovlivněno jeho metabolizmem. Tudíž je výhodnější se zdržovat vedle jiné stínky, z níž se vypařuje voda, než být sám v sušším vzduchu. Podobná logika stojí za tzv. sociální termoregulací, kterou prosluli například tučňáci císařští (Ancel a kol. 2015). Tato ekologická souvislost platí nespecificky a je zřejmě hlavním důvodem k tvorbě vícedruhových smíšených agregací suchozemských stejnonožců (Cloudsley-Thompson a Constantinou 1987). Ačkoliv stejnonožci profitují ze snížení evaporace, jejich agregační chování je řízeno spíše thigmokinezí, než hygrokinezí, neboli založení agregace ovlivňují hlavně taktilní signály (Freidlander 1965). V praxi to znamená, že v laboratorních podmínkách se budou například tvořit agregace stínek spíše v suchém rohu než na navlhčeném středu experimentálního boxu. Odfiltrovat v laboratoři thigmokinezi při studiu agregačního chování lze například využíváním kruhových arén (Devigne a kol. 2011, Broly a kol. 2012).

Život v agregacích také může fungovat i jako antipredační strategie. Stejný princip využívají kopytníci, kteří se sdružují do stád, respektive ryby sdružující se do hejn. Podstatou je, že pokud se kořist shlukuje, tak má predátor nižší pravděpodobnost, že nějakou kořist potká, než když je tato roztýlena rovnoměrně (Krause a Ruxton 2002). To platí i za předpokladu, že stádo či hejno je nápadnější, než jedinec. Pokud totiž predátor napadne stádo kořisti, obvykle se mu podaří ulovit jen jednoho či několik málo jedinců. Srovnáme-li tudíž pravděpodobnost přežití střetu s predátorem jedince ve velké skupině či jedince při osobním setkání s predátorem, je výhoda „ukrytí se v davu“ zjevná. Každý, kdo se někdy pokusil nasbírat větší množství živých stejnonožců a snažil se vysbírat pinzetou stínky z odhalené agregace, jistě potvrdí, že většina jedinců má dost času na útěk.

Na druhou stranu živočichové platí za život v agregaci vyšší kompeticí o potravu (Krause a Ruxton 2002). Tento aspekt má svou platnost i u suchozemských stejnonožců. Byť se obvykle uvádí, že detritofágové nejsou limitováni dostupností potravy, je dobře známo, že různé druhy mají specifické potravní preference (Carefoot 1973, Rushton a Hassall 1987, Szlávecz a Maiorana 1991, Nair a kol. 1994, Zimmer a Topp 2000) a dostupnost nejoblíbenější potravy proto může být omezená. Je možné, že tento trade-off stojí za existujícím sociálním limitem velikosti agregací, který aktuálně doložili francouzští výzkumníci (Broly a kol. 2012, 2014), velikost ideální agregace stínek obecných je okolo 70 jedinců, přičemž agregace 50-60 stínek má nejnižší průměrné ztráty vody. Zajímavé je, že tato sociální regulace velikosti agregace platí jak pro (stabilnější) jednodruhové agregace, tak pro smíšené agregace více druhů (Broly a kol. 2016). A nutno podotknout, že tento závěr není artefaktem zjednodušených laboratorních podmínek, ale že se nám jej podařilo zopakovat v experimentech s taktilními podněty i více úkryty (Jeníková 2013, Truhlářová 2016).

Antipredační strategie

Přechod na souš pomohl suchozemským stejnonožcům uniknout jejich dosavadním predátorům. Nicméně na souši se postupně setkávali s jinými typy predátorů, jako jsou pavouci, mravenci, stonožky a další draví členovci, ale také obojživelníci, plazi, ptáci a drobní savci. Většina z nich se živí stejnonožci příležitostně, jediný specializovaný predátor stínek a svinek jsou pavouci šestiočky rodu *Dysdera* (Řezáč a Pekár 2007, Řezáč a kol. 2008). Proti predátorům si stejnonožci vyvíjeli různé strategie. Jednou z nejjednodušších je dříve popsané agregační chování, při němž se jedinec schovává v davu a využívá roptýlení pozornosti predátora při odhalení větší skupiny stejnonožců.

Suchozemští stejnonožci jsou poměrně bezbranní, nedisponují silnými kusadly, pevnou kutikulou, či mohutnými trny. Některé svinky sice jsou dlouze otrněné, jedná se však o drobné formy a význam jejich excesivních „ostnů“ je pro biology záhadou (Schmalfuss 1984) a dosud nebyl experimentálně zkoumán. Většina stejnonožců disponuje tzv. repugnatorickými žlázami, které ústí na laterálním okraji pereonitů a na uropodech (Gorvett 1956). Stejnonožci vylučují kapičky lepkavé hmoty z těchto žláz obvykle až po větším ohrožení, jako je manipulace predátora se zvířetem, či kousnutí. Experimentálně lze vyvolat vyloučení této látky pomocí mačkání tvrdou pinzetou či píchnutím entomologickým špendlíkem (Gorvett 1956, Deslippe a kol. 1996), které může připomínat píchnutí pavoučí chelicerou. (Některé další manipulace, jako například silné podchlazení, také vyvolají exkreci z repugnorických žláz, zjevně se však jedná o nespecifickou reakci.) Gorvett (1956) předpokládá, že tento sekret může být tvořen na obranu proti pavoukům a experimentálně také dokládá, že většina pavouků stejnonožce jako kořist odmítá a i když je chytí, po vyloučení sekretu je zase pouštějí. Podobný efekt má tento sekret na mravence; jelikož je lepivý, mravenci okamžitě pouštějí kořist a začínají se čistit (Deslippe a kol. 1996). Sekret obsahuje složky bílkovinné povahy a dosud nebyl analyzován a jeho složení není známo.

Ačkoliv produkce odpudivého zápachu je dobrý způsob pro odrazení útočníka, absence jakýchkoliv pachů je strategie možná ještě účinnější. Syntéza proteinových sekretů je metabolicky náročná, takže při napadení větším počtem mravenců může být stínka přeci jen přemožena a ulovena. Absence povrchových pachů je naopak strategií myrmekofilního specialisty berušky mravenčí (*Platyarthrus hoffmannseggi* Brandt, 1833). Přestože velká část myrmekofilů se snaží napodobovat pach mravenců, je absence pachu účinnější v tom smyslu, že umožňuje využívat pohostinství prakticky všech druhů mravenců v areálu výskytu této berušky (Parmentier a kol. 2017).

Dobrou strategií, jak uniknout predátorovi, je přesvědčit jej, že nejsem kořist. Jednou z klíčových informací, kterou predátoři o kořisti mají, je její pohyb. Pokud tudíž kořist „hraje mrtvého brouka“, daří se jí nepovzbuzovat lovecký instinkt predátora a s poměrně velkou pravděpodobností může být jeho pozornost odlákána někam jinam (Jakob a Long 2016). U hmyzu je tato strategie často doprovázena spadnutím z vegetace do opadu, kde se špatně hledá. V této souvislosti je vhodné připomenout, že suchozemští stejnonožci rádi v noci lozí po vertikálních površích (stromy, stěny domů) a že i u nich může tato strategie způsobit pád na zem, daleko od predátora. Obecně se tato strategie nazývá tonická imobilita, přičemž specifická verze typická pro stejnonožce čeledí Armadillidae, Armadillidiidae, Budellundiellidae, Eubelidae, Sphaeroniscidae či Tylidae se nazývá volvace. Jedná se o pasívní antipredační strategii (Langerhans 2007), která predátorovi znemožňuje nalezení (zakutálené) kořisti, manipulaci s ní či její pozření. Tuto strategii nemusejí využívat všichni jedinci v populaci, u zatím prozkoumaných druhů se reaktivita (tj. navození tonické imobility) pohybovala mezi 20–90 % (Quadros a kol. 2012, Tuf a kol. 2015a).

Pokud tonická imobilita vede ke vzdálení se predátorovi (tj. zapadnutí do opadu, spadnutí ze stromu či zdi), je tato strategie velmi účinná. Pokud však je tonická imobilita využita na rovném povrchu (např. na zemi), může být velmi neúčinná – mravenci stínku (nikoliv však svinku) jednoduše transportují do mraveniště, jako tomu činí s nalezenými mrtvolkami. Stejnonožci, kteří se potkají s predátorem na zemi, se proto snaží uprchnout. Při útěku využívají strategii, která se nazývá *turn alteration*, což by bylo možné přeložit jako kličkování (Watanabe a Iwata 1956, Kupfermann 1966, Hughes 1967). Význam systematického kličkování je velký, zabraňuje při útěku nepřehledným terénem, kde je nemožné držet přímý směr (například kamenitý terén, drny trávy na louce atp.), aby se prchající živočich vrátil zpět, tj. aby „chodil v kruhu“ (Ono a Takagi 2006). Systematické střídání odbočení vlevo a vpravo je tudíž nejlepší aproximací k přímému pohybu a proto se kličkování považuje za „korekční chování“ – zvíře systematicky koriguje svůj směr pohybu tak, aby se dostalo co nejdále. Kličkováním stejnonožci reagují při nepříznivých podmínkách, jako je vysoká teplota, nedostatek potravy a podobně. V těchto případech je vhodnou strategií dostatečně se vzdálit od nevyhovujího místa. Kličkování lze studovat v laboratoři například pomocí složeného T-labyrintu, kde musí stejnonožec několikrát po sobě odbočit. Intenzita kličkování v labyrintu tudíž reflektuje míru stresu, kterému je stejnonožec vystaven. Tímto způsobem bylo také doloženo, že stejnonožci citlivě reagují na přítomnost predátora, tj. šestiočky, a dokáží jej odlišit od jiných „podobných“ ale neškodných stimulů (Carbines a kol. 1992).

Kognitivní schopnosti suchozemských stejnonožců

Schopnost stejnonožce rozpoznat povahu krátkodobého stimulu (vystavení přítomnosti šestiočky, respektive mouchy v malé temné krabičce) naznačuje relativně vysokou úroveň kognitivních schopností těchto živočichů. Rozdíl v reakci na šestiočku a mouchu byl měřen pomocí míry kličkování v labyrintu (Carbines a kol. 1992), což je obecně dobrá metoda vyjádření intenzity stresu. Recentní studie naznačují, která vodítka stejnonožci využívají k rozpoznávání predátorů. Kelly Zimmerman a Scott Kight (2017) testovali stínku obecnou a svinku obecnou v přítomnosti pavoučího predátora a manipulovali s přítomností vizuálních, chemických i seismických vodítek. Zjistili, že oba druhy stejnonožců registrovali hlavně vizuální a seismické signály. Přestože jejich experiment měl jisté metodologické nedostatky (seismické stimuly byly zvýrazněny umístěním experimentu na blánu bubnu, přičemž reakcí kořisti byla její nehybnost – tuto nehybnost však mohla způsobit i jen prostá snaha nedávat o sobě vědět okolí vlastním pohybem), kognitivní schopnosti stejnonožců jsou zjevně poměrně vysoké a je třeba jim věnovat více pozornosti.

S kognitivními schopnostmi souvisí také agregační chování zmíněné výše. Toto chování totiž může být adaptivní v tom smyslu, že zjednodušuje vyhledávání vhodného úkrytu. Běžný den stínky spočívá ve schovávání v úkrytu s vyšší vzdušnou vlhkostí přes den a ve večerní aktivitě mimo úkryt, během které je nutno najít vhodnou potravu a samozřejmě také úkryt pro další den, respektive několik dní, jak doložil Piet den Boer (1961). Stejnonožci totiž nejsou svým úkrytům příliš věrní a spokojí se s jiným vhodným úkrytem, na který narazí (Dangerfield a Hassall 1994, Hassall a Tuck 2007). Volba vhodného úkrytu je přitom zásadní, jelikož nevhodný úkryt může způsobit přílišné ztráty vody. Kognitivní přístup, během kterého stejnonožec používá velikost agregace jako vodítko k posouzení kvality úkrytu, je zjevně důvodem k tvorbě agregací o ideální velikosti okolo 70 jedinců, jak je popsal Pierre Broly a kol. (2012, 2016).

Nejen pro studium tvorby agregací by bylo vhodné umět rozpoznávat jednotlivé stínky v experimentu a sledovat případné individuální rozdíly v jejich chování. Identifikace jedince je také nezbytným předpokladem pro studium personality živočichů. V některých případech je možné chovat jedince odděleně a rozpoznávat je tak podle kódů umístěných na jejich chovných boxech, při studiu interakcí je však tento přístup nepoužitelný. V několika studiích se výzkumníci pokusili rozlišovat jednotlivé stínky pomocí barevných značek na těle jedince (např. Brereton 1957). Nám se však podařilo doložit, že takové značení je nejen nestabilní (Tuf a kol. 2013), ale i významně pozměňuje chování stejnonožců (Drahokoupilová a Tuf 2012).

Zajímavé etologické experimenty prováděl belgický psycholog Patrick Anselme (2013a, 2013b), který se rozhodl studovat zvědavost u stínek obecných. Studovat zvědavost přitom vůbec není jednoduché, pokud zvířeti nabídneme nové prostředí a to jej začne prozkoumávat, nemusí to být hned důkazem zvědavosti. Důvodem může být pouhá snaha uniknout ze starého, nevyhovujícího prostředí. Abychom odlišili tuto reakci, musíme znát nároky studovaných druhů na jejich prostředí.

Suchozemští stejnonožci jsou přitom vhodnou modelovou skupinou, jelikož víme, že preferují vlhčí místa před suchými, úkryty před otevřeným prostranstvím, přítomnost skupiny jiných příslušníků svého druhu (či alespoň vůni jejich exkrementů) před „stínkoprázdnými“ (a zápachu prostými) prostory. Anselme proto připravil chytře vymyšlené experimenty, ve kterých prokázal, že stínky ve výběrových testech upřednostňují prozkoumávání neznámého prostředí, a to dokonce tehdy, když staré prostředí poskytovalo vhodnější podmínky (vyšší vlhkost papíru a/nebo nabídnutý úkryt). Lze tudíž shrnout, že zvědavost je suchozemským stejnonožcům vlastní. Vždyť lze (s nadsázkou) tvrdit, že právě zvědavost stála za tím, že předci dnešních suchozemských stejnonožců před 300 miliony lety vylezli z moře na neznámou souš (Broly a kol. 2013).

Kognitivní aspekty má také systematické kličkování, což je výše zmíněná (nejen) antipredační strategie suchozemských stejnonožců. Experimentátoři totiž při pokusech s probíháním labyrintů obvykle zaznamenávají pouze výsledný cíl, do kterého stejnonožec dorazí. (Tyto cíle se liší mírou pravidelnosti střídání odboček vpravo a vlevo.) Každý, kdo sledoval živočichy v bludišti, však ví, že subjekt občas „změní názor“, vrátí se k nejbližší odbočce a vydá se na druhou stranu. Toto chování dosud systematicky zkoumal pouze Toru Moriyama (Moriyama a kol. 2015), kterého zajímalo, s čím souvisí pravděpodobnost, že se svinka vrátí a změní směr odbočení. Zjistil, že hlavním důvodem je směr předchozího odbočení, neboli že svinka opraví svou odbočku tak, aby odpovídala vzoru kličkování čili střídání pravých a levých odboček. Zjistil také, že pokud se svinka vrátí a změní směr odbočky, je potom podstatně „opatrnější“ na dalších odbočkách a systematičtěji kličkuje (Moriyama a kol. 2015). Sytematičnost tohoto počínání naznačuje záměr svinky dostat se co nejdále od rušivého vjemu, jakkoliv tento závěr může vypadat antropomorfně.

Zkoumání kognitivních schopností různých druhů živočichů v poslední době napomáhá odhalit „osobnostní rysy“ různých jedinců. Tzv. personalita živočichů je studována v posledních ca 20 letech, ačkoliv drtivá většina výzkumníků se věnuje obratlovcům. O personalitě lze u živočichů hovořit, jsou-li splněny tři podmínky: 1) musejí existovat rozdíly v chování mezi jednotlivci v populaci, 2) tyto rozdíly musejí být stabilní napříč různými situacemi a 3) chování jednotlivců musí být stabilní v čase (Reale a kol. 2007). Časový aspekt je přitom nejhůře prokazatelný, obvyklé jsou studie s opakováními v řádu dnů či týdnů, delší časový horizont se u bezobratlých živočichů studuje jen velmi zřídka. Koncept personality byl aplikován na relativně široké spektrum bezobratlých včetně korýšů (např. Briffa 2013, Biro a kol. 2014, Brodin a Drotz 2014, Yli-Renko a kol. 2015). Nám se podařilo doložit individuální rozdíly v odvaze stínek obecných, kterou jsme kvantifikovali pomocí tonické imobility. Dráždili jsme stínky opakovaně různými podněty a měřili jejich reaktivitu, senzitivitu a dobu setrvávání ve strnulosti (Tuf a kol. 2015a). Doložili jsme, že jen některé stínky jsou „odvážné“, což znamená, že potřebují více opakování konkrétního stimulu k navození relativně krátké strnulosti, tento „povahový rys“ přitom nesouvisel s velikostí daného jedince.

Koncept personality je stále relativně kontroverzní, hlavně z důvodu dosavadní nekonzistentnosti přístupů a definic. I přes uvedenou kontroverzi však existuje hypotetické evoluční vysvětlení adaptivnosti existence odlišných personalit u živočichů. Základním konceptem je přitom trade-off mezi dvěma (či více) odlišnými behaviorálními strategiemi, kde jedinci s nevyhraněným intermediátním chováním mají nižší fitness. Příkladem může být vztah mezi aktivitou a přežíváním u mořských stejnonožců druhu *Idotea baltica* (Pallas, 1772), který zkoumali recentně ve Finsku (Yli-Renko a kol. 2015). Dosud bylo známo, že aktivnější stejnonožci tohoto druhu mají rychlejší růst, větší jedinci jsou plodnější než malí a zároveň aktivnější samci mají během rozmnožování větší šanci potkat samice a mají tudíž vyšší reprodukční úspěch. Překvapivé proto bylo, když Finky zjistily, že méně aktivní stejnonožci přežívali zimu s trojnásobně vyšší pravděpodobností než stejnonožci středně aktivní a hodně aktivní. Zde je právě onen předpokládaný trade-off mezi plodností a přežíváním, který umožňuje „vybrat" si nevratnou cestu, jak zvýšit svou fitness: buď sázkou na velikost a aktivitu, jež zvýší pravděpodobnost zplodit mnoho potomků, či sázkou na nízkou aktivitu, která zvýší šanci se období rozmnožování vůbec dožít. Odsud zřejmě pramení ony rozdíly mezi jednotlivci v chování, které jsou považovány za aspekt jejich personality (Yli-Renko a kol. 2015).

Publikace autora k dané tematice, jež jsou součástí této práce

Drahokoupilová, T., **Tuf, I.H.** (2012): The effect of external marking on the behaviour of the common pill woodlouse *Armadillidium vulgare*. *ZooKeys*, 176: 145-154. **(Příloha 6)**

Krumpálová, Z., **Tuf, I.H.** (2013): Differences in diurnal activity of ground living spiders: Mechanisms of strategy based on the body size. *Polish Journal of Ecology*, 61: 575-586. **(Příloha 8)**

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Přílohy

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Příloha 2: **Tuf, I.H.**, Chmelík, V., Dobroruka, I., Hábová, L., Hudcová, P., Šipoš, J., Stašiov, S. (2015): Hay-bait traps are useful tool for sampling of soil dwelling millipedes and centipedes. *ZooKeys*, 510: 197-207. ($\mathrm{IF}_{2015}=0.938$)

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Poznámka

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Different collecting methods reveal different ecological groups of centipedes (Chilopoda)

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ABSTRACT. Soil invertebrates are studied by a number of methods. Here we used zoological methods (soil sampling, pitfall trapping, litter sifting, and hand collecting) to sample centipede communities in four floodplain forests (Czech Republic, Europe) and compared the efficiency of these methods. Heat-extraction from soil samples was the most effective, followed by pitfall trapping. The centipedes found by us can be divided into five ecological groups: 1) larger, abundant lithobiomorphs, 2) larger, scarcer lithobiomorphs, 3) smaller, soil lithobiomorphs, 4) abundant geophilomorphs, and 5) scarcer geophilomorphs.

KEY WORDS. Heat extraction; litter sifting; pitfall trapping; soil sampling.

Soil macrofauna (i.e., soil invertebrates ranging from 2-20 mm in size) is an important part of terrestrial ecosystems; some soil species are decomposers of dead organic matter, whereas others are predators. Several standard methods have been used for collecting faunistical and coenological data. The easiest and the oldest method is hand collecting using tweezers. Though very adequate for obtaining live animals, this method is time-consuming and difficult to quantify. Another method consists of Barber traps (Barber 1930), better known as pitfall traps. The latter are widely used in ecological studies for their simplicity, time efficiency and the possibility to leave them in the field for a long time. These traps consist of plastic or glass jars that are embedded deep in the soil and are filled with a solution to preserve the specimens that fall in them (e.g., formaldehyde, ethyleneglycol). Unfortunately, Barber traps mostly catch surface-dwelling species. Adis (1979) and Woodcock (2005) concluded the following about pitfall traps: the size of the sample these traps collect is affected by a number of environmental factors (e.g., climate, microclimate, biotope, age of wood, type of soil surface and soil), as well as trap parameters (diameter, shape of mouth, depth, cover, fixation solution, pattern of traps distribution, digging method). They stressed that traps need to be chosen according to the biotope and type of research.

To study animals inhabiting soil (not only the soil surface), it is necessary to sample soil layers. Litter and/or soil sifting is the easiest choice to get soil animal samples (Górny & Grüm 1993). Sifting has the advantage of sampling microsites that are not accessible by other quantitative methods, for example brittle wood, local litter heap etc. Its major disadvantages are that they can damage the invertebrates (e.g., centipedes or diplurans have specific characters on appendages or legs) and are size-selective (the size of the mesh is a compromise between animal size and size of material particles). There are two ways to compare the species's spectrum recorded by individual samples collected by sifting: 1) according to the sampled area and depth of the sifted soil layers (e.g., sifted sample of original size of 25 x 25 x 5 cm); or 2) according to the volume of sifted material (e.g., two litres of sifted material form favourable microsite as decaying stub). The most useful method for quantitative studies is soil sampling. Even though it is a difficult method (due to time and weight aspects), it is the only way to guarantee that all animals from a given surface are sampled, since its success does not depend on the activity of the animals (Zou et al. 2012). After sifting or soil sampling, animals and soil matter need to be separated. There are several methods (e.g., Górny & Grüm 1993) to do this, which are clustered into two categories: passive and active. Passive methods separate animals and soil particles according to their different physical (shape, size) or chemical (consistency) characteristics. The samples are processed by (i) hand sorting, (ii) sorting by sieve-system (dry or wet sieving, Morris 1922) or (iii) separated in liquids of specific consistency (e.g. Raw 1955). The active methods benefit from the movement of animals in the soil sample. Invertebrates are unearthed by applying (iv) electrical, (v) temperature, (vi) light or (vii) chemical stimuli. The most frequent is the use of positive geotaxis – a decrease in soil humidity and an increase in soil temperature cause the movement of invertebrates to the deepest soil layer. The first thermoextractors were made by Berlese (1905), and several modifications of it have been designed since (e.g., Kempson et al. 1963).

Centipedes are adequate to ascertain the effectiveness of soil macrofauna sampling methods. Generally, they are divided into two large groups that differ in biology. Lithobiomorpha and Scolopendromorpha centipedes inhabit the soil surface and litter layers. They predate on springtails, potworms, small spiders and harvestmen, and insect larvae. Geophilomorpha, by contrast, are associated with the upper soil layers and hunt small earthworms and potworms, also consuming dead organic matter. In Central Europe, the numbers of species in both groups are similar (Tuf & Tufová 2008). For this reason, different methods of sampling need to be used to estimate species diversity there. Some researchers have described centipede assemblages as epigeic and edaphic in part depending on whether the material was obtained using pitfall traps or soil sampling (e.g., Wytwer 1995, Wytwer & Tajovský 2009). However, our knowledge about the efficiency of different methods is limited. No important differences have been found among some methods (Smith et al. 2008) and no significant differences have been detected between the efficiency of pitfall traps and litter sifting (Prasifka et al. 2007). This study aimed to find the best combination of methods to ascertain the centiped species' spectrum in Central European forests.

MATERIAL AND METHODS

Centipedes were collected by two quantitative (pitfall trapping, soil sampling) and two qualitative (hand collecting, litter sifting) methods. Pitfall traps (PT) were made by using glass jars (0.7 l) with properly fitting plastic cups (0.25 l) filled with 4% formaldehyde liquid; traps were covered by bark or metal hoods (approximately 2 cm above surface) and inspected approximately every three weeks. Soil samples (SS) were collected by circle metal probe made from toothed metal tube engraving intact soil samples. Obtained samples (area 1/16 m^2 and depth 10 cm, leaf litter included) were immediately transported to the laboratory in plastic bags. Litter sifting (LS) was done using sifter with mesh size 5 mm; favourable microsites and microhabitats were selected and sampled. Sifted litter and soil (mainly fermentation layer) material of approximately 3 l was transported immediately to the laboratory in plastic bags. Centipedes were hand collected (HC) by using tweezers in favourable microsites such as fallen trunks or branches, under barks, stones and moss cushions, for approximately one hour in each locality, per visit. Soil samples and siftings were successively cultivated in heat-extractors for ten days (60 W bulb, mesh size 5 mm, thickness of material inside funnel up to 10 cm – Tuf & Tvardík 2005) to total dryness. The material collected by all methods was sorted, identified and stored in the author's private collection in 70% ethanol. The classification of centipedes into the following categories, relic/adaptable/eurytopic, is based on their distribution on natural/antropogenic habitats in the Czech Republic (Tuf & Tufová 2008).

Communities of centipedes from four localities in the Litovelské Pomoraví Protected Landscape Area (PLA), near the city of Olomouc in the Czech Republic, Europe, were sampled. All these localities were situated in hardwood floodplain forests (vegetation association *Querco-Ulmetum*). The herbal layer included *Anemone nemorosa*, *Polygonatum* spp., *Lathyrus vernus*, and *Maianthenum bifolium*. The dominant moss was *Eurhynchium hians*. The alluvial soil was loamy-sandy to loamy in the localities, with pH 4.8-5.The annual precipitation was around 520 mm, mean annual temperature was 9.1°C, the altitude of localities was about 200-230 m a.s.l. The following four localities were studied: (i) U zámecké Moravy Natural Monument (MOR; 49°43'4.256"N, 17°1'57.580"E, inside Hejtmanka Natural Reserve now), (ii) Vrapač National Nature Reserve (VR1; 49°42'40.581"N, 17°2'15.038"E), (iii) another site in Vrapač National Nature Reserve (VR2; 49°42'16.703"N, 17°2'33.423"E), (iv) forest mosaic near Horka nad Moravou (HNM; 49°39'12.592"N, 17°12'13.723"E). At MOR, VR1 and VR2, ten pitfall traps were installed during the season from April-September 2001 where four soil samples were taken using litter sifting and hand collecting. Litter sifting as well as hand collecting were applied randomly at the localities in an area of ca one hectare, and all the sifted material was combined into one mixed sample. At VR2 centipedes were collected and evaluated by Tajovský (Pižl, V. & Tajovský, K. unpubl.) using five traps (from May to September 1997), extraction of five soil samples (May and September 1997) and hand collecting. HNM was sampled by 18 traps (January 1998 to March 2001), 15 soil samples (taken monthly) and by occasional hand collecting. The localities can be divided based on the length of the study (shorter/longer than one vegetation season): short-term (MOR and VR1) and long-term studied (VR2 and HNM).

RESULTS

Evaluation of methods

The combination of all studied methods yielded 11 centipede species at locality MOR; 8 species at locality VR1; 15 species at locality VR2 and 12 species at locality HNM (without litter sieving), respectively (Fig. 1). A comparison between the short-term and long-term sampling has shown that the probability to detect rarer centipedes by any method increases with time. The greatest numbers of species were obtained at all localities by heat extraction of soil samples (more than 60%). Second came pitfall trapping and litter sifting (Fig. 1).

Beside the species' spectrum sampled by soil samples, 1, 2 or 3, other species were recorded by pitfall traps exclusively. *Lithobius erythrocephalus* C.L. Koch, 1847, *Lithobius nodulipes* Latzel, 1880 and *Lithobius piceus* L. Koch, 1862 were not present in soil samples and *Lithobius forficatus* (Linaeus, 1758) was rarely extracted from soil samples (Table 1). Litter sifting was also useful in the short-term; 2 species were collected by this method, at MOR – *Lithobius agilis* C.L. Koch, 1847 and *Strigamia transsilvanica* (Verhoeff, 1928). In general, litter sifting collected few centipedes (20-55%, Fig. 1), showing that this method is inefficient; similarly, hand collecting was also ineffective. At three localities (VR1, VR2, HNM) all species were recorded by the combination of soil samples and pitfall traps (Table 1). Other combinations (with litter sifting or hand collection) were not as successful.

Table 1. Species spectrum recorded by individual methods on studied localities. Methods: (SS) soil sampling, (PT) pitfall trapping, (LS) litter sifting, (HC) hand collecting.

	MOR				VR1				VR2				HNM		
	SS	PT	LS	HC	SS	PT	LS	HC	SS	PT	LS	HC	SS	PT	HC
Lithobius aeruginosus L. Koch, 1862	+	–	–	–	–	+	–	–	+	–	–	–	+	–	–
Lithobius agilis C.L. Koch, 1847	–	–	+	–	–	–	–	–	+	+	–	–	+	+	+
Lithobius austriacus Verhoeff, 1937	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–
Lithobius curtipes C.L.Koch, 1847	–	–	–	–	–	–	–	–	+	+	–	–	+	+	+
Lithobius erythrocephalus C.L. Koch, 1847	–	+	–	–	–	–	–	–	–	+	–	–	–	+	+
Lithobius forficatus (Linaeus, 1758)	–	+	–	+	+	+	–	–	+	+	–	+	+	+	+
Lithobius micropodus (Matic, 1980)	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–
Lithobius mutabilis L.Koch, 1862	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
Lithobius nodulipes Latzel, 1880	–	–	–	–	–	–	–	–	-	+	–	–	–	–	–
Lithobius piceus L. Koch, 1862	–	–	–	–	–	–	–	–	-	+	–	–	–	–	–
Geophilus electricus (Linnaeus, 1758)	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–
Geophilus flavus (DeGeer, 1778)	+	–	+	–	+	–	–	–	+	–	–	+	+	+	+
Geophilus insculptus Attems, 1895	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–
Geophilus proximus C.L. Koch, 1847	+	–	–	–	–	–	–	–	+	–	–	–	+	–	–
Schendyla nemorensis (C.L. Koch, 1836)	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+
Strigamia acuminata (Leach, 1814)	+	–	+	+	+	–	+	–	+	+	+	–	+	+	+
Strigamia crassipes (C.L. Koch, 1835)	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
Strigamia transsilvanica (Verhoeff, 1928)	–	–	+	–	–	–	–	–	–	–	–	–	+	+	–
Number of species	7	3	6	4	7	5	4	–	12	10	3	4	11	10	8
Number of species together	11				8				15				12		

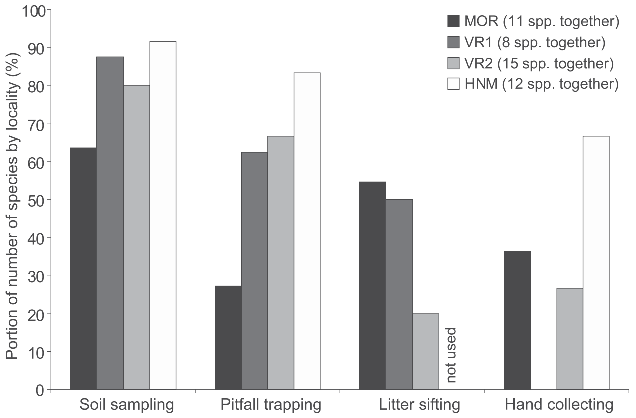

Figure 1. Comparison of relative efficiency of methods for centipede collection. Per cent proportion of species found by individual methods in studied localities.

In summary, the need to use more than one method to uncover the centipede species spectrum was identified (Table 2). All species (18) were only recorded when a combination of pitfall trapping and soil sampling were used; individual methods recorded only 15, 15, 7 and 8 species. Pitfall trapping and heat extraction of soil samples were the only methods capable of recording relic (less abundant) species (Table 3).

Table 2. Numbers of species recorded by individual methods and by combinations of two methods.

	Soil sampling	Pitfall trapping	Litter sifting	Hand collecting
Soil sampling	15	18	15	16
Pitfall trapping		15	15	15
Litter sifting			7	10
Hand collecting				8

Groups of trapped centipedes

Joint evaluation of the material collected from all four localities in Litovelské Pomoraví PLA has revealed specific patterns of recording species by individual methods (Table 3). Separate groups are characterised according to their preferred environment (soil surface vs. soil layers) and their frequency in samples (abundant vs. less abundant species). Five groups can be recognized, as follow: 1. larger, abundant lithobiomorphs: group of abundant ground-dwelling species of the order Lithobiomorpha with larger body size (more than approximately

Table 3. Main groups of centipede species according to similar pattern of efficiency of individual methods (number of individuals) in Litovelské Pomoraví PLA. Rarity – ecological valence in Central European conditions (Tuf & Tufová 2008): (R) relic species, (A) adaptable species, (E) eurytopic species.

Group	Rarity	Species	Soil sampling	Pitfall trapping	Litter sifting	Hand collecting
Larger abundant lithobiomorphs	E	*Lithobius forficatus*	24	332	–	21
	E	*Lithobius mutabilis*	1135	3940	38	38
	A	*Lithobius agilis*	8	19	8	5
Larger scarcer lithobiomorphs	E	*Lithobius erythrocephalus*	–	5	–	5
	R	*Lithobius nodulipes*	–	1	–	–
	A	*Lithobius piceus*	–	5	–	–
	A	*Lithobius austriacus*	3	13	–	–
Smaller soil lithobiomorphs	A	*Lithobius micropodus*	84	7	6	–
	E	*Lithobius curtipes*	47	6	–	1
	A	*Lithobius aeruginosus*	9	5	–	–
Abundant geophilomorphs	A	*Strigamia transsilvanica*	36	3	3	–
	E	*Strigamia acuminata*	76	18	62	11
	E	*Geophilus flavus*	1160	3	15	15
	E	*Schendyla nemorensis*	1540	16	15	42
Scarcer geophilomorphs	A	*Geophilus insculptus*	2	1	–	–
	A	*Geophilus electricus*	8	–	–	–
	R	*Geophilus proximus*	29	–	–	–
	A	*Strigamia crassipes*	1	–	–	–

12 mm). These species were recorded by all methods, but fell more often in pitfall traps. 2. larger, scarcer lithobiomorphs: a group of rare (with low dominances) but large species from the order Lithobiomorpha. These species were recorded by pitfall trapping exclusively; 3. smaller, soil lithobiomorphs: group of centipede species with small body size (less than 12 mm) inhabiting cavities and burrows in the soil, offering more favourable conditions (higher humidity, lower temperature fluctuation) and low epigeic activity. These species were recorded mainly by extraction of soil samples and several individuals were collected in pitfall traps; 4. abundant geophilomorphs: group of centipede species from order Geophilomorpha with abundant populations. These species were extracted from soil samples, and fell in traps also relatively frequently. The probability of recording these species using other methods (sifting, hand collecting) was relatively high as well; 5. scarcer geophilomorphs: the second group of geophilomorphs, which were relatively rare and were recorded exclusively in soil samples.

DISCUSSION

Efficiency of methods

We compared four main methods of soil macrofauna sampling to ascertain how well they determine the local centipede species' spectrum. In our results, the most useful methods were pitfall trapping and extraction of soil samples. Their combination recorded all species in the localities sampled, particularly in the long-term. Litter sifting and hand collecting of centipedes can be complementary methods in short term studies since they collect a large portion of the species' spectrum, but do not efficiently record less abundant species. Similarly to our results, a combination of sifting and hand-collecting were the most effective methods for sampling centipedes in the African savannah with prevailing scolopendromorphs (Druce et al. 2004).

It is necessary to note that our soil samples were heat-extracted by modified Berlese-Tullgren funnels. Soil samples can be processed not only by this Berlese-Tullgren device, but by using Winkler xereclector or to be handsorted. Published comparisons favour Berlese-Tullgren funnels (Smith et al. 2008, Sabu & Shiju 2010).

Centipedes are not the only soil animals that are regularly sampled. Terrestrial isopods (Isopoda: Oniscidea) or millipedes (Diplopoda), for example, also have diversified life strategies (Schmalfuss 1984, Hopkin & Read 1992); some species live exclusively in soil, whereas others are mostly ground-dwelling. Both millipedes and woodlice are among the most important decomposers in terrestrial ecosystems. Thus, to assess their local diversity, a combination of different methods may be necessary. For example, to sample millipedes, a combination of hand collecting and litter sifting are recommended (Snyder et al. 2006), or hand collecting by an experienced handpicker, including the deeper strata (Mesibov et al. 1995). Similarly, spiders inhabit different strata (litter, soil, trees) and it is useful to combine several methods to assess their species diversity (Hövemeyer & Stippich 2000).

Ecological groups of centipedes

Centipedes can be divided into five ecological groups according to their probability of being recorded by a particular collecting method. There are two main groups with different biologies – the more surface-dwelling and litter-dwelling lithobiomorphs and the geophilomorphs, which are mainly associated with soil strata (Poser 1990). Lithobiomorphs usually represent a very high proportion of pitfall trap catches (e.g., Fründ et al. 1997, Grgič & Kos 2009). These two groups can be divided according to their trapability; abundant species are collected by all methods, and less frequent species are recorded only by one method (i.e. by pitfall traps in Lithobiomorpha and soil sampling in Geophilomorpha). The fifth group is composed of small, more endogeic lithobiomorphs – they are caught by pitfall traps and are also extracted from soil samples. These species (and younger specimens of larger lithobiids) live in soil crevices and fissures trying to protect themselves against cannibalism of larger centipedes (Rawcliffe 1988) and the drier conditions on the surface.

Since there are several ecological groups of centipedes, a combination of methods is necessary to sample them all. Our results have shown that the most useful methods are pitfall trapping and heat extraction of soil samples. Both methods are relatively inexpensive. Pitfall traps are made from common glass or plastic pots usually and Tullgren apparatuses can be simply created from plastic pails (Tuf & Tvardík 2005).

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Příloha 2

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Hay-bait traps are a useful tool for sampling of soil dwelling millipedes and centipedes

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Abstract

Some species of centipedes and millipedes inhabit upper soil layers exclusively and are not recorded by pitfall trapping. Because of their sensitivity to soil conditions, they can be sampled quantitatively for evaluation of soil conditions. Soil samples are heavy to transport and their processing is time consuming, and such sampling leads to disturbance of the soil surface which land-owners do not like. We evaluated the use of hay-bait traps to sample soil dwelling millipedes and centipedes. The effectiveness of this method was found to be similar to the effectiveness of soil sampling. Hay-bait traps installed for 8–10 weeks can substitute for direct soil sampling in ecological and inventory studies.

Keywords

Diplopoda, Chilopoda, soil sampling, agroecosystem, soil fauna

Introduction

Soil macrofauna is commonly used for monitoring or evaluation of sites. Besides ground beetles (e.g. Hůrka et al. 1996, Kotze et al. 2011), spiders (e.g. Buchar and Růžička 2002, Maelfait et al. 2004) or woodlice (e.g. Souty-Grosset et al. 2005, Tuf and Tufová 2008), centipedes and millipedes are sampled frequently too (Tuf and Tufová 2008, Dunger and Voigtländer 2009). Nevertheless, not all species of centipedes and millipedes are ground-dwelling with many species inhabiting the upper soil layer exclusively (Lee 2006, Barber and Keay 1988). Soil dwelling animals can be sampled using litter/soil sieving, soil sampling or hand-collecting. Sieved material and soil samples have to be hand-sorted or processed using heat extractors, e.g. Tullgren funnel or Kempson apparatus (Tuf and Tvardík 2005). Handling of soil samples can be difficult due to the higher weight of samples (one sample of size 25 × 25 × 10 cm weighs around 6 kg). Litter/soil sieving can reduce the weight of samples, nevertheless as with hand-collecting, it is time consuming and attention-intensive. Moreover, soil sampling can cause damage to the site; pot-holes created by a soil corer can endanger people passing the site and can increase water erosion on slopes. These pot-holes are definitely not popular among land-owners of the sampled sites. For these reasons (severity of sampling, damage of ground), we have attempted to evaluate the effectiveness of sampling centipedes and millipedes using hay-bait traps. The aims of this research were 1) comparison of the efficiency of hay-bait trapping, soil sampling and pitfall trapping and 2) to find the optimal length of exposure of hay-bait traps for maximum efficiency.

Material and methods

Field study

The research was done at three sites in the Czech Republic from May to July 2013. The first site was an alfalfa field (49°34.41'N, 17°17.17'E) on the border of the town of Olomouc. This large field of ca 250 square metres is surrounded by other fields (with cereals) and a railway embankment. In the previous year it had also grown alfalfa. The field is under conventional management including use of herbicides and ploughing.

The second site was an old meadow (50°26.85'N, 15°0.00'E) being mown once to twice per year for the last 30 years. This meadow of ca 500 square metres is surrounded by fields and gardens with mixed wood across the road and is ca 6 km north-east of the town of Mladá Boleslav. The third site studied was a mixed forest (49°15.66'N, 17°17.72'E) 6 km south-west of the town of Kroměříž. The forest is classified as *Fageto-Quercetum illimerosum trophicum*; dominant trees are oaks, hornbeams and some pines, with *Rubus fruticosus*, *Galium odoratum* and *Galium aparine* as dominants of undergrowth. The soil surface of this forest is covered by a rather thick layer of oak leaf litter.

In the Czech Republic generally, the weather conditions during the study period were characterised by average or slightly increased temperatures and higher than average precipitation in May-June, and a very hot July in contrast to long-term average values. The previous winter season was rather warmer and with higher precipitation (ref. historical territorial data at www.chmi.cz).

Soil macrofauna, including millipedes and centipedes, was sampled using three methods at each site. Pitfall traps (10 traps consisting of glass jars with inserted plastic pots of diameter 7.5 cm filled with 2 dl of 4% formaldehyde in water with some detergent, metal covers) were arranged in 2 lines of 5 traps with a span of 10 m, and inspected at 2-week intervals. Five soil samples (25 × 25 × 10 cm including litter layer) were obtained using a spatula, three times per study (i.e. 15 soil samples per site) and transported to the laboratory in plastic bags. Hay-bait traps were made from a wire gauze (2 cm mesh) shaped as a simple pocket of size 25 × 25 cm. Each pocket was marked by a code written on the band. These pockets were filled with hay (commercial hay mixture for feeding rodent pets) and submerged into water for 2 hours before installation. Altogether, 60 hay-bait traps were placed horizontally at each site in a following scheme: 5 lines of 12 traps (2-5 cm under soil surface) over a length of 2 m with 10 m between lines. All traps were installed at the same time and 5 traps were taken away each week during the course of the study lasting for 12 weeks. Hay-traps were transported into the laboratory inside separate plastic bags.

Sample processing

Soil samples and hay-traps were heat-extracted immediately in the laboratory using simple Kempson devices (Tuf and Tvardík 2005). Hay-traps were extracted for a week, soil samples for 2 weeks, both under electric 60W-bulbs. Extracted animals from both soil samples and hay-traps were sorted to higher taxonomic groups and millipedes and centipedes were identified to the species level.

Data analyses

We tested the effects of trapping time and methods on species richness by repeated-measures on traps with nested design. The traps were nested in each of the three study sites (field, meadow, forest). Explanatory variables in the model were trapping time and trapping methods. The response variable was defined as a number of species per trap for particular time and place. Habitat type was used as random variable. We used a mixed model to estimate the correct error term and degrees of freedom. To test this effect, a generalized linear mixed model (glmmPQL, part of R package MASS) was used with negative binomial error distribution and log link function (Bates et al. 2014).

To test if one level of a particular factor (trapping method and study site) is more variable than other levels of the same factor, a permutation test was used (permutest.betadisper, part of R package vegan). This permutation based method tests pairwise comparisons of group mean dispersions. It is based on the t-statistic computed on pairwise group dispersions. A distance matrix was computed based on "Bray-Curtis" index of dissimilarity (vegdist, part of R package vegan). Then the function "betadisper" (part of vegan package in R) was used to calculate variance for each group of samples. Variance was computed as average distance of group members to the group centroid.

Rarefaction curves were constructed to show how the species richness varies for the same sample size between the three trapping methods. Function "rarefy" (part of vegan package in R software) computed the expected species richness and standard deviation in random subsamples of a particular sample size from the community. Data were analysed using R software (R Development Core Team 2011).

Results

Altogether, we obtained 541 millipedes from 17 species and 435 centipedes from 13 species (Table 1). Based on the number of recorded animals, the richest site was the forest (553 myriapods) and poorest site was the field (100 myriapods). Number of species showed the same pattern: 21 myriapod species in the forest and 6 in the field. Soil sampling was the least efficient for sampling species (9 millipede and 7 centipede species) as well as individuals (36 and 100 individuals respectively), whereas pitfall traps and hay-bait traps were similar in their efficiency: 14–15 millipede and 9–10 centipede species; for number of individuals, see Table 1.

Methods at individual sites were evaluated according to their efficiency using rarefactions (Fig. 1). Bait traps sampled higher numbers of species in contrast to other methods in the field site, meanwhile increasing sampling effort (number of sampled animals) was connected with a bigger species list in bait traps as well as in pitfall traps in forest.

Differences between species lists at all sites and lists sampled by individual methods were compared by pairwise comparisons and differences confirmed between all pairs of sites (Table 2a). Nevertheless, the same analysis revealed there was no statistically significant difference between the suite of species sampled by hay-bait traps and soil samples (Table 2b).

Evaluation of colonization of hay-bait traps (Fig. 2) showed that the highest diversity as well as abundance of collected myriapods in these traps is after 7 weeks following installation in field, or 9-10 weeks following installation in forest or meadow. A longer period of exposure leads to a decrease of both parameters of myriapod communities. Generalized linear mixed models reveal that changes in abundance during exposure was significantly influenced by the second power of time (LRT = 6.43, $p = 0.040$, AIC = 667.83). The analogous model for diversity confirmed significant changes during time (LRT = 5.81, $p = 0.042$, AIC = 543.38) which were site dependent too (LRT = 6.74, $p = 0.034$, AIC = 544.12).

Table 1. List of millipedes obtained using three methods from three biotopes (ind./10 pitfall traps/12 weeks, ind./60 bait traps and ind./0.94m^2 respectively.

	Pitfall traps			Hay-bait traps			Soil samples			Total pitfall traps	Total hay-bait traps	Total soil samples
	field	**meadow**	**forest**	**field**	**meadow**	**forest**	**field**	**meadow**	**forest**			
Glomeris connexa C. L. Koch, 1847	-	9	1	-	-	1	-	-	-	10	1	0
Blaniulus guttulatus (Fabricius, 1798)	-	2	-	-	31	1	-	2	-	2	32	2
Brachyiulus bagnalli (Curtis, 1845)	2	-	-	5	-	-	-	-	-	2	5	0
Cylindroiulus boleti (C.L. Koch, 1847)	-	-	3	-	-	-	-	-	-	3	0	0
Cylindroiulus caeruleocinctus (Wood, 1864)	1	-	-	-	-	-	-	-	-	1	0	0
Enantiulus nanus (Latzel, 1884)	-	-	64	-	-	32	-	-	4	64	32	4
Julus scandinavius Latzel, 1884	-	-	-	-	-	1	-	-	-	0	1	0
Leptoiulus proximus (Němec, 1896)	-	-	2	-	-	1	-	-	-	2	1	0
Megaphyllum projectus Verhoeff, 1894	-	-	2	-	-	2	-	-	-	2	2	0
Ommatoiulus sabulosus (Linnaeus, 1758)	-	-	10	-	-	11	-	-	4	10	11	4
Ophiulus pilosus (Newport, 1842)	27	-	-	59	-	-	2	-	-	27	59	2
Unciger foetidus (C.L. Koch, 1838)	-	9	36	-	30	26	-	3	2	45	56	5
Brachydesmus superus Latzel, 1884	-	-	-	-	-	-	-	3	-	0	0	3
Polydesmus complanatus (Linnaeus, 1761)	-	3	1	-	2	3	-	-	1	4	5	1
Polydesmus denticulatus C.L. Koch, 1847	-	8	-	-	7	-	-	-	-	8	7	0
Polydesmus inconstans Latzel, 1884	-	1	-	-	39	-	-	6	-	1	39	6
Strongylosoma stigmatosum (Eichwald, 1830)	-	-	47	-	-	26	-	-	9	47	26	9
Diplopoda	30	32	166	64	109	104	2	14	20	228	277	36
Clinopodes flavidus C.L. Koch, 1847	-	-	2	1	-	9	-	-	-	2	10	0
Geophilus electricus (Linnaeus, 1758)	-	-	-	-	-	-	-	9	-	0	0	9
Geophilus flavus (DeGeer, 1778)	-	-	10	-	9	20	1	30	-	10	29	31
Geophilus truncorum Bergsoe & Meinert, 1866	-	-	-	-	-	-	-	1	-	0	0	1
Schendyla nemorensis (C.L. Koch, 1836)	-	-	23	-	11	60	-	26	-	23	71	26
Strigamia transsilvanica (Verhoeff, 1928)	-	-	4	-	-	2	-	-	-	4	2	0
Lithobius aerugineus L. Koch, 1862	-	-	41	-	-	39	-	-	-	41	39	0
Lithobius austriacus (Verhoeff, 1937)	-	-	-	-	-	2	-	-	-	0	2	0
Lithobius dentatus C.L. Koch, 1844	-	-	2	-	-	1	-	-	-	2	1	0
Lithobius erythrocephalus C.L. Koch, 1847	-	-	1	-	-	-	-	-	-	1	0	0
Lithobius forficatus (Linnaeus, 1758)	-	-	-	-	-	1	-	-	1	0	1	1
Lithobius microps Meinert, 1868	-	4	-	-	47	-	-	31	-	4	47	31
Lithobius mutabilis L. Koch, 1862	-	-	3	-	-	3	-	-	1	3	3	1
Lithobius spp.	-	-	14	2	-	24	-	-	-	14	26	0
Chilopoda	0	4	100	3	67	161	1	97	2	104	231	100

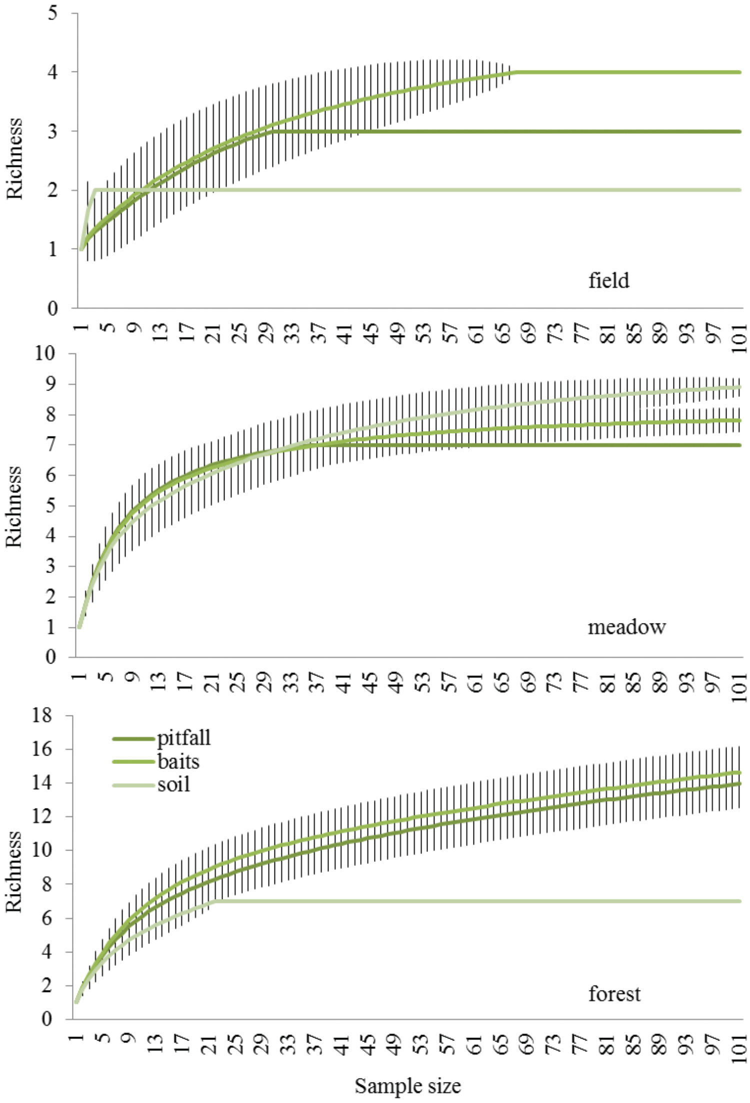

Figure 1. Rarefactions of estimated species richness (i.e. number of species) in increasing size of random samples (i.e. number of individuals), comparison of effectiveness of sampling by individual methods at different sites. Vertical lines represent standard errors.

Table 2. Pairwise comparisons of species lists collected (a) at different sites and (b) by different methods. (Observed p-value below diagonal, permuted p-value above diagonal).

a)	field	forest	meadow	b)	hay-bait	pitfall	soil
field	-	0.001	0.048	hay-bait	-	0.003	0.917
forest	0.000	-	0.001	pitfall	0.003	-	0.043
meadow	0.041	0.004	-	soil	0.911	0.052	-

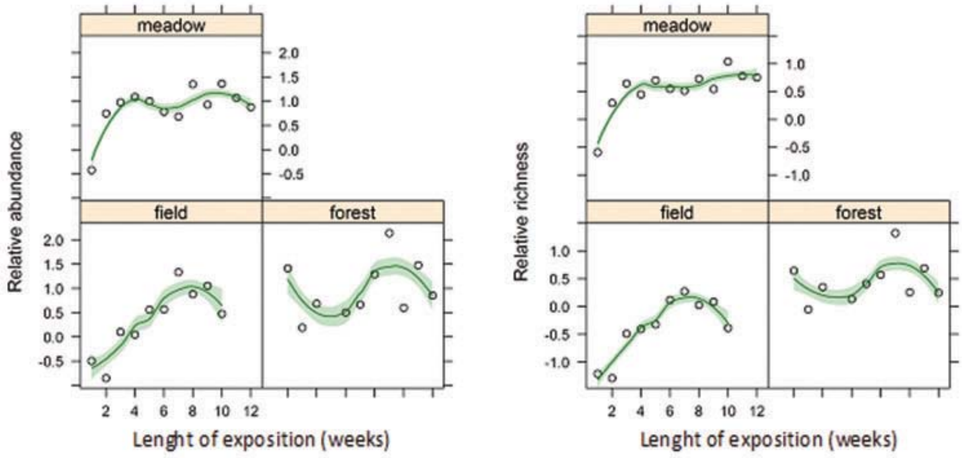

Figure 2. Changes in myriapod communities inside hay-bait traps installed in three biotopes during the 12 week trapping period. Qualitative as well as quantitative parameters are shown for these communities. Open dots are observed parameters, whereas solid lines represent models of succession including standard errors (green shading).

Discussion

Centipedes and millipedes live on the soil surface and inside soil. We can find them through the whole soil gradient to a depth of one meter (e.g. Ilie 2003) although they are abundant in upper layers predominantly. This is the reason why pitfall traps are not sufficient for sampling the whole community adequately. We evaluated efficiency of hay-bait traps for sampling soil-dwelling millipedes and centipedes with the time consuming soil sampling (connected with destruction of the soil surface and transport of heavy samples to the laboratory).

Bait traps were used for sampling invertebrates, mainly beetles, in caves originally (Barber 1931). Bait traps are much more common for carnivorous or necrophagous species; baits are represented by pieces of flesh, fish or cheese above a fixation solution surface, or direct addition of beer to a solution. Straw, wood or yeast is placed in caves occasionally as the baits for detritivores (Mock pers. comm.). Nevertheless, baits are not working there as traps, as they need to be visited and inspected continuously to collect attracted animals to avoid them to leave baits.

The first documented version of bait traps for millipedes was a shingle trap by Barber (1997) filled with kitchen tissue and potatoes. He used this trap to sample millipedes and isopods at a shingle beach in England. Similar kinds of bait traps, containing sweet potatoes or corn, were used by Brunke et al. (2012) for sampling *Cylindroiulus caeruleocinctus* in Canadian fields. Almost the same traps are used with the name litter bags for studying decomposition of different kinds of litter and/or by different size groups of decomposers (according to diameters of holes in the traps). Litter bags are also used for sampling soil mesofauna or microarthropods (e.g. Wiegert 1974).

Prasifka et al. (2007) used litter bags to sample ground dwelling invertebrates; they installed litter bags at the soil surface as well as below the soil surface in a corn field. Above-ground bait traps were attractive for centipedes (millipedes were not recorded in this research). Apart from these publications, we did not found any records of the use of bait traps for sampling millipedes or centipedes.

Hay-bait traps vs. soil samples

If we are interested in using hay-bait traps as an adequate (or even better) substitute for soil sampling, we have to compare species lists of millipedes and centipedes trapped by these methods. There were only three species recorded exclusively from soil sampling, i.e. missing in hay-bait traps: millipede *Brachydesmus superus* and centipedes *Geophilus electricus* and *G. truncorum*. The minute millipede species lives preferably in clay soils with litter (Lee 2006) usually in huge quantities. This species is a dominant species recorded by pitfall traps in cities (Riedel et al. 2009), so its absence in pitfall traps at the meadow site is probably caused by its low abundance. *Geophilus truncorum* was recorded once only, so it is hard to evaluate effectiveness of sampling of so “rare” a species. Nevertheless, both geophilomorphs (*G. electricus* and *G. truncorum*) are known as predators of earthworms (Sergeeva et al. 1985, Keay 1986); for this reason, hay-bait may be not attractive for them as they follow earthworms into their corridors in soil. So, to collect *G. electricus*, soil sampling or direct hand collecting seems to be necessary. Other geophilomorphs (*C. flavidus*, *S. nemorensis*, *S. acuminata*, *G. flavus*) are common species, which are frequently found by individual hand-collecting; they live in soil near the surface, under logs, bark and stones (e.g. Barber and Keay 1988). Their presence in shallow hay-bait traps is not surprising as these species were sampled by pitfall trapping too.

One millipede species, *Julus scandinavius* was recorded exclusively in a hay-bait trap, but as one specimen only was found no generalization can be made. Many more species were found in both hay-bait traps and pitfall traps but not in soil samples. Nevertheless, hay-bait traps are not a substitute method to pitfall trapping as there were significant differences between species lists recorded by these methods (see Tab. 2), but it can definitely substitute the soil samples.

Colonisation of hay-bait traps

Centipedes, and especially millipedes, are attracted into the hay-bait traps. The possible reason can be as a food source and/or sustainable shelter with higher humidity. At least for millipedes, food source seems to be the more probable explanation; wet cloth method (offering higher humidity) did not record any millipedes in African savannah ecosystems (Druce et al. 2004). More probably, millipedes and centipedes are attracted by food availability, as it can be associated with hay decomposition and colonization of the traps. Smaller decomposers colonising baits are welcomed food for carnivorous centipedes (e.g. Perry et al. 1997).

Eight to ten weeks seems to be the optimal exposure time for hay-bait traps in Central European conditions. A similar result was found by Ožanová (2001) using grass traps (a small heap of mowed grass on the surface of meadow), with a much higher number of species after 7 weeks than for a shorter exposure time. Although Prasifka et al. (2007) did not evaluate the effect of length of exposure time of bait traps, it is evident from their results that below-ground traps were more effective after 8 weeks than after 6 weeks. It supports our results that the best length of exposure of bait traps is from 8 to 10 weeks, although we aware of difficulties with this generalization. The best length of exposure is not dependent only on a type of habitat, but also on climate conditions (rainy or dry weather) and time of year when exposed. Traps installed in Central European conditions in late autumn or in winter or during dry hot summer can be colonised in different ways as invertebrates change their activity and position in the soil profile during the year (David 1984, Geoffroy 1985, David et al. 1996, Tuf 2002). The general recommendation for using these traps when installed in spring is to use them for 8–10 weeks. Timing of installation and the length of time exposed in field sites will need to coordinate with agricultural activities such as sowing and harvest times.

Conclusion

Centipedes and millipedes inhabit the soil surface as well as the soil profile. For a complete knowledge of myriapod fauna, pitfall trapping needs to be combined with a method to collect soil dwelling species, e.g. soil sampling. Hay-bait traps were tested for their ability to replace soil sampling. Our results showed that hay-bait traps are attractive to myriapods and can have a similar sampling effort as soil sampling. The optimal length of exposure of hay-bait traps in soil seems to be ca 2 months (8–10 weeks).

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Příloha 3

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Vertical distribution of spiders in soil

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Abstract. Research studies of the shallow subterranean habitats as environments for arthropods have been sparse up to this point. Using subterranean traps, we studied the distribution of spiders in soil profile over a depth span of 5–95 cm at six sites. Although almost 40% of individual specimens (1088 in total) were obtained from the epigeon (5 cm depth), spiders colonized all parts of the soil profiles examined. Beside ground-dwelling species with significant preferences for the upper layers, some species (*Porrhomma microphthalmum* (O. Pickard-Cambridge 1871), *Centromerus cavernarum* (L. Koch 1872), *Cicurina cicur* (Fabricius 1793), *Dysdera lantosquensis* Simon 1882, and *Nesticus cellulanus* (Clerck 1757)) commonly inhabited the whole range of the profiles studied, without any depth preference. In contrast, depigmented and microphthalmous *Porrhomma microps* (Roewer 1931) and *Maro* sp. exclusively inhabited deep soil layers adjoining void systems in bedrock.

Keywords: Mesovoid shallow substratum, superficial underground compartment, subterranean environment, Araneae

The soil is an aphotic environment inhabited by casual transmigrants as well as fauna adapted for subterranean life (e.g., microphthalmy, depigmentation etc.). Soil porosity limits the size of its inhabitants; nevertheless, soil spaces vary in dimension from sand fissures to cave systems (Christian 1999). Three distinct types of habitats in relation to the soil can be formally distinguished: 1) epigean, inhabited by surface dwelling animals; 2) endogean, with mainly edaphic animals; and 3) hypogean, with subterranean species inhabiting void systems, including caves (Giachino & Vailati 2010).

Our knowledge of endo- and hypogeic animals is limited, due to difficulties in collecting samples. Invertebrates inhabiting a subterranean environment can be studied with the use of only a few methods. One such method is the use of pitfall traps, modified in various ways (e.g., a collar around the nose, perforation of walls), dug to different depths and exposed for an extended period of time (Růžička 1982, 1988; Yamaguchi & Hasegawa 1996). Another type is Barber's Shingle Trap (Barber 1997), with bait inside and a long hose exposed at the depth studied. A frequently used method involves traps installed in drills (Illie 2003; Illie et al. 2003; Negrea 2004). One clever modification of drill trapping is the subterranean trap designed by Schlick-Steiner and Steiner (2000) that collects animals in one drill at various depths.

Beside an extensive knowledge of cave spiders (e.g., Paquin & Dupérré 2009), we have some information on spiders in scree slopes (Růžička et al. 1995; Růžička 1999a, 1999b, 2002; Růžička & Klimeš 2005). In stony alpine debris, spiders are generally most abundant in the upper layers, but differ significantly depending on locality (Schlick-Steiner & Steiner 2000). A similar pattern of abundance was found in the vertical distribution of spiders in peat bogs (Biteniekytė & Rėlys 2006). However, we have limited knowledge about spiders inhabiting soil, as endogeic and hypogeic spiders have only been studied recently in Bulgaria (Deltshev et al. 2011). The present study aims to reveal whether spiders inhabit deep soil layers and to characterize the vertical distribution of spider communities at several sites with different soil types in Central Europe.

METHODS

Site description.—The research was carried out at six sites in the Czech Republic; three of them (*Above cave, Rock, Debris*) were situated in Central Moravia near the town of Hranice na Moravě (320 m a.s.l.). The other three sites (*Beech wood, Quarry, Valley*) were situated 10 km east of the town of Skuteč, on the border of Žďárské vrchy Protected Landscape Area (Eastern Bohemia, approx. 450 m a.s.l.).

Above cave (49°31′N, 17°44′E)*:* This site is situated above Zbrašov Aragonite Caves. The cave system is linked with stony debris above through shafts, ending approximately one meter below the surface. The upper soil layer was covered by leaf litter from deciduous forest. The debris was partially filled by soil particles created by interskeletal erosion forming lithosol soil type.

Rock (49°32′N, 17°44′E)*:* This site is one kilometer from *Above cave* under a limestone rock face in deciduous forest. In this renzic leptosol soil type, the upper organic layer is about 5 cm thick, the A-horizon with a mixture of organic and inorganic particles (about 15 cm thick) passing to the C-horizon with broken-down lime bedrock (stones several centimeters in diameter) and clay.

Debris (49°32′N, 17°44′E)*:* This site is only about 50 m from the *Rock* site on a slope in deciduous forest. The soil is similar to the previous one, but with larger stones (20–30 cm).

Beech wood (49°50′N, 16°3′E)*:* This site with cambisol soil type has a thick layer of leaf litter covering an A-horizon (ca 15 cm) passing to a 50 cm-thick cambic horizon above arenaceous marl bedrock.

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Table 1.—List of spider species in subterranean traps, with the number of trapped specimens and their depth range (uppermost to undermost record). Nomenclature of spiders according to Platnick (2010) with two exceptions: *Dysdera lantosquensis* sensu Řezáč et al. (2008) and *Porrhomma microps* (= *lativelum*) sensu Růžička (2009).

Family / Species	# Specimens	Depth distribution
Dysderidae		
Dysdera lantosquensis Simon 1882	25	5–95 cm
Harpactea lepida (C.L. Koch 1838)	101	5–95 cm
Harpactea rubicunda (C.L. Koch 1838)	3	5–15 cm
Nesticidae		
Nesticus cellulanus (Clerck 1757)	28	5–85 cm
Theridiidae		
Pholcomma gibbum (Westring 1851)	14	5–95 cm
Robertus lividus (Blackwall 1836)	13	5–35 cm
Robertus truncorum (L. Koch 1872)	2	15 cm
Linyphiidae		
Asthenargus perforatus Schenkel 1929	1	15 cm
Bathyphantes gracilis (Blackwall 1841)	1	5 cm
Centromerus cavernarum (L. Koch 1872)	28	5–75 cm
Centromerus silvicola (Kulczyński 1887)	37	5–55 cm
Ceratinella brevis (Wider 1834)	16	5–15 cm
Diplocephalus picinus (Blackwall 1841)	5	5–25 cm
Diplostyla concolor (Wider 1834)	7	5–15 cm
Entelecara acuminata (Wider 1834)	1	55 cm
Maro sp.	2	45–65 cm
Micrargus herbigradus (Blackwall 1854)	5	5–15 cm
Microneta viaria (Blackwall 1841)	6	5 cm
Neriene emphana (Walckenaer 1841)	1	25 cm
Oedothorax apicatus (Blackwall 1850)	223	5–55 cm
Palliduphantes alutacius (Simon 1884)	83	5–95 cm
Porrhomma microphthalmum (O. Pickard-Cambridge 1871)	185	5–85 cm
Porrhomma microps (Roewer 1931)	21	25–85 cm
Porrhomma oblitum (O. Pickard-Cambridge 1871)	1	15 cm
Saaristoa firma (O. Pickard-Cambridge 1905)	1	25 cm
Saloca diceros (O. Pickard-Cambridge 1871)	15	5–95 cm
Tenuiphantes flavipes (Blackwall 1854)	21	5–35 cm
Tenuiphantes tenebricola (Wider 1834)	1	5 cm
Walckenaeria atrotibialis (O. Pickard-Cambridge 1878)	2	5 cm
Walckenaeria dysderoides (Wider 1834)	1	15 cm
Walckenaeria furcillata (Menge 1869)	3	5–15 cm
Walckenaeria obtusa Blackwall 1836	2	5 cm
Walckenaeria vigilax (Blackwall 1853)	5	15 cm
Araneidae		
Araneus diadematus Clerck 1757	1	5 cm
Lycosidae		
Trochosa terricola Thorell 1856	2	25–35 cm
Agelenidae		
Histopona torpida (C.L. Koch 1834)	7	5–85 cm
Malthonica silvestris (L. Koch 1872)	6	5–65 cm
Cybaeidae		
Cybaeus angustiarum L. Koch 1868	8	5–75 cm
Hahniidae		
Hahnia nava (Blackwall 1841)	1	25 cm
Dictynidae		
Cicurina cicur (Fabricius 1793)	123	5–95 cm
Amaurobiidae		
Amaurobius fenestralis (Ström 1768)	2	5 cm
Callobius claustrarius (Hahn 1833)	2	5–15 cm
Coelotes terrestris (Wider 1834)	9	5–25 cm
Eurocoelotes inermis (L. Koch 1855)	12	5 cm
Liocranidae		
Apostenus fuscus Westring 1851	5	5–15 cm
Clubionidae		
Clubiona brevipes Blackwall 1841	3	5 cm
Salticidae		
Ballus chalybeius (Walckenaer 1802)	3	5 cm
Neon reticulatus (Blackwall 1853)	9	15–25 cm

Quarry (49°50′N, 16°2′E)*:* This site was situated in an abandoned basalt quarry. The soil profile consisted entirely of pieces of basalt about 10 cm in size. The upper soil layers, including vegetation, were removed during excavation.

Valley (49°50′N, 16°2′E)*:* This site is ca 1 km from the preceding one, situated in the Krounka stream basin. This debris slope is covered by deciduous forest and large stones overgrown with mosses. The homogenous soil profile comprised of basalt stones is about 20–25 cm in size with space partially filled by organic material from trees and inorganic particles created by erosion to a depth of one meter.

Sampling.—Spiders were collected using subterranean traps (Schlick-Steiner & Steiner 2000). The trap, made of rigid plastic, consists of a tube (10 cm in diameter) with three fissures (ca 4 mm wide, 6–7 cm long) at 10 cm intervals. The traps are over one meter long, and the last sampling fissure was 95 cm deep. A hole, about 1.5 × 0.7 m and 1.3 m deep was dug at each site, and soils of different layers were separated carefully using plastic sheets. Three tubes were put in the hole in line with each other, 50 cm apart, and the hole was then filled with soil in the proper order. A set of ten removable plastic containers (250 ml) situated on a central metal axis was placed in each tube; the position of the containers corresponded to the fissures in the tube. Through this arrangement, the containers collected animals entering the tube through fissures at particular depths. The traps were filled with a 4%

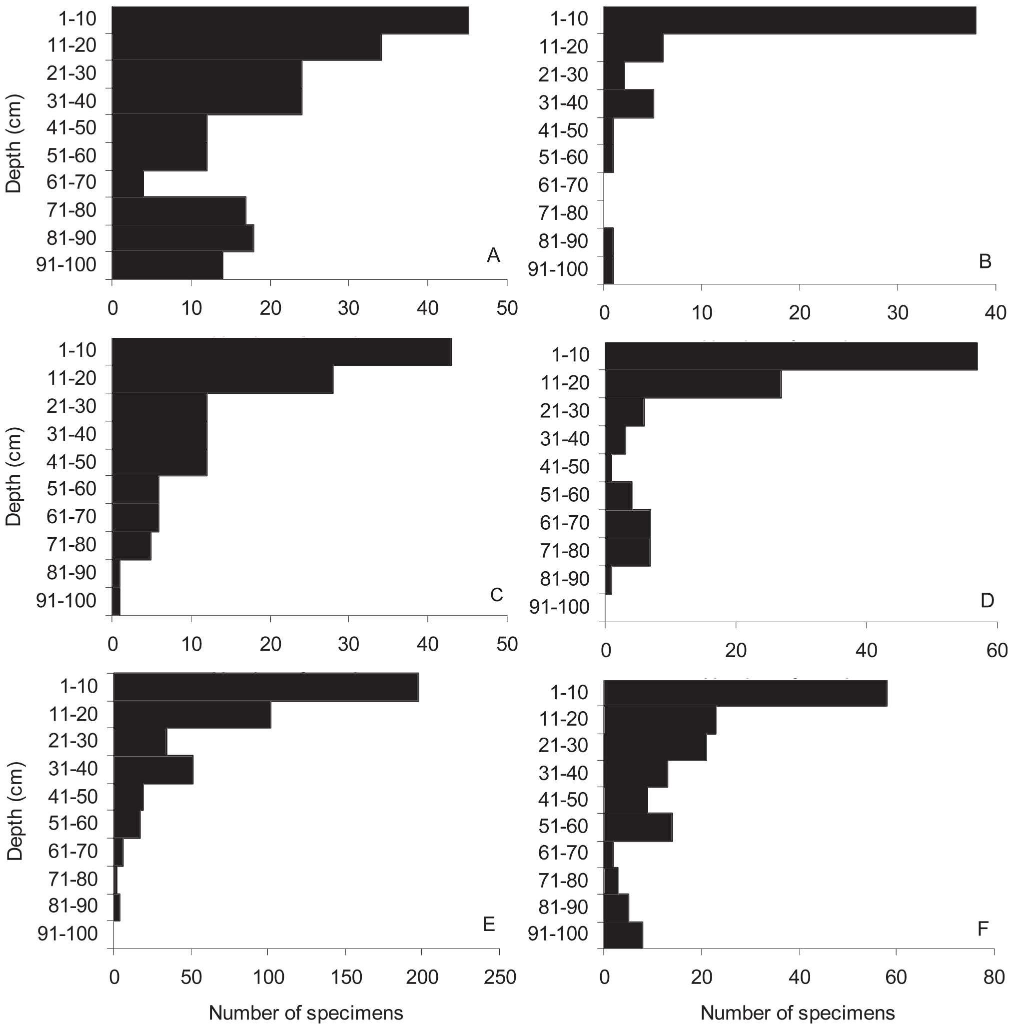

Figure 1.—Depth distribution of spider individuals at sites studied (number of all collected specimens). A – *Above cave* (altogether 205 individuals, 17 spp.), B – *Rock* (55 individuals, 12 spp.), C – *Debris* (126 individuals, 20 spp.), D – *Beech wood* (113 individuals, 17 spp.), E – *Quarry* (433 individuals, 9 spp.), F – *Valley* (156 individuals, 18 spp.).

formaldehyde solution and emptied at six-week intervals between 7 March 2005 and 17 March 2007. Voucher specimens are deposited in the collection of V. Růžička at the Institute of Entomology, Biology Centre, AS CR in České Budějovice.

Data analysis.—In the analysis, we used only species with more than 10 individuals and/or species found in more than four sites. RDA (Canonical redundancy analysis) was used to study the effect of depth and individual site. The significance of the first axis was determined by a Monte Carlo permutation test (499 permutations). Data standardized by error variance were used for RDA. Generalized linear models (GLM) with Poisson errors were used to study the relationship of each species and environmental variables (site, depth). CANOCO software was used for these analyses (Ter Braak, Šmilauer 1998).

RESULTS

The most numerous taxa collected were Collembola (44.7%), followed by Diptera (13.0%), Oniscidea (12.5%), Coleoptera (10.8%), Araneae (10.0%), and other rare taxa (Diplopoda 2.7%, Acarina 2.0%, Chilopoda 1.3%, Pseudoscorpiones 1.1%, Formicidae 0.8%, Dermaptera 0.6%, and Opiliones 0.4% respectively). Altogether, 1088 spider specimens of 48 species were trapped (Table 1). Among 14 spider families, the Linyphiidae was the richest in species (26 species), followed by the Amaurobiidae (4 species), Theridiidae and Dysderidae (3 species each) and Agelenidae and Salticidae (2 species each). We only evaluated the distribution of the 17 most numerous species statistically.

Quarry was the site with the greatest number of trapped spiders (433 individuals, 9 species); the highest number of species was recorded at *Debris* (20 species). The most numerous catches were in the upper level, at a depth of 5 cm (almost 40% of the individuals), and the lowest were at a depth of 95 cm (2.5%). Beside the most abundant spiders at the uppermost level, no common pattern was typical for all sites (Fig. 1). The number of spider species below 55 cm was highest in the *Above cave* site (over 30%), followed by *Debris*, *Beech wood*, and *Valley* sites (about 20%), followed by *Rock* and *Quarry* sites (only approximately 6%).

The RDA model revealed significant differences among the distribution of species ($F = 9.07$, $P < 0.01$). The sum of all

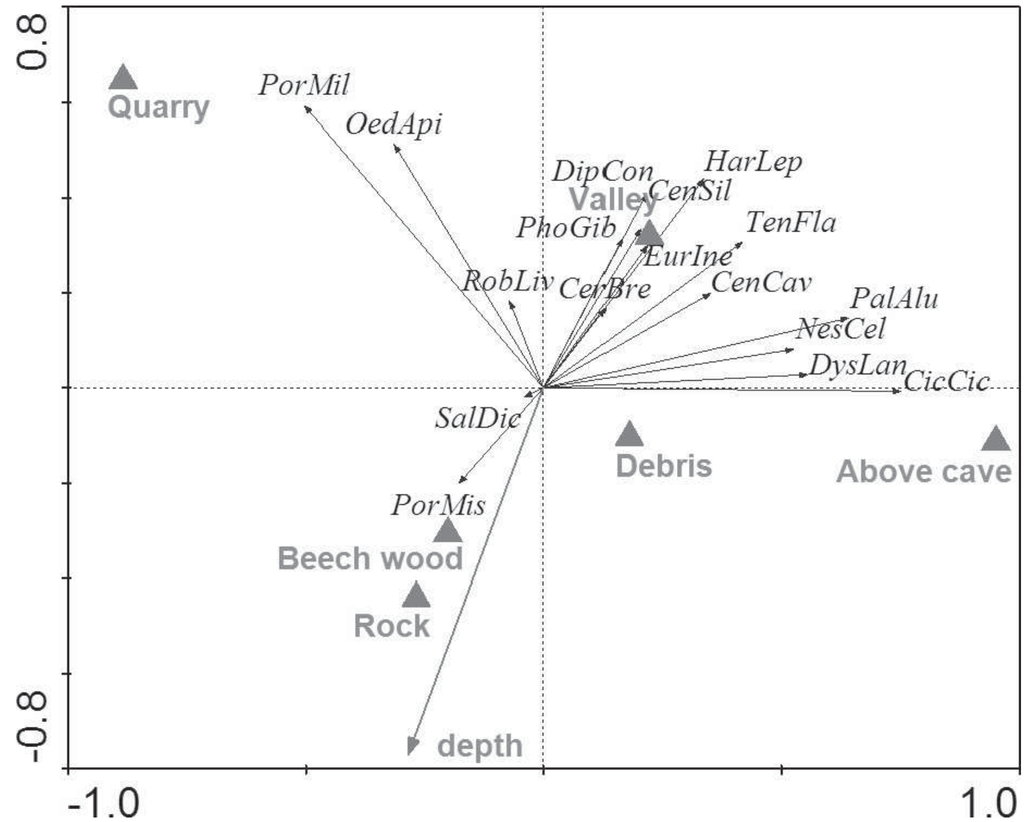

Figure 2.—RDA ordination biplot illustrating distribution of spiders in relation to sites and depth. Abbreviations: CenCav – *Centromerus cavernarum*, CenSil – *Centromerus silvicola*, CerBre – *Ceratinella brevis*, CicCic – *Cicurina cicur*, DipCon – *Diplostyla concolor*, DysLan – *Dysdera lantosquensis*, EurIne – *Eurocoelotes inermis*, HarLep – *Harpactea lepida*, NesCel – *Nesticus cellulanus*, OedApi – *Oedothorax apicatus*, PalAlu – *Palliduphantes alutacius*, PhoGib – *Pholcomma gibbum*, PorMil – *Porrhomma microphthalmum*, PorMis – *Porrhomma microps*, RobLiv – *Robertus lividus*, SalDic – *Saloca diceros*, TenFla – *Tenuiphantes flavipes*.

canonical eigenvalues explains 50.7% of variability. Although there is no common pattern of depth distribution of spiders, depth is a significant general predictor ($F = 7.74$, $P < 0.01$), and distribution patterns and community compositions differ among sites ($F = 3.18$, $P = 0.04$). The ordination diagram shows that *Porrhoma microps* (Roewer 1931) is positively correlated with soil depth (Fig. 2).

GLM modeling of the response of the 17 dominant spider species to depth showed significant pattern for ground-dwelling species mainly; the spiders inhabiting the entire profile do not significantly prefer any depth (Table 2). These 17 species can be separated into four categories according to their depth distribution and preferences (distributions of 11 species with more than 20 trapped specimens are displayed in Fig. 3):

1. Exclusively surface dwelling species: *Eurocoelotes inermis* (L. Koch 1855), *Diplostyla concolor* (Wider 1834), *Ceratinella brevis* (Wider 1834)
2. Surface-dwelling species (significant preference for upper layers) penetrating into deeper layers: *Centromerus silvicola* (Kulczyński 1887), *Oedothorax apicatus* (Blackwall 1850), *Tenuiphantes flavipes* (Blackwall 1854), *Harpactea lepida* (C.L. Koch 1838), *Robertus lividus* (Blackwall 1836), *Palliduphantes alutacius* (Simon 1884), *Saloca diceros* (O. Pickard-Cambridge 1871)
3. Species inhabiting whole soil profile (without preference for any depth): *Pholcomma gibbum* (Westring 1851), *Porrhomma microphthalmum* (O. Pickard-Cambridge 1871), *Centromerus cavernarum* (L. Koch 1872), *Cicurina cicur* (Farricius 1793), *Dysdera lantosquensis* Simon 1882, *Nesticus cellulanus* (Clerck 1757)
4. Species inhabiting exclusively (any) of the deeper layers: *Porrhomma microps*. Two other species, too rare for statistical evaluation, were found in the deeper layers: *Entelecara acuminata* (Wider 1834) at 55 cm and *Maro* sp. at 45 cm and 65 cm (identification is complicated by expanded palps; these two specimens have slightly reduced eyes, unlike species of *Maro*, posterior median eyes 1.2 diameters apart).

DISCUSSION

Studies of the deep soil layer environment have been scarce due to the difficulty of sampling these arthropod communities. We present evidence for the occurrence of spiders (invertebrates larger than typical soil invertebrates such as mites and collembolans) in soil layers down to one meter in depth.

Vertical distribution of spiders in the soil profile differed according to the habitat type. Although we were not able to evaluate the soil porosity due to the presence of large stones (making it impossible to take intact soil samples), we assume that there were relatively large spaces at some study sites (e.g., in fractured, arenaceous marl bedrock). This seems to be an important factor for the vertical distribution of spiders.

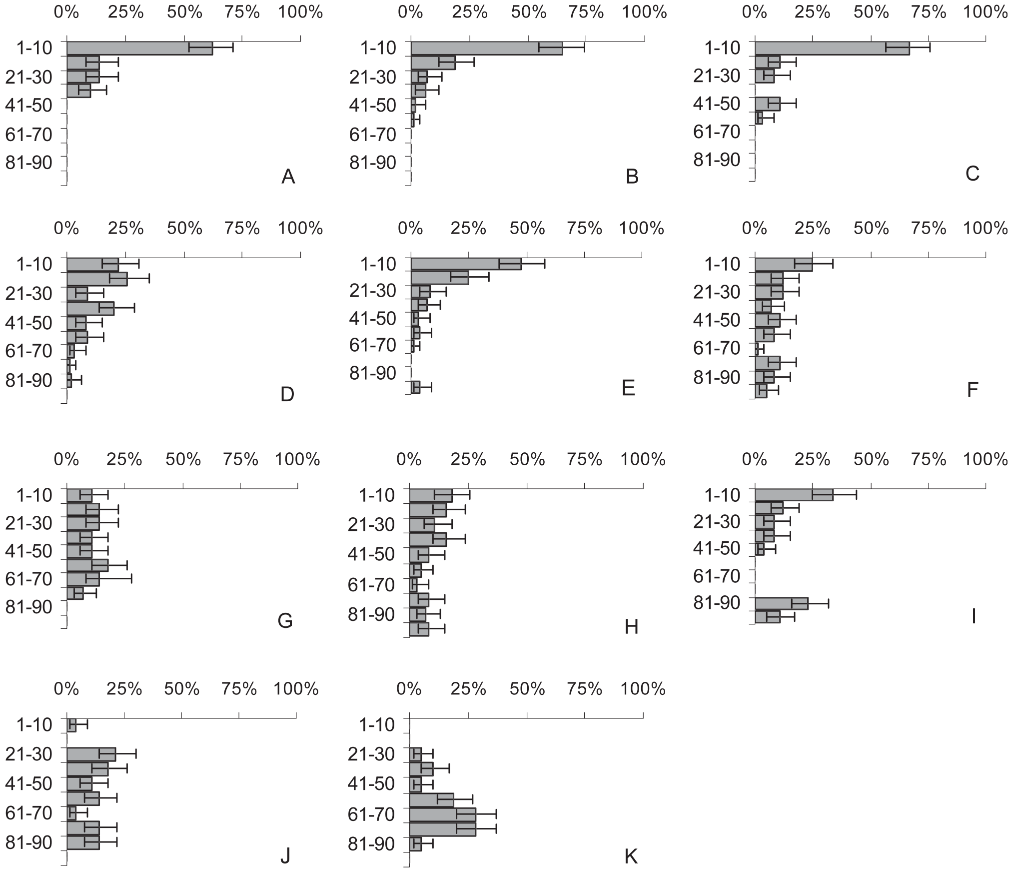

Figure 3.—Depth distribution of 11 species (more than 20 trapped specimens apiece). Bars represent mean proportions, whiskers are 95% confidence intervals. A – *Tenuiphantes flavipes* (mean record at 12 cm), B – *Oedothorax apicatus* (mean record at 11 cm), C – *Centromerus silvicola* (mean record at 10 cm), D – *Porrhomma microphthalmum* (mean record at 27 cm), E – *Harpactea lepida* (mean record at 19 cm), F – *Palliduphantes alutacius* (mean record at 38 cm), G – *Centromerus cavernarum* (mean record at 39 cm), H – *Cicurina cicur* (mean record at 40 cm), I – *Dysdera lantosquensis* (mean record at 40 cm), J – *Nesticus cellulanus* (mean record at 48 cm), K – *Porrhomma microps* (mean record at 61 cm).

Spiders were found to inhabit deeper soil layers in scree slopes with large soil spaces (*Above cave*, *Debris*, *Valley*, *Beech wood*, Fig. 1). Spiders were less common in the deeper soil layers in sites with small spaces and small stones. The *Beech wood* site was a different case, hosting spiders in the deep soil layer, which likely did not penetrate it from the surface. Spiders found here were microphthalmous species that can inhabit the subterranean environment created by systems of voids (MSS) in arenaceous marl bedrock exclusively. Presence of MSS is evident at sites *Above Cave* (corresponding with cave environment) and also *Valley* (Fig. 1).

Several species exhibit a clear tendency to live in deep soil layers. These belong to the families Linyphiidae, Dictynidae, and Nesticidae. Small body size results in a large ratio of surface area to volume, and vulnerability of desiccation. The deeper layers of soil can protect these individuals against desiccation. Such a pattern was described by Wagner et al. (2003) in the litter at a microscale level.

An affinity to a broad spectrum of subterranean habitats is found in species of the genus *Porrhomma*. A species recorded in this study, *P. microps*, has been repeatedly found in caves in Italy and in leaf litter in Germany (Růžička 2009). Although it also inhabits leaf litter in floodplain forests of the Czech Republic (Buchar & Růžička 2002), it was also recently found

Table 2.—Categorization of species by their affinity to depth and results of GLM model (Note: only values related to depth are presented).

Species	Category	F	P
Ceratinella brevis	1	3.78	0.03
Diplostyla concolor	1	24.91	$< 1.0e-6$
Eurocoelotes inermis	1	20.18	$< 1.0e-6$
Centromerus silvicola	2	5.12	0.01
Harpactea lepida	2	12.56	0.00
Oedothorax apicatus	2	3.51	0.04
Palliduphantes alutacius	2	3.62	0.03
Robertus lividus	2	4.71	0.01
Saloca diceros	2	3.17	0.05
Tenuiphantes flavipes	2	13.93	0.00
Centromerus cavernarum	3	1.75	0.18
Cicurina cicur	3	1.45	0.24
Dysdera lantosquensis	3	2.21	0.12
Pholcomma gibbum	3	2.97	0.06
Porrhomma microphthalmum	3	2.06	0.14
Nesticus cellulanus	3	0.96	0.39
Porrhomma microps	4	1.67	0.20

in karst and pseudokarst caves. *Porrhomma egeria* Simon 1884 was recorded in basalt scree slopes at a depth of about 1 m (Růžička et al. 1995) and in the block accumulations and crevice caves in a decaying gneiss massif at depths greater than 5 m (Růžička 1996). A troglomorphic population of *Porrhomma myops* Simon 1884 was recorded in caves and in andesite scree slopes at a depth of 40–100 cm (Růžička 2002), whereas an edaphomorphic population of this species was described from a deep soil layer (35–95 cm) in floodplain forest (Růžička et al. 2011). *Porrhomma microcavense* Wunderlich 1990 was recorded in an arenaceous marl layer (Kůrka et al. 2006). This rock is known to form extensive underground void systems, and we consider these void systems to be ideal locations for the future research of invertebrates in shallow subterranean habitats. This assumption is supported by a record of microphthalmous *Maro* sp. in a beech forest on arenaceous marl bedrock during our research.

Another species, *Zangherella relicta* (Kratochvíl 1935) (Anapidae) was described from caves in Montenegro, and recently it was found at several localities in Bulgaria, where it occurs exclusively in mountain scree slopes at depths of 40–50 cm (Deltshev et al. 2011). All these findings document individual phases of the evolutionary process leading to colonization of subterranean environment over the entire depth profile of the terrain (Růžička 1999a; Culver & Pipan 2009; Giachino & Vailati 2010).

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Příloha 4

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Can montane and cave centipedes inhabit soil?

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Abstract: Communities of centipedes (Chilopoda) were studied at three localities in eastern Bohemia (Czech Republic) near the town of Skuteč using modified subterranean traps. Centipedes were trapped separately from depths of 5, 15, 25, 35, 45, 55, 65, 75, 85, and 95 cm to allow evaluation of the vertical distribution in the soil. Presence of centipedes in deeper soil profiles is related to their ability to colonize the subterranean environment. *Lithobius tenebrosus fennoscandius*, *L. lucifugus*, *L. macilentus*, *Cryptops parisi*, *Strigamia acuminata*, and *S. transsilvanica* inhabited deeper soil layers preferentially. This study shows that soil can be inhabited by cave centipedes or centipedes with montane distribution.

Key words: Chilopoda, milieu souterrain superficiel, mesovoid shallow substratum, superficial underground compartment, subterranean environment

There are many species inhabiting soil as spaces in soil offer free ecological niches (Růžička, 1999). Nevertheless, life underground is enabled by specific adaptations. Christiansen (1992) summarized exaptations as the key factor necessary for colonization of subterranean habitats, although we do not know for which environmental factors these preadaptations were evolved. Vandel (1965) suggested that cave species probably evolved from surface-dwelling species (terrestrial as well as aquatic ones). Recent studies support an alternative view: adaptations to life in soil are good preadaptations to life in caves as well as in the mesovoid shallow substratum (MSS, i.e. spaces in soil and bedrock mainly; Ortuño et al., 2013). Růžička (1999) showed that some troglomorphic species of spiders in Central Europe are related to species inhabiting forest soils, screes, rock fissures, mountain snowfields, and peat bogs. The vertical distribution of spiders in the soil was recently studied in forests (Deltshev et al., 2011; Laška et al., 2011; Růžička et al., 2011), peat bogs (Biteniekytė and Rėlys, 2006), and screes (Culver and Pipan, 2009; Pipan et al., 2011). Nevertheless, the vertical distribution of centipedes in soil and the MSS has rarely been studied (Nitzu et al., 1999; Ilie, 2003; Rendoš et al., 2012, 2016) and then at the community level only, so we do not have information about the distribution of individual species in the soil profile. The aim of this study is to describe the vertical distribution of individual centipede species at three localities with different soil conditions.

The study was done at three localities in eastern Bohemia, 10 km northwest of Skuteč. The first locality, called *beech*, was in this type of wood near the village of Hluboká. The soil was covered by approximately 15 cm of leaf litter. The clay layer of 30 cm lay on cracked marl bedrock. The second locality was situated in slate *quarry* near the village of Hněvětice. This slate quarry soil profile was almost homogeneous, created by bigger stones (approx. 10–15 cm) with some amount of soil among them. The third locality was located in the *valley* of the Krounka River between Hněvětice and Předhradí. There was a scree slope of big stones (20–30 cm) partially filled by soil and detritus.

Centipedes were sampled using subterranean traps according to the alpine model of Schlick-Steiner and Steiner (2000), similar to that of López and Oromí (2010). Traps were made from long tubes buried in a vertical position from the soil surface to a depth of 130 cm. Walls of the tube were perforated in intervals of 10 cm. Inside the tubes there were systems of 10 pots with fixation solution, which collected the animals entering into tubes at depths of 5, 15, 25, 35, 45, 55, 65, 75, 85, and 95 cm, respectively. As a fixation solution, 4% formaldehyde was used. At each locality, three traps were installed in one hollow. The distance between traps was 50–60 cm. During installation of traps, the hollow was filled by material (soil, stones) considering the original stratification of stones. Traps were

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inspected once every 6 weeks from March 2005 to March 2006.

In total, 63 centipedes were collected by subterranean traps during 54 weeks or 1 year. This material comprised 11 species of centipedes (and 5 individuals of juveniles of the genus *Lithobius*). The highest number of species of centipedes inhabited the locality *valley* (7 species), while the locality *quarry* was inhabited by *Lamyctes emarginatus* only (Table).

Localities differed strongly in centipede vertical distribution patterns. Whereas *L. emarginatus* was recorded in *quarry* from upper layers exclusively (up to 15 cm), centipedes inhabited deep layers at *beech* (up to 75 cm) as well as at *valley* (whole profile). The highest number of species (5) was recorded in the deepest layer at *valley*.

Species associated with upper soil layers (i.e. depths of 5 and 15 cm) were *L. nodulipes*, *L. mutabilis*, and *L. agilis*, while *L. lucifugus*, *L. tenebrosus fennoscandius*, and both

Table. Vertical distribution of trapped centipedes (number of individuals / 3 traps / 1 year) and time pattern of presence at relevant depths (month span) at three studied localities.

	beech					*quarry*	*valley*							
depth	*Lithobius agilis* L.Koch, 1847	*Lithobius forficatus* (Linnaeus, 1758)	*Lithobius lucifugus* L.Koch, 1862	*Lithobius mutabilis* L.Koch, 1862	*Cryptops parisi* Brölemann, 1920	*Lamyctes emarginatus* (Newport, 1844)	*Lithobius lucifugus* L.Koch, 1862	*Lithobius macilentus* L.Koch, 1862	*Lithobius mutabilis* L.Koch, 1862	*Lithobius nodulipes* Latzel, 1880	*Lithobius tenebrosus fennoscandius* Lohmander, 1948	*Strigamia acuminata* (Leach, 1815)	*Strigamia transsilvanica* (Verhoeff, 1928)	Σ
5 cm	-	-	3	-	-	3	1	-	2	-	1	-	3	13
			5–7			6–7	5–6		5–6		10–11		10–11	
15 cm	2	-	-	1	-	4	1	-	2	1	-	-	-	11
	3–4			7–8		6–8	5–6		10–11	5–6				
25 cm	-	-	1	1	-	-	-	-	-	-	1	-	-	3
			9–10	7–8							10–11			
35 cm	-	-	-	-	1	-	1	-	-	-	-	-	-	2
					10–11		9–10							
45 cm	-	-	-	-	1	-	-	2	-	-	1	1	-	5
					10–11			9–10			9–10	10–11		
55 cm	-	-	1	-	-	-	-	-	-	-	-	-	-	1
			7–8											
65 cm	-	-	-	-	-	-	2	-	1	-	1	-	1	5
							6–8		10–11		6–7		1–2	
75 cm	-	1	-	-	-	-	-	-	-	-	2	1	1	5
		12–2									7–8	11–12	1–2	
85 cm	-	-	-	-	-	-	2	-	-	-	1	-	-	3
							6–8				7–8			
95 cm	-	-	-	-	-	-	2	1	-	-	1	4	2	10
							7–8	9–10			7–8	11–2	11–2	
Σ	2	1	5	2	2	7	9	3	5	1	8	6	7	58

recorded species of the genus *Strigamia* were present at lower depths (i.e. below 55 cm) predominantly. Centipedes were trapped frequently during summer (June–August) and winter (October–December) (Table).

Sampling of centipedes using three subterranean traps for 1 year yielded 63 trapped individuals. This number of trapped centipedes seems to be relatively low compared to common Barber traps (e.g., Tuf, 2015), but adequate in comparison to other published studies about subterranean traps: one unidentified centipede was trapped by several such traps during 2 weeks in Steinernes Meer in Carinthia, Austria (Schlick-Steiner and Steiner, 2000) and 69 specimens were trapped during 1 year in three traps in Western Carpathians Mts., Slovakia (Rendoš et al., 2012, 2016).

Beside the eurytopic species *Lithobius forficatus*, three species of the genus *Lithobius*, order Lithobiomorpha, were frequent in the whole profile and/or were more frequent in depths of 30 cm and more than in upper layers: *Lithobius tenebrosus fennoscandius*, *Lithobius lucifugus*, and *Lithobius macilentus*.

The presence of *L. tenebrosus fennoscandius* in the *valley* locality is remarkable, because this species was known from the Czech Republic from the Giant Mountains (Tajovský, 2000) and the Děčínský Sněžník (Hoher Schneeberg) Mountains (Tajovský, 1998) only, i.e. typical montane species distribution. The species is known from Scandinavia, as its name suggests. Recently was recorded in the MSS in Hranický kras and an abyss in Moravský kras Czech Republic, as well (Mikula, unpublished data; Růžička et al., 2016). It seems to be evident that this species from Nordic countries is able to survive in Central Europe in montane and subterranean conditions only, probably because of its preference for lower temperatures. Its presence in the MSS and deeper soil profiles can be a reason for underestimation of its distribution. Moreover, the nominate subspecies was recorded in soil layers in Banat and Cloşani, Romania, as well (Ilie, 2003).

Lithobius lucifugus is well known from different karstic areas (e.g., Folkmanová, 1951; Ilie et al., 2003; Ilie, 2004); this species shows an ability to inhabit caves as well as different underground artificial spaces (Novák and Dányi, 2010; Dvořák and Dvořáková, 2015; Růžička et al., 2016). The third species, *L. macilentus*, has been known from Czech caves for a long time (Vališ, 1904). All these species found in deep soil are cave species or montane species: the MSS offers a suitable substitute environment for species preferring lower temperatures. According to data of the Czech Hydrometeorological Institute, all these species were trapped during relatively warmer months in June–September (http://portal.chmi.cz/historicka-data/pocasi/uzemni-teploty?l=en) or dryer months of June and September–November (http://portal.chmi.cz/historicka-data/pocasi/uzemni-srazky?l=en). It seems evident that soil can be a refuge for these species, similarly as some talus slopes can be refuges for cold-adapted boreal species (Růžička et al., 2015).

Other species recorded in deeper layers, *Strigamia acuminata* and *Strigamia transsilvanica*, represent the order Geophilomorpha, the members of which are adapted to living in soils correspondingly with its English name, "soil centipedes" (see Tuf, 2015). Their prolonged segmented soft body with short legs is adapted to crawling through soil crevices. This shape of body is a direct adaptation to soil lifestyle by the increasing of body segment number during evolution (Minelli et al., 2009). Both these species were categorized as chasmatophiles, too, i.e. species inhabiting the entrance part of caves preferentially (Gulička, 1985). The species *Crytops parisi*, order Scolopendromorpha, also has a prolonged body and, like the previously mentioned species, it is a chasmatophile recorded in the MSS (Gulička, 1985; Mikula, unpublished data). Both species of *Strigamia*, as well as *L. forficatus*, common inhabitants of the soil surface (Tuf, 2015), were found in deep layers during winter. Such seasonal vertical migrations in soil were reported for these species also at a small scale of 0–5 cm and 5–10 cm (Tuf, 2002).

The presence of *Lamyctes emarginatus* at the locality *quarry* is remarkable. This species usually inhabits wetlands, banks of rivers, fields, and other disturbed localities (Andersson, 2006), which are typically very poor centipede communities consisting of a few species only. Its presence at *quarry* as an exclusive member of the centipede community is more evidence of its low ability to compete with other centipedes. Its "preference" for disturbed habitats is very probably caused by its inability to survive in the abundant presence of other centipedes.

The MSS and soil are inhabited by remarkable species of centipedes, which need stable temperature conditions; this may be a reason why we can find here some cave species as well as montane species, which try to avoid changes of temperature on the soil surface. Species with low ability to compete with other centipede species can also find suitable refuge in the MSS. We should pay attention to this subterranean environment so as to not underestimate the overall distribution of centipedes and mainly centipedes on the border of its distribution ranges; other studies using these subterranean traps are therefore desirable.

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Příloha 5

Dányi, L., Tuf, I.H. (2016): Out of Africa: The first introduced African geophilomorph centipede record from a European greenhouse (Chilopoda: Geophilidae). *Zootaxa*, 4067: 585-588. (IF_{2015}=0.994)

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As the corresponding author on the paper above, hereby confirms that candidate Ivan Hadrián Tuf has contributed to the work as stated below:

1) The percentage of candidate´s contribution to the paper is ca 50%

2) The candidate contributed to conception and design of the manuscript, collected material, cooperated on its identification, contributed on discussion and critically reviewed manuscript.

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Out of Africa: The first introduced African geophilomorph centipede record from a European greenhouse (Chilopoda: Geophilidae)

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In Europe, 184 species of Geophilomorpha are recognised (Bonato & Minelli 2014) of which eight are evaluated as alien to the region (Stoev *et al.* 2010, Decker *et al.* 2014). Four of these have been reported from greenhouses exclusively: *Mecistocephalus guildingii* Newport, 1843, *Mecistocephalus maxillaris* (Gervais, 1837), *Tygarrup javanicus* Attems, 1929 and *Pectiniunguis pauperatus* Silvestri, 1907. In this paper, we report another species, *Polygonarea silvicola* Lawrence, 1955, which is the first Geophilomorpha species of unambiguous African origin in Europe. Description of the specimen found in Olomouc (Czech Republic) is provided. Co-occurrence of another African species, the lithobiomorph *Lamyctes africanus* (Porath, 1871) (also new for Czech Republic) is also reported here.

Material and methods

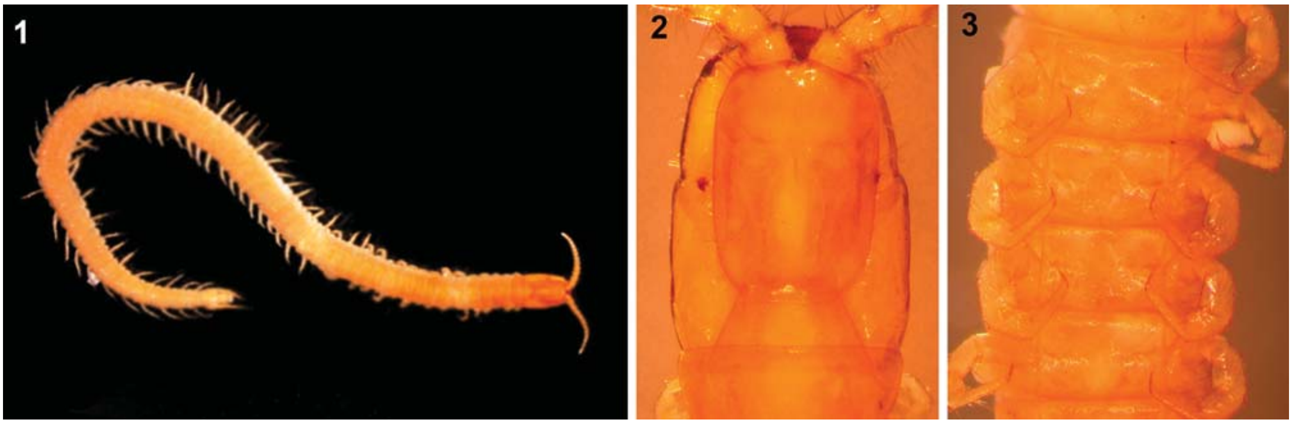
Material examined. *Polygonarea silvicola* Lawrence, 1955, 1 female, Palm House of the Exhibition Centre Flora Olomouc, Czech Republic, 15.IV.2013, lgt. I.H. Tuf (deposited in the Soil Zoology Collection of the Hungarian Natural History Museum, Budapest, Hungary (HNHM)). *Lamyctes africanus* (Porath, 1871): 8 females, same collecting data (5 specimens in HNHM, 3 in I.H. Tuf's collection at Palacký University, Olomouc).

For light microscopy, specimens were dissected with the method described by Pereira (2000) then cleared in potassium-hydroxide, mounted in Euparal, and examined under a Leica DM 1000 microscope with phase contrast optics. Line drawings were prepared with a drawing tube. Terminology for external anatomy follows Bonato *et al.* (2010).

Taxonomic part

Polygonarea silvicola Lawrence, 1955 (Figures 1–15)

Polygonarea silvicola Lawrence, 1955: 141, Figs 6d–f



FIGURES 1–3. *Polygonarea silvicola* Lawrence, 1955: 1, habitus, dorsal view; 2, head, dorsal view; 3, sternites 7–10, ventral view.

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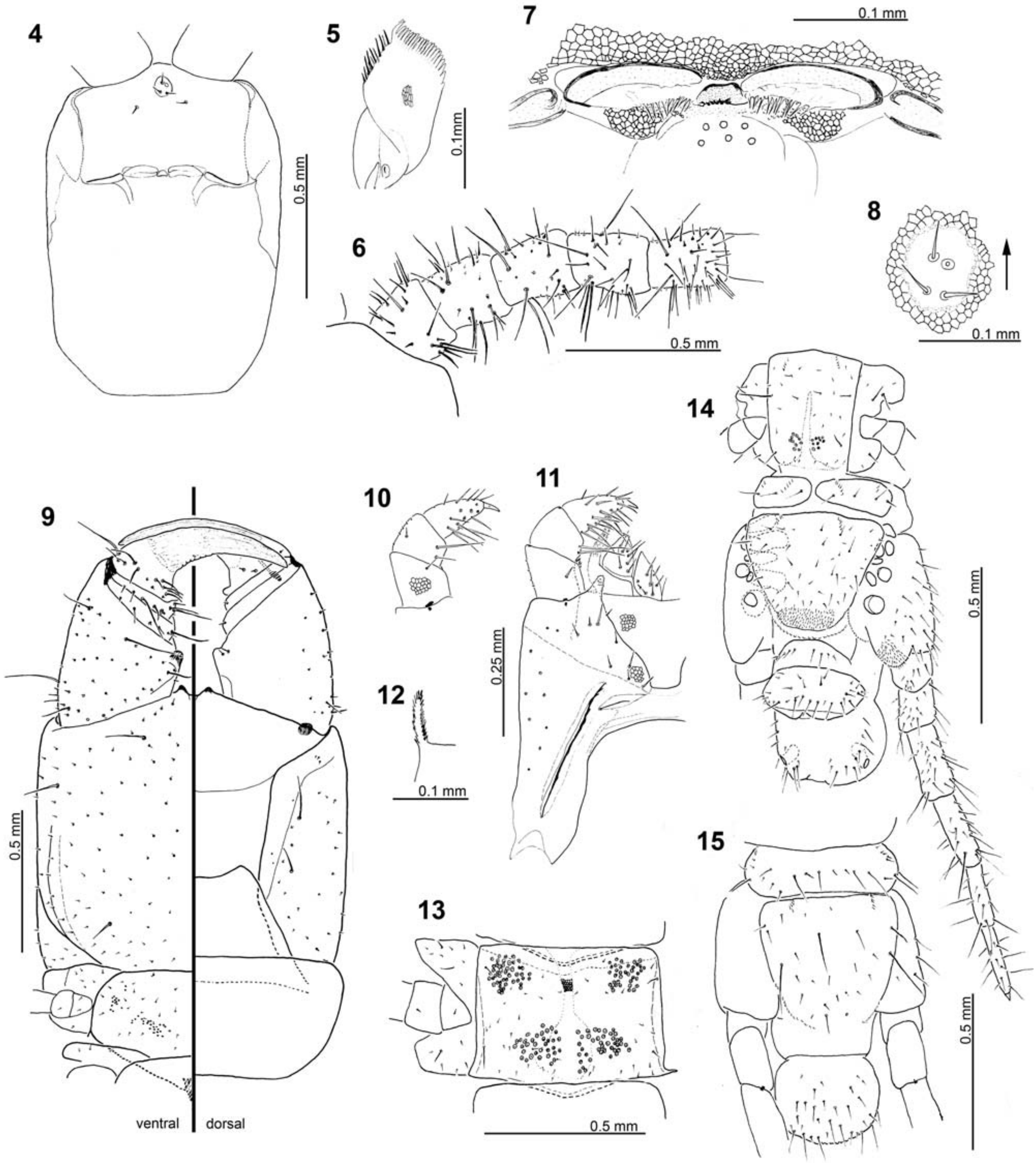

FIGURES 4–15. *Polygonarea silvicola* Lawrence, 1955: 4, head capsule (forcipule and maxillae removed), ventral view; 5, distal part of mandible, antero-ventral view; 6, basal part of left antenna, ventral view; 7, labrum, ventral view; 8, clypeal area, ventral view; 9, forcipule, left side with ventral view, right side with dorsal view; 10, second maxillary telopodite, dorsal view (areolation drawn in part, same scale as for Fig. 11); 11, left side of maxillae, ventral view (areolation drawn in part); 12, first maxillary coxosternal lappet, ventral view; 13, 12th sternite, ventral view (areolation drawn in part, margin of stronger areolated area marked with dotted line); 14, posterior end of body, ventral view (setae on left leg omitted); 15, posterior end of body, dorsal view (setae of legs omitted).

Description of the specimen from Olomouc. Forty-nine leg-bearing segments, body length 29 mm. Habitus and colour of preserved specimen in alcohol as in Fig. 1.

Antennae about 1.5 times as long as the cephalic plate, distally attenuate, with setae irregularly arranged on articles 1–2, and with some longer setae forming a rather irregular whorl on articles 3–4 as in Fig. 6. Cephalic plate about 1.5

times as long as wide, shape as in Fig. 2 and Fig. 4. Clypeus with four setae located on the clypeal area and two setae on the middle part, the remaining clypeal surface without setae (Fig. 4). Clypeal area as in Fig. 4 and Fig. 8, surface without areolation. Labral mid-piece with 6 sclerotized teeth, side pieces with about 11+12 membraneous fimbriae that are very delicate and almost transparent (Fig. 7). Mandible with a pectinate lamella of ca. 22 hyaline filaments, and with hyaline and more sclerotized (spine-like) fringes as in Fig. 5. First maxillae with well-developed lappets both on coxosternite and telopodites (Figs 11–12). First maxillar coxosternite without setae; coxal projections and telopodites as in Fig. 11. Second maxillae medially joined through a very narrow, hyaline and non-areolate isthmus only (Fig. 11), process of antero-internal corners of coxosternum well developed (Fig. 11). Second maxillary coxosternum and telopodites with setae and apical claw as in Figs 10–11. Epipharynx with 6 pores.

Forcipular segment: when closed, the telopodites over-reach the anterior margin of the head (Fig. 2). Forcipular tergite trapeziform, with anterior margin concave (Fig. 9). Coxosternum with incomplete chitinous lines, middle part of the anterior border with two denticles, ochreous in colour (Fig. 9). Trochanteroprefemur with a well developed tooth on the distal part of the medial edge, femur and tibia unarmed, tarsungulum with a denticle (Fig. 9). Posterior edge of the ungular blade weakly serrulate. Calyx of poison gland and chaetotaxy of forcipule as in Fig. 9.

Sternites with pore fields present from the first to the penultimate sternite. Four groups of pores on sternites 1–46 as in Figs 9 and 13, and in two subsymmetrical areas on sternites 47–48 (Fig. 14). Anterior ca. 16–17 sternites with posterior triangular protuberance running under the next segment's metasternite (Figs 9, 13), especially well developed and sclerotized in sternites 1–3 (Fig. 9). Sternites with medial longitudinal depression (Fig. 3) and with stronger areolation in this part (Fig. 13).

Ultimate leg-bearing segment without separate intercalary pleurites (Fig. 15). Ultimate presternite divided along the sagittal plane (Fig. 14). Shape and chaetotaxy of tergite and sternite as in Figs 14–15. Ultimate coxopleura with a distinct protuberation at their distal ventral margin, tightly covered with numerous small setae similarly to the ultimate sternit's posterior part (Fig. 14). Six and seven coxal pores close to the sternal margin and partly covered by the sternit, opening separately but grouped in a weakly developed longitudinal depression (Fig. 14). Ultimate legs composed of seven articles with ratios and ventral chaetotaxy as in Fig. 14. Ultimate pretarsus represented by a claw (Fig. 14). Postpedal segments as in Figs 14–15, gonopods uniarticulate, anal pores present.

Remarks on morphology. *P. silvicola* was known till now from only one specimen, the female holotype. Our specimen fits well the detailed original description given by Lawrence (1955) and only differs in the following characters: 1) number of pairs of legs is 49 versus 55 in holotype; 2) 6 teeth on labral mid-piece versus 7–8 in holotype;. 3) 1+1 setae on the middle part of clypeus versus 2+4 in holotype.

The difference in the number of pairs of legs can be explained by individual variability, since similar intraspecific differences were found in other species (Lawrence 1955). The number of clypeal setae and labral teeth increases with age in many species of Geophilidae, thus the probably younger stadium of the studied specimen (indicated also by its smaller size) might explain the differences in these features. Although the arrangement of setae on the basal antennal articles does not fit *silvicola* in the key given by Lawrence (1955) for the genus, it is in agreement with the text of the original description published in the same paper.

Discussion

Polygonarea silvicola Lawrence, 1955 was known till now only from South Africa (Natal) and thus it is the first geophilomorph species introduced into Europe with an unambiguous African origin (cf. Stoev *et al.* 2010). Considering the subfamily Chilenophilinae as a whole, our record is the first in Europe that is probably not only about specimens that were captured just when accidentally arrived, as in the only other known case with *Steneurytion* Attems, 1909 in the United Kingdom (see Bonato & Minelli 2014). We could not get any information about the origin of the plants in the Palm House where the specimen was collected, but a fresh import of South African material is not very probable. This might indicate the possibility of an established population of the species either in the Palm House or in one of the European exotic plant trade centres from where their plants were bought. In the latter case, an overlooked presence of this species in other greenhouses in the Czech Republic (or even in other European countries) cannot be ruled out.

In addition to *P. silvicola*, further support of the African origin of some imports is the presence of another African centipede, *Lamyctes africanus* (Porath, 1871) (Lithobiomorpha: Henicopidae), collected in the same Palm House on the same occasion. *Lamyctes africanus* also has its type locality in South Africa, but it has already been reported from South-west Australia, Île St Paul, Hawaii and Denmark (Enghoff *et al.* 2013).

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Příloha 6

Drahokoupilová, T., Tuf, I.H. (2012): The effect of external marking on the behaviour of the common pill woodlouse *Armadillidium vulgare*. *ZooKeys*, 176: 145-154.
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RESEARCH ARTICLE

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The effect of external marking on the behaviour of the common pill woodlouse *Armadillidium vulgare*

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Abstract

Zoologists distinguish individual animals using marking techniques. Generally they test the potential influence of marking on survival only; the influence on behaviour is usually neglected. We evaluated the influence of two external marking techniques (nail polish and queen-bee marker) on the behaviour of common pill woodlouse, *Armadillidium vulgare*. The behaviour was examined from two points of view: (1) activity during 24 hours and (2) specific expressions of behaviour (exploring, feeding, resting and hiding) over a 24 hour period. We compared behaviour among woodlice marked with nail polish and queen-bee marker with the unmarked control group during a nine-day experiment. Although we did not find any influence of marking on survival, there was an evident influence on behaviour in most cases. Generally, in the groups of marked individuals of *A. vulgare* there were large differences observed against the control group in the overall activity. Activity of marked individuals was significantly reduced and they preferred hiding. The influence of polish and marker on the overall frequencies of behavioural categories was evident, mainly in feeding, resting and hiding. The influence on the frequency of exploring was significant in the polish marked group only.

Keywords

Diurnal activity, external marking, influence on behaviour, daily pattern, Isopoda, Oniscidea

Introduction

From time to time zoologists need to distinguish individuals of model species. Individual identification is important in ecological studies (e.g. migration or population size) as well as in ethological studies (e.g. home range or social hierarchy). Researchers are able to use individual phenotypic/genotypic differences to identify individuals of some vertebrate species (cf McGregor and Peake 1998) but this approach is a waste of time in studies of animals with short life spans such as many invertebrates. Several methods of marking invertebrate animals have been developed. Internal marking methods used in invertebrates are based generally on colouring and are suitable mainly for unpigmented animals (e.g. termites, tiny spiders or woodlice). Other internal marking methods are based on using isotopes (radioactive or stable ones) but they are limited mainly to population studies (Southwood and Henderson 2000). Paris (1965) also used this method in a study of common pill woodlouse dispersal. More frequently external marking methods are used in studies of invertebrates. They are especially used for marking of adult insects. Beside scarification (e.g. deformations of beetle elythrae by rasper or laser) and tagging (labels with code on locusts, molluscs etc.), painting is one of the most popular methods of external marking. Painting of woodlice has been used during laboratory and field studies of their life history (Lawlor 1976, Madhavan and Shribs 1981), shelter fidelity (Brereton 1957, den Boer 1961) and vagility (Paris and Pitelka 1962). A typical substance used for marking woodlice has been "enamel", substituted by nail polish in the study of Madhavan and Shribs (1981).

Acceptable methods for animal marking should not affect survival (such as increasing probability of predation or infection, or causing intoxication) or behaviour of marked individuals. The potential influence of marking on survival of marked animals is often evaluated but the influence on behaviour is generally neglected (cf Gallepp and Hasler 1975). Hence we decided to investigate if external marking could influence the behaviour of the common pill woodlouse, *Armadillidium vulgare* (Latreille, 1804) using two external marking methods: nail polish and queen-bee marker. Our study also aimed to investigate the potential influence of marking on survival.

Materials and methods

Biological material and marking process

Common pill woodlice, *Armadillidium vulgare,* were hand-collected in Olomouc City (Czech Republic). Collected animals of similar size were sorted out and reared in plastic boxes under room conditions (approx. temperature 21°C, almost 100% air humidity in boxes, natural summer photoperiod, sufficient raw potato food, stones as shelters). Three groups of 40 individuals were chosen for the experiment. Both first and second group were marked, the third group was left unmarked and served as a control.

The two external markings selected for the experiment were nail polish (60 seconds RIMMEL London^{TM}) and queen-bee marker (Uni Paint Marker^{TM}). The fast-drying nail polish was selected to reduce the probability of bonding tergites or sticking of an individual to the surface. Animals were picked up gently with two fingers, marked quickly with a small dot of marking agent on the first pereion segment and placed back into the box. The control group was also manipulated (i.e. picked up and placed in a box, but without marking agent).

Experimental design

The experiment was performed during August 2009. Individuals from polish-marked, marker-marked and control groups were placed in groups of 4 to a box (box size 20×20×10 cm with 0.5 cm layer of plaster of Paris). A box with 4 randomly chosen individuals from one group was considered as one sample. Each box was divided into thirds: the first third contained 3 shelters made from dark but see-through red plastic, the second third contained 40 g of fine soil and the last third contained 3 pieces of potatoes as food. After sunset a red coated flashlight was used to minimize the disturbance of individuals. There were 10 repetitions of each treatment, i.e. 30 boxes altogether. After the marking process, individuals were left to acclimatize in the experimental boxes for 2 days. Observations were performed for 24 hours on the 3rd, 6th and 9th day after marking. The actual behaviour of each individual was recorded once each hour with the naked eye. Active behavioural categories were recorded as: *exploring* (walking), *monitoring* (staying with moving antennae), *cleaning* (clearing of antennae or legs), *interacting* (contact with another individual outside soil or shelter) or *feeding* (feeding on potato, excrements or soil, drinking or defecation). Inactive behavioural categories were recorded as *hiding* (inactivity in soil or in shelter) or *resting* (inactivity on surface).

Statistical analysis

The effect of marking on survival of woodlice was tested by comparing the number of dead individuals from groups using a Fisher's Exact Test. To study behavioural responses to treatment, each behavioural category was defined as proportion of individuals from the group of 4 individuals in the same box exhibiting this particular type of behaviour. The four commonest (see below) categories of behaviour were evaluated, i.e. *feeding*, *exploring*, *resting* and *hiding*. Because time of day clearly acts as a strong confounding variable with a non-linear effect on behaviour of animals during the day, we decided to include this variable in the model structure. We analysed the effect of treatment (3 levels: control, marker and polish) on proportions of the exhibited type of behaviour by fitting generalized additive models (GAMs) which are capable of accounting for nonlinearity imposed by time of day, thereby leaving residuals for category testing. We set binomial error distribution and logit link function to model the effect of both predictors. We used package *mgcv* in program R (Wood 2006) which is exceptional by solving the smoothing parameter estimation problem as part of the estimation procedure. This procedure also provides approximate p-values for the null hypotheses that each term is zero. We modelled behavioural activities for the 3rd, 6th and 9th day separately. The smoothing term for time of day was always significant justifying the presence of this variable in the model. The effect of marking on *activity* (we analysed the main active categories, feeding and exploring, jointly) was visualized in program Oriana for Windows and also analysed with GAMs.

Results

We did not find any difference between survival of woodlice from the control group when compared with woodlice from the polish-marked group (3 *vs* 1 dead individual in these groups; *p*=0.615) or with woodlice from the marker-marked group (3 *vs* 0 dead individuals; *p*=0.241).

In total, 8640 records of behaviour were collected, but some behaviour categories were recorded rarely (*cleaning* 25 times, *interacting* 37 times, *monitoring* 88 times). Influence of marking on behaviour was evident in most cases at first sight: animals looked apathetic (i.e. they moved slowly and were less disturbed during manipulations than the controls).

There are differences evident between *activity* of woodlice from control group and woodlice from both marked groups (Fig. 1). Woodlice were active mainly dur-

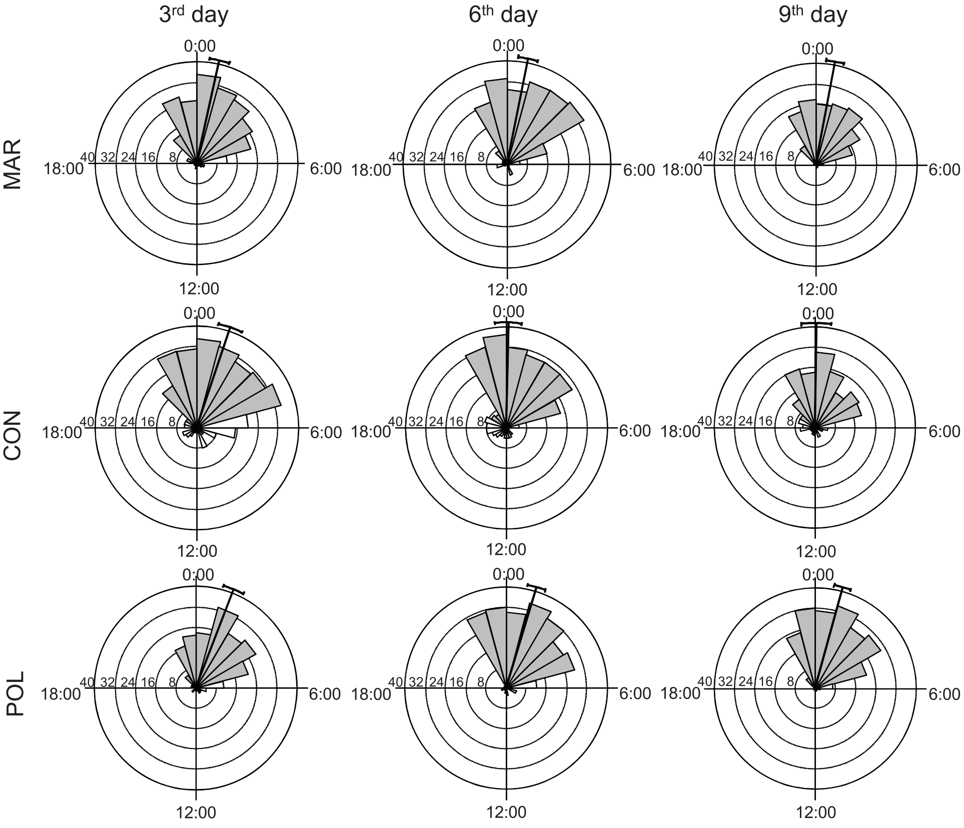

Figure 1. Time-distribution of active behavioural categories (feeding and/or exploring) of *A. vulgare* from all groups in observational days. Legend: CON – control, MAR – marker-marked, POL – polish-marked, grey triangles mark night-time activity, black line running from the centre of the diagram to the outer edge marks mean time of activity and the arcs extending to either side represent the 95% confidence limits.

ing night, although a few unmarked individuals were active during the daylight as well. Their activity generally started between 21:00 and 22:00 and finished at 05:00. Peaks of activity were between 00:00 and 01:30 (Fig. 1). Activity of woodlice from both marked groups was significantly lower in all observation days (with the exception of polish-marked group in the last day, Table 1) and showed the same daily pattern.

All main behavioural categories were recorded with a significant 24 hour pattern. The typical daily patterns of behavioural categories of *A. vulgare* were visualized without effect of marking and effect of experimental day using GAMs (Fig. 2).

Resting of woodlice was recorded mainly before sunrise (c. 05:00–06:00, Fig. 2). Woodlice from both marked groups rested significantly less during the whole experiment (Figs 3a–c, Tab. 1). Resting was the least frequent behaviour category among evaluated ones; woodlice were recorded resting 846 times. *Feeding* was generally the second most frequented category (1023 recorded acts) of behaviour, woodlice fed regularly at c. 00:00–05:00 (Fig. 2). Nevertheless feeding was significantly decreased by marking; individuals from both marked groups fed less in contrast to unmarked ones in all three days (Figs 3d–f, Tab. 1). *Exploring* behaviour

Table 1. Statistical tests for each level of treatment that the estimate differs from zero. Whereas parameter estimate for control group was estimated as intercept, parameters for level marker and polish represent pure effects. Significance testing was carried out after accounting for variation imposed by time of day. Behavioural category activity represents joined evaluation of both active categories (i.e. feeding and exploring) (see Fig. 1).

		activity		resting		feeding		exploring		hiding	
		z value	*p*	*z value*	*p*	*z value*	*p*	*z value*	*p*	*z value*	*p*
3rd day	*control* (intercept)	-9.30	< 0.001	-17.87	< 0.001	-17.17	< 0.001	-19.33	< 0.001	-4.68	< 0.001
	marker (x *control*)	-6.43	< 0.001	-8.52	< 0.001	-4.51	< 0.001	-1.45	0.147	12.34	< 0.001
	polish (x *control*)	-8.91	< 0.001	-5.22	< 0.001	-6.09	< 0.001	-2.64	0.008	11.93	< 0.001
6th day	*control* (intercept)	-11.93	< 0.001	-19.50	< 0.001	-16.58	< 0.001	-15.69	< 0.001	0.77	0.444
	marker (x *control*)	-4.48	< 0.001	-2.61	0.009	-3.18	0.001	0.09	0.932	6.00	< 0.001
	polish (x *control*)	-3.34	< 0.001	-5.33	< 0.001	-5.30	< 0.001	3.75	< 0.001	7.18	< 0.001
9th day	*control* (intercept)	-15.06	< 0.001	-19.15	< 0.001	-16.88	< 0.001	-19.06	< 0.001	7.59	< 0.001
	marker (x *control*)	-4.49	< 0.001	-4.83	< 0.001	-4.19	< 0.001	-1.02	0.306	7.39	< 0.001
	polish (x *control*)	-1.12	0.264	-4.67	< 0.001	-4.42	< 0.001	3.22	0.001	4.45	< 0.001

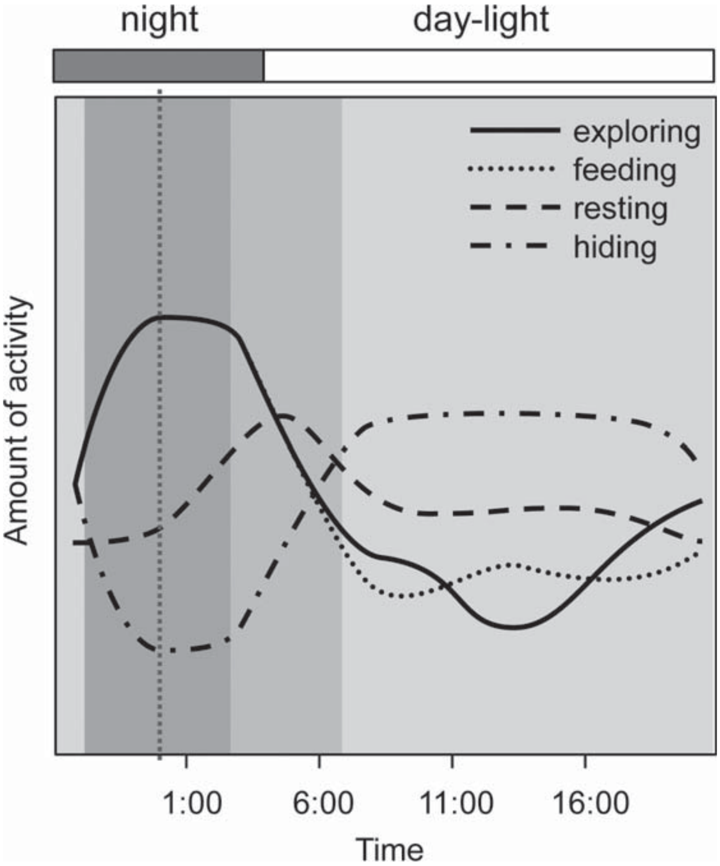

Figure 2. Daily patterns of behavioural categories as modelled by fitting GAM to illustrate a high degree of non-linearity in the response (logits). Compound graph from curves expressing frequency of exploring, feeding, resting and hiding of *A. vulgare* in a mean day

of woodlice (recorded 982 times) showed a typical and significant daily pattern in spite of marking; woodlice were exploring boxes during night and feeding at the same time (Fig. 2). Although there were no significant differences in the frequency of exploring between woodlice from marker-marked group and woodlice from control group, woodlice marked by nail polish exhibited significantly less exploring in the 3rd day and more exploring in following days (Figs 3g–i, Table 1). *Hiding* was the most frequent behaviour (5523 recorded acts). Woodlice were hidden especially during daylight (c. 06:00–21:00, Fig. 2). Marked woodlice were hidden in shelters significantly and strikingly more frequently compared with unmarked woodlice (Figs 3j–l, Table 1).

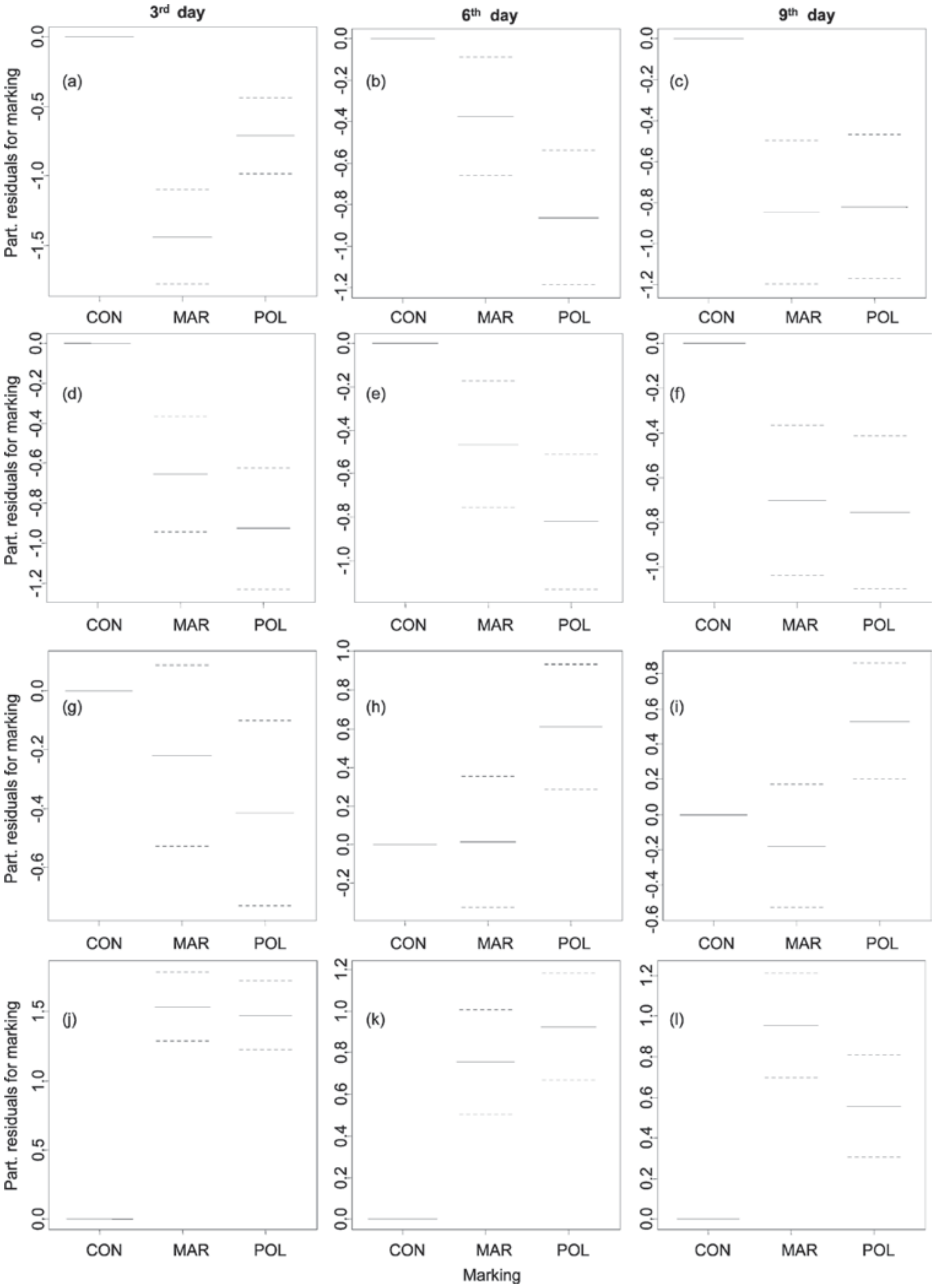

Figure 3. Influence of marking on frequency of *resting* (a), (b), (c), on *feeding* (d), (e), (f), on *exploring* (g), (h), (i), and on *hiding* (j), (k), (l) of *A. vulgare* in 3rd, 6th and 9th day analyzed by GAMs (confidence intervals dotted). Legend: CON – control, MAR – marker-marked, POL – polish-marked.

Discussion

We evaluated the effect of two external marking agents (nail polish and queen-bee marker) on behaviour and survival of the common pill woodlouse *Armadillidium vulgare*. Neither agent had any effect on survival of woodlice, but influence on behaviour was evident in almost all studied cases. Woodlice of both marked groups were less active, with less feeding and more hiding in contrast to those from the control group. Woodlice marked by nail polish also exhibited less exploring at 3rd day.

Den Boer (1961) used marking of woodlice (*Porcellio scaber* Latreille, 1804) by "shellac-solution in alcohol with pigment" to study shelter fidelity. He marked woodlice found on trees and tried to observe them again an hour later. He saw only about 10–20% of them (even though he prevented them escaping from the trees using tree-banding grease) and he concluded that the marked woodlice were hidden in shelters on tree trunks. Similarly our marked woodlice from both groups exhibited more hiding over the whole experiment. Their hiding behaviour could be connected with aggregation as result of attraction between conspecifics (Devigne et al. 2011) as well as looking for excrement as suitable source of food (Hassall and Rushton 1982). Greater exploring behaviour of unmarked woodlice at start of experiment can be associated with active interest in the new neighbourhood, marked animals were more apathetic.

Paris and Pitelka (1962) using marked woodlice (*A. vulgare*) found that the population is very fluid. They observed only a few marked individuals in bait traps the day after marking. At first sight, this is contrary to our results. Nevertheless from the activity pattern of *A. vulgare* it is evident that they are hiding during daylight and feeding/exploring during night. Paris and Pitelka checked their traps during nights, i.e. during feeding/exploring. Probably the marked animals were hidden somewhere else and did not enter trap due to lower activity and lower level of feeding.

Common pill woodlice were significantly less active due to marking. Cuticle of terrestrial isopods is relatively permeable to water, they avoid desiccation by finding a locality with suitable humidity, e.g. shelter during daytime (Hornung 2011). In our parallel study with the pill millipede, *Glomeris tetrasticha* Brandt, 1833, marked individuals were also significantly less active than unmarked ones. Moreover, this effect of marking on activity was much more intensive compared with the results presented here about *A. vulgare* (Drahokoupilová and Tuf 2011). Perhaps we could search for the reasons in anatomy. The thin cuticle of *G. tetrasticha* is very permeable for water (Edney 1951) in comparison with thicker cuticle of *A. vulgare*. We suppose some chemicals from polish and marker might break through cuticle into haemolymph of pill woodlice as well as pill millipedes. Lower activity and higher resting could have been a result of some poisoning overshoot. This question should be explored. The queen-bee marker probably did not affect behaviour of marked bees, because the dot of marking agent is not in contact with cuticle but usually only with hairs (Sammataro and Avitabile 1978). The lack of evidence for effect of marking on survival of woodlice should be interpreted carefully. Firstly, we evaluated effect of marking on survival and behaviour for 9 days only. We do not know if marked woodlice will show higher mortality later or not. Late increased mortality could be caused e.g. by reduced feeding activity of marked woodlice. Secondly, we found an effect of external marks of nail polish on survival of woodlouse *P. scaber* in a longer experiment recently (Tuf et al., in prep.).

Our observations about night activity of *A. vulgare* are supported by previous studies. Refinetti (2000) found that *A. vulgare* shows strongly nocturnal activity under a natural light-dark cycle, more or less controlled by an endogenous timer (Cloudsley-Thompson 1956, Smith and Larimer 1979).

We conclude that common pill woodlice should not be externally marked by nail polish or by queen-bee marker. Both marking agents cause lower activity of marked woodlice and their usage, for example in capture-mark-recapture studies, can provide biased or wrong results.

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Příloha 7

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Personality affects defensive behaviour of *Porcellio scaber* (Isopoda, Oniscidea)

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Abstract

We evaluated individual behavioural patterns of isopods expressed as tonic immobility following some intrusive treatments. Common rough woodlice, *Porcellio scaber*, were kept individually in plastic boxes and tested for tonic immobility repeatedly. Reactivity, sensitivity (number of stimuli needed to respond), and endurance of tonic immobility (TI) according three types of treatments (touch, squeeze, drop) were evaluated. Touch was the weakest treatment and it was necessary to repeat it a number of times to obtain a response; while squeeze and drop induced TI more frequently. Nevertheless, duration of the response persisted for a longer time with the touch treatment. Within each set of the three treatment, the strongest response was the third one, regardless of treatment type. Duration of reaction was affected by the size of the woodlouse, the smallest individuals feigning death for the shortest time. Despite body size, we found a significant individual pattern of endurance of TI among tested woodlice, which was stable across treatments as well as across time (5 repetitions during a 3 week period). *Porcellio scaber* is one of the first species of terrestrial isopods with documented personality traits.

Keywords

Anti-predatory behaviour, death feigning, thanatosis, predation, behavioural trait

Introduction

Generally, when animals encounter their predator they (1) run away, (2) attack it or (3) stay invisible and/or look unpalatable.

Anti-predatory behaviour including boldness can be a part of animal personality. Personality of animals has been routinely studied during the last twenty years, although the study of personality in vertebrates prevails. Behavioural traits, which are consistent over time in individuals and as a response to different situations, have been described as a personality (Reale et al. 2007). The concept of personality has been used for a relatively broad spectrum of invertebrates including crustaceans (e.g. Briffa 2013, Biro et al. 2014, Brodin and Drotz 2014), but not explicitly studied in terrestrial isopods previously. The main behavioural traits found in Crustacea are boldness (Brifa et al. 2008; Hazlett and Bach 2010; Brifa and Twyman 2011; Brifa 2013), voraciousness (Biro et al. 2014) and activity (Yli-Renko et al. 2014).

Change in anti-predatory behaviour during growth and development of animal can challenge stability over time of the behavioural traits mentioned above. Examination of animal personality traits must consider consistency over two different time intervals: short intervals to determine whether behaviour is sufficiently consistent to be included in a study of personality, and longer intervals to determine how behaviour changes over the course of a lifetime (Stamps and Groothuis 2010).

During their evolution, terrestrial isopods colonised land and they were faced with new types of stresses, including new types of predators (Broly et al. 2013). The anti-predator mechanisms used by woodlice include escape, armour, cryptic colouration, chemical protection, acoustic warning, feigning death and/or specific posture (Witz 1990). Some of these strategies are not direct adaptations against predators, but evolved as parts of their terrestrial life-style. For example, escape is simply an extension of the ability to move as necessary to find food and mates, while armour is usually found in isopods living in (semi) dry conditions, which need to minimize water loss using thick cuticle (e.g. Smigel and Gibbs 2008; Csonka et al. 2013) and evolved as a defensive reaction. Chemical defensive secretions are a direct adaptation against predators being at least spider- (Gorvett 1956) and ant-repulsive (Deslippe et al. 1996; Yamaguchi and Hasegawa 1996).

Terrestrial isopods developed behavioural protection known as tonic immobility or death feigning, which is related also to behaviour known as "taking specific posture". In general, the main difference between these categories is that "taking posture" is aimed for protection against being swallowed by a predator (e.g. Honma et al. 2006) and "feigning death" increases the probability to be ignored by predators with sight as the prevailing sense. This behaviour includes the so-called conglobation or volvation, behaviour typical for members of some isopod families such as Armadillidae, Armadillidiidae, or Cylisticidae, as well as for pill millipedes (Glomerida) and giant pill millipedes (Sphaerotheriida), some soil mites (Oribatida), and cuckoo wasps (Chrysidoidea). Conglobation involves the body being rolled into non-perfect or perfect ball with legs, antennae and ventral body surface more or less hidden. Non-perfect conglobation (e.g. typical for the genus *Cylisticus*) is less effective as uropods and antennae are not well protected. Nevertheless, tonic immobility is a much more general behaviour than conglobation and it is used by isopods (Quadros et al. 2012). Tonic immobility in non-conglobating forms of isopods is characterised by the contraction of the body and the contraction and folding of the legs towards the ventral side while holding the antennae folded or extended backwards and pressed against the dorsal part of the first pereonites (see fig. 1 in Quadros et al. 2012). During this posture the organism lacks motional responsiveness to external stimulation. Differences between death feigning and conglobation (called also shrinking in Anura) were discussed in the case of amphibians, but for isopods these differences are of marginal importance (Toledo et al. 2010).

The usefulness of feigning death as an anti-predatory behavioural strategy can theoretically be dependent on body size of an animal. If smaller animals can be easily overlooked by predator, the frequency of using this strategy by small animals can be higher than by bigger animals. This pattern was confirmed in some studies (Hals and Beal 1982; Quadros et al. 2012) but not found in other ones or in other species (Hazlett and Bach 2010; Quadros et al. 2012) for several crustaceans including terrestrial isopods.

We studied anti-predatory behaviour of the Common rough woodlouse *Porcellio scaber* Latreille, 1804, and we added a new parameter to standard experimental design – repetitions at the individual level. With this modification we were able to study the stability of behavioural traits, i.e. animal personality. The main aims of this research were: Are there any patterns in death feigning (tonic immobility, TI) behaviour? Is TI affected by type of treatment or its order? Is there a body-size pattern of behaviour among woodlice suggesting any developmental changes of its behaviour? Despite size of body, is there an individual specific pattern of behaviour among woodlice, i.e. are we able to evaluate their boldness on personal level?

Methods

Subjects and housing conditions

Several hundreds of Common rough woodlice, *Porcellio scaber*, were collected in the environment of Kutna Hora, Czech Republic (urban green areas and gardens) during June 2013. Following transport to laboratory, they were not sexed, but sorted in three size categories by length (small < 7 mm, medium 7–12 mm, and large > 12 mm). Size of woodlouse is related to its age (Zimmer 2002). Fifty individuals of each size category were inserted into small non-transparent plastic boxes (area 33 cm^2) each with a thin plaster of Paris layer on the bottom. Each isopod individual had its own identifying code (ID) marked on its box. These codes enabled analyses of the stability of its behaviour (personality). Isopods were fed on potatoes and plaster was kept moist; natural (room) temperature regime was maintained at 21–26 °C.

Procedure

Behavioural experiments followed the design used by Quadros et al. (2012); each isopod was exposed to several treatments. One experimental set contained three types of treatments to induce tonic immobility (TI): touch, squeeze, and drop. The touch stimulus was applied as gentle nudge to the isopod with forceps. The squeeze stimulus was applied as a firm grab to the isopod body by entomological soft-metal forceps, when one prong was undercutting the ventral part of the body and the other part was applied on the dorsal part. The drop stimulus was similar to squeeze one, though followed by lifting to *ca* 10 cm and then letting it drop back in the box.

The first treatment was applied and if TI was induced, its duration was measured. If necessary, the stimulus was repeated up to 5 times in order to induce TI. If TI was not induced, lack of reaction was recorded. We let individual woodlouse rest for approximately 30 minutes and applied the second treatment in the same way and the third treatment after a further half hour, respectively (Fig. 1). ID of woodlouse, order of types of treatments, sensitivity or promptness of TI induction (i.e. number of stimuli needed) or non-reactivity; and endurance of TI (i.e. time from start of TI to the first movement of antenna or leg) was measured in each experimental set. Each individual was involved in five experimental sets with 4 day intervals between experimental sets. The order of stimuli was changed systematically to distinguish the effect of type of stimulus from an effect of order of stimuli.

Data analysis

We tested the effects of the three types of treatment (touch, squeeze and drop) on reactivity (presence/absence of reaction to stimulus, i.e. probability of inducing TI), sensitivity

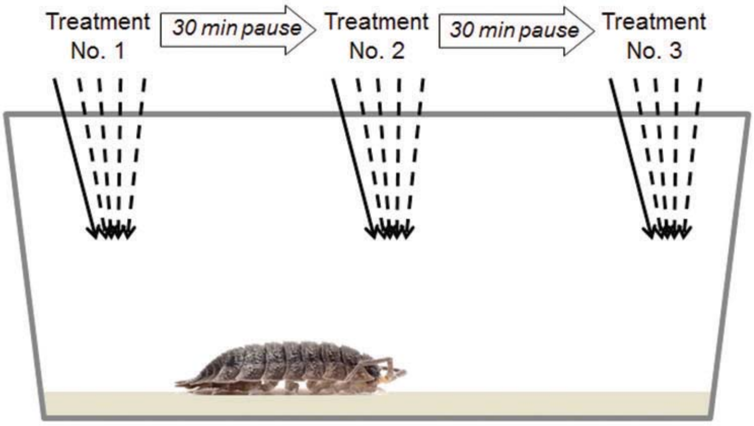

Figure 1. Design of one experimental set. Dashed arrows symbolise repeated stimuli applied if previous stimulus did not evoke tonic immobility. Experimental sets were applied repeatedly over a three week period; each individual was exposed to five experimental sets with 4 days intervals between.

(number of stimuli needed to induce TI) and endurance of TI. Experimental sets which failed to induce TI were excluded from next data analyses. To determine the effect of different types of treatments we conducted repeated measures ANOVA. The error term of ANOVA reflects that we had the type of treatment nested within individuals of woodlice (ID). Data were not normally distributed therefore we transformed data by decimal logarithm. For multiple comparisons we used a pairwise t-test with adjusted p-values by the Holm correction. We used the F test to check the significance of the explanatory variables. Kendall's coefficient of concordance was computed in order to determine the consistency of between-individual differences in the three types of treatment. We also used the correlation of TI endurance among different type of treatment. Significance of correlations was tested by using Kendall method with Bonferroni correction.

Results

Three isopods died after the first experimental set, but data are available to evaluate from 738 experimental sets; TI as a reaction to at least one treatment was recorded in 334 sets (45% of sets) in 35 woodlice (23% of individuals). TI was induced by all treatments during the same experimental set in 41 experiments (6%) in 25 woodlice, with only one individual showing TI at each of the 15 treatments (i.e. through all five experimental sets).

If a woodlouse reacted to a treatment in an experimental set, the probability of reaction was influenced by type of treatment ($F_{2,298} = 1165.00$, $p < 0.001$, Fig. 2b); in those experimental sets isopods reacted to drop and squeeze in all cases, but to touch in *ca* 20% only. If isopods reacted to treatment by TI, duration of TI significantly depended on the type of treatment ($F_{2,298} = 2.97$, $p = 0.052$, Fig. 2d), too: with touch followed by the longest TI. Also reactivity, i.e. number of stimuli needed to induce TI, was significantly dependent upon the type of treatment ($F_{2,298} = 517.00$, $p < 0.001$, Fig. 2f); if the global probability to react to touch is the lowest, more stimuli of touch were necessary to induce TI.

To avoid misunderstandings relating to the effect of treatment type and its order in the experimental set, the order of the applied treatments was changed. Without respect to type of treatment, the third treatment was the most probable to be followed by TI ($F_{2,298} = 81.00$, $p < 0.001$, Fig. 2a). Nevertheless the endurance of TI shortened significantly during experimental sets ($F_{2,298} = 9.63$, $p < 0.001$, Fig. 2c). On the other hand, number of stimuli needed to induce TI was significantly related to the order of the treatment ($F_{2,298} = 16.55$, $p < 0.001$, Fig. 2e).

Although there were no significant differences among body-size categories of *P. scaber* in the probability of inducing TI ($F_{1,148} = 0.73$, $p = 0.395$), the longest TI duration was performed by medium body sized woodlice ($F_{1,148} = 6.75$, $p < 0.05$, Fig. 3).

Personality, i.e. individual stability of duration of TI was confirmed by Kendall's concordance analysis for the whole reactive group of isopods ($W = 0.73$, $p < 0.001$); there were individual patterns of endurance of TI irrespective of type of treatment or

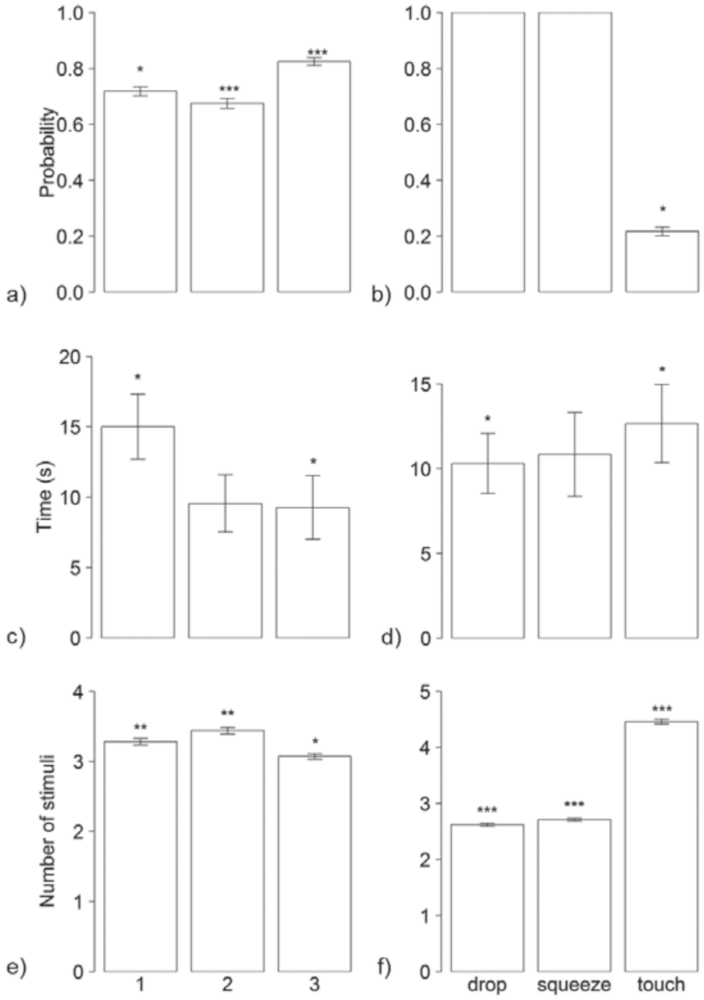

Figure 2. Tonic immobility of *Porcellio scaber* induced by different treatments: **a** probability of inducing TI by the first, the second and the third treatment **b** probability of inducing TI by different treatments **c** endurance of TI following the first, the second and the third treatment **d** endurance of TI following different treatments **e** sensitivity, i.e. promptness of inducing TI by the first, the second and the third treatment **f** sensitivity, i.e. promptness of inducing TI by different treatments. (*** $p < 0.001$; ** $p < 0.01$; * $p \leq 0.05$)

Table 1. Correlations between durations of TI of *Porcellio scaber* induced by different treatments: D – drop, S – squeeze, T – touch. (*** $p < 0.001$; ** $p < 0.01$; * $p \leq 0.05$)

	all animals			**large-size animals**			**medium-size animals**			**small-size animals**		
	D	**S**	**T**	**D**	**S**	**T**	**D**	**S**	**T**	**D**	**S**	**T**
D	-	0.55***	0.45***	-	0.40*	0.32*	-	0.71**	0.56**	-	0.44*	0.44*
S		-	0.49***		-	0.61*		-	0.52**		-	0.32**
T			-			-			-			-

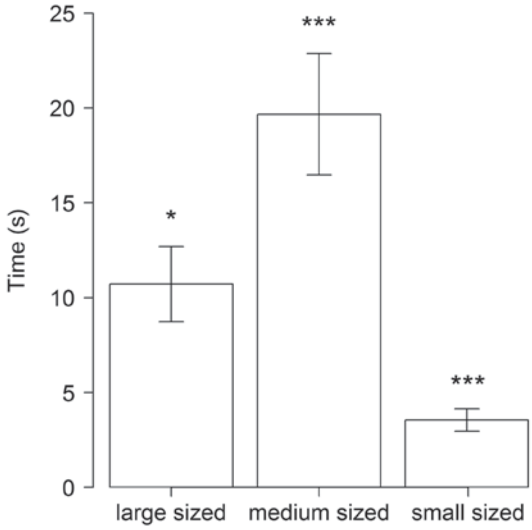

Figure 3. Endurance of tonic immobility of *Porcellio scaber* of different body sizes induced by treatments. (*** $p < 0.001$; ** $p < 0.01$; * $p \leq 0.05$)

its order. To avoid obfuscation of personality and size-dependent differences in behaviour, concordance analyses for individual size categories were calculated and revealed significant stability of endurance of TI inside all body-size categories (large size: $W = 0.68$, $p < 0.001$; medium size: $W = 0.82$, $p < 0.001$; small size: $W = 0.65$, $p < 0.001$). Stability of patterns of durations of TI can be visualised by correlations between endurances of different TI values (Fig. 4). Correlations between duration of TI were significant for *P. scaber* analysed as a whole group as well as between different body-size groups (Table 1).

Discussion

We evaluated reactivity, sensitivity, and duration of tonic immobility of *Porcellio scaber*. It is difficult to evaluate the functional significance of anti-predatory behaviour, as there are several interfering behaviours which affect probability of an animal being recognized, captured and consumed by predators (Lind and Cresswell 2005). These behaviours can have evolved independently; nevertheless it is impossible to measure the independent effect of one of those behaviours on the fitness of target animals. For this reason we cannot say that tonic immobility increases the fitness of *P. scaber*.

Generally, the reactivity was relatively low (23% of isopods). It is known that tonic immobility is not the main anti-predatory strategy for *P. scaber*, as a clinger ecomophological type (Schmalfuss 1984). They use sticking against a surface, or run away more frequently to escape predators (77% of woodlouse in our study tried to run away exclusively), or uses chemical protection (Gorvett 1956; Deslippe et al. 1996).

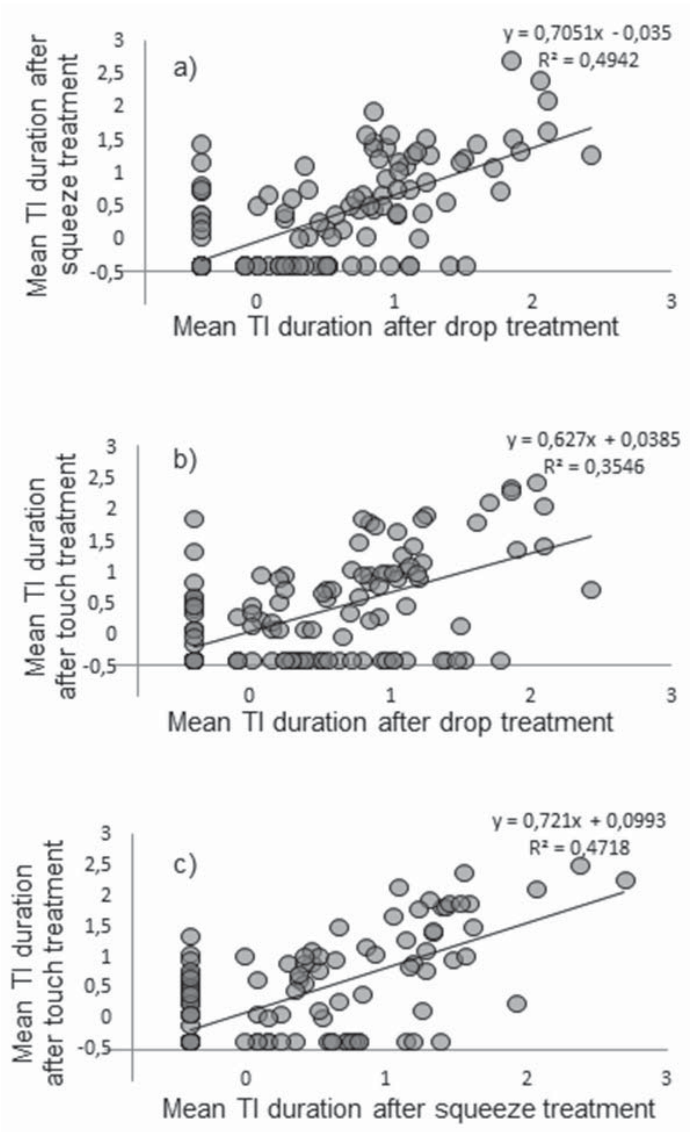

Figure 4. Correlations between duration (in seconds) of TI of *Porcellio scaber* induced by the different treatments: **a** correlation between duration of TI induced by squeeze and drop **b** correlation between duration of TI induced by touch and drop **c** correlation between duration of TI induced by touch and squeeze. Data were transformed by decimal logarithm.

Nevertheless, Quadros et al. (2012) found *Porcellio dilatatus* to be a highly responsive species (89% specimens used TI).

Reactivity of isopods was affected by the type of treatment. Whereas drop and squeeze were followed by TI regularly, touch was not an effective treatment for TI in some specimens. The explanation can be found in the manipulation of isopods by different kinds of predators (e.g. Sunderland and Sutton 1980, Dejean 1997, Řezáč and Pekár 2007, Quadros et al. 2012). Despite the lack of experimental verification of tonic immobility as defence behaviour against predators and regarding the size of *P. scaber*, we can hypothesise that drop treatment is probably more similar to manipulation by some vertebrate visual predator (birds, amphibians, or lizards). Squeeze can be similar to manipulation by some small vertebrate or large invertebrate predators (e.g. small rodents or shrews, ground beetles of the genus *Carabus*), whereas touch resembles the manipulation of small invertebrate predators (spiders, centipedes, ants, etc.). According to these categories of predators, TI following drop can be very useful, if the isopod is lost by predator in leaf litter. Big predators do not loose time for looking for one small prey item. They probably continue walking and searching for another prey. Similarly, TI as response to squeeze can also help the attacked isopod to survive, if the predator is not able to manipulate the immobile prey very well. By contrast, the most effective defensive strategy against small invertebrate predators is the secretion of chemicals (Deslippe et al. 1996), which may not be so effective against larger predators.

It is necessary not to forget that *P. scaber* is strongly thigmotactic (e.g. Friedlander 1964) and lives in large aggregations (Broly et al. 2012): this is important for two aspects concerning its TI reaction. First, touch is a common stimulus in the way of life of woodlice. In aggregates, there are many conspecific individuals around; reacting by TI to each touch becomes meaningless. For this reason, low reactivity and low sensitivity to touch is understandable. But if touch is repeated several times (it was necessary to repeat it more times than drop or squeeze), endurance of TI is longer than TI following drop or squeeze. This is probably because of the foraging mode of the predator: small invertebrate predators such as spiders or ants can manipulate small isopods for a longer time and can wait for the first movement (providing time to attack the un-armoured ventral side). Larger predators do not waste time by waiting; they swallow prey immediately if they notice and catch it.

Another advantage of aggregates is the higher probability of being passed over by a predator among running conspecifics (Miyatake et al. 2009). If larger predator turns over the shelter of a group of isopods (e.g. stone or bark on dead stump), it can be useful to stay in TI and wait until the predator is lured away by other, running members of the aggregation. It can be gainer strategy even if the woodlouse is lost by the predator (drop or squeeze). In addition, the shorter duration of TI can be more useful if the lured-away predator is coming back to search for the last prey items. It can be an explanation for higher reactivity and shorter endurance for TI following drop and squeeze stimuli.

Studies have shown changes in behaviour according to the type of disturbing treatment. Carbines et al. (1992) studied the character of escape mechanisms of isopods from predators. They measured turn alteration in a simple labyrinth and related it to the probability of survival (as direction of run). If the treatment was harmless cotton-wool fluff, the probability of survival was much lower than if *Dysdera* spider predators were the agent of disturbance. It means an authenticity of stimulus affected its defensive behaviour; perhaps over time in our prolonged experiment the authenticity of disturbance was decreasing. During one experimental set in our study, the reactivity increased in the third treatment while in the third treatment duration of TI was reduced. This resembles a situation when the isopod is (hypothetically) able to evaluate the meaningless stimulation of the experimenter and learn "to escape" from this situation by a more prompt TI response for a shorter time. As this "explanation" is rather implausible, shorter duration of TI in the last stimulus can be explained also by quick habituation of *P. scaber* to stable environmental cues, as was described by Anselme (2013). Habituation, i.e. changes of response to repeated stimulus was reported also for the aquatic crab *Chasmagnathus granulatus* (Tomsic et al. 2009).

Although our research is not the first to look into TI in terrestrial isopods, the results presented here enable to test repeatability of responses of individual isopods, i.e. its personality. The concept of personality was used for behavioural studies of some Crustacean species, mainly Decapoda, i.e. in crabs, hermit crabs, crayfishes (e.g. Briffa 2013, Biro et al. 2014, Brodin and Drotz 2014), as well as Isopoda (Yli-Renko et al. 2014). Among terrestrial isopods, the only study dealing with personality known to the authors was done by Matsuno and Moriyama (2012). They found a correlation between the walking speed and endurance of conglobation in some specimens of the Common pill bug *Armadillidium vulgare*. Nevertheless, this "stable internal factor" was found only in specimens showing a stable-style end of conglobation: specimens that finished conglobation in two trials by leg movement or antenna movement consistently, were more "brave" (shorter duration of tonic immobility) and ran faster compared to leg-antenna "alternators" (i.e. specimens ending conglobation by antenna movement and leg movement in two trials). We also found correlations in individual specimens for duration of TI across different types of treatment and these correlations were found over three weeks (five experimental sets with 4 day breaks), meaning that there were some consistently more "bold" woodlice (short TI) and some more "shy" woodlice (long TI).

Correlations between length if TI, even if there is a decrease of endurance of TI during one experimental set, can be caused by habituation of isopods to repeated treatment as well as their sensitivity to new type of treatment. Anselme (2013) found that *P. scaber* individuals are able to habituate to an environment in around 10 minutes. Over this time, they become less interested in stable stimulus and their activity decreased. In the same study woodlice preferred new stimuli (such as a new texture of substrate) if it was provided (Anselme 2013), or a random pattern of known stimuli (Anselme 2015), so there is some evidence of "curiosity" in *P. scaber* (although not studied at individual level).

Documented "boldness", as a parameter of personality of *P. scaber*, is independent of size (age) of specimen. Hals and Beal (1982) reported that the largest specimens (> 1 cm of length) of *P. scaber* reacted by TI at less intensity compared to smaller specimens (< 1 cm). Similarly Quadros et al. (2012) found the same pattern in reactivity for *Balloniscus sellowii*. However we did not find significant differences in reactivity of woodlice in our three body-size groups, although there are differences in endurance of TI among groups. The longest reaction time was measured in medium-sized woodlice

(7–12 mm) and shortest in small-sized woodlice (< 7 mm). One explanation could be sought in terms of changes of the effectiveness of TI as a defence mechanism against predators. TI is not necessary for large woodlice against medium-sized and smaller predators, because large woodlice are less catchable and can use chemical defence: their glands are well developed and able to produce sufficient amount of secretions (Gorvett 1956, Sutton 1970) in comparison to the less developed glands in smaller stages of *P. scaber*. As well TI would not be a successful protection for the smallest woodlice against predators such as *Carabus* or centipedes, as they are easy to manipulate. Indeed, the mortality of juvenile stages of isopods is estimated to reach 80% (Sutton 1970) and 11–51% decrease in populations is caused by predation upon juveniles by invertebrates (Sunderland and Sutton 1980). This indicates TI can be a useful strategy mainly for medium-sized *P. scaber* specimens.

Besides finding differences in endurance of TI between body size groups, we also identified personal behavioural patterns in all tested individuals, as well as variation within these body-size groups. These findings are not able to resolve if personality is changing during individual development or not. Although behavioural traits can be stable across short time intervals, changes to personality due to development can cause inconsistency in responses to stimuli over longer time intervals (Stamp and Groothuis 2010). We did not evaluate if traits remained the same over long time intervals, but this type of stability was not proved for marine isopod *Idothea baltica* recently (Yli-Renko et al. 2015). Investigation of long-time stability of behavioural traits in terrestrial isopods should be a possible goal of future studies.

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Příloha 8

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As the corresponding author on the paper above, hereby confirms that candidate Ivan Hadrián Tuf has contributed to the work as stated below:

1) The percentage of candidate´s contribution to the paper is 50%
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Regular research paper

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CIRCADIAN RHYTHMS OF GROUND LIVING SPIDERS: MECHANISMS OF COEXISTENCE STRATEGY BASED ON THE BODY SIZE

ABSTRACT: Circadian rhythms of activity are one of the many cases of the multidimensional mechanisms of species coexistence. Except of others, the mechanisms of coexistence strategy of spiders involve habitat, seasonal occurrence, food offers and body size of spiders. Circadian rhythm of activity of ground living spiders in floodplain forest and clearcut along the Morava River in the Litovelské Pomoraví Protected Landscape Area (Czech Republic, Central Europe) was studied. Activity of whole community was asymmetrical, diurnal activity was more frequent than nocturnal. Abundant species were analysed closely. Patterns of similarity in syntopic spider groups suggested the body size is significant factor influencing their circadian activity. We found out the predominantly small species achieved bimodal pattern of activity, influenced by the different activity of males and females. Generally we can conclude that spiders smaller than 5 mm were active during late night-morning and spiders bigger than 5 mm were active during afternoon and evening. This pattern was associated with changes of temperature of soil surface – big spiders were active during warmer parts of day. The presented data provide evidence of body-size differences among the spiders enable their coexistence in assemblages.

KEY WORDS: biological hours, strategy, diurnal, nocturnal, spider activity

1. INTRODUCTION

Animal activity is periodically synchronized with the environmental day-night rhythm. Light and humidity are important environmental factors influenced the spider circadian rhythms (Platen 1988). Uetz (1977) argues that habitats can be arranged as a gradient of litter spatial pattern, which has been shown to be an important factor influencing the structure of spider communities. According to Shoener (1974), circadian rhythm should be greater factor than habitat specialization. Temporal stratification may be an important means of reducing competition (Breymeyer 1966, Luczak 1959). Flatz (1987) found that the circadian activity of spider species is asymmetrical: lycosid spiders are active mainly during the day, linyphiids during the midnight or in the early morning. Lycosid spiders *Pardosa lugubris* and *Pardosa amentata* were diurnal active under natural changes of temperature conditions (Granström 1977), but became arrhythmic after being kept under constant temperature. Temperature plays a more important role in open areas than in forests. In the extreme condition on Mount Rainier, Mann *et al.* (1980) found that the habitat conditions, occurrence of food and reproductive satiation appear to be the major controller of circadian activity pattern. Uetz (1977) argues that interspecific competition is a logical explanation, since wandering spiders are polyphagous and would not likely be influenced by seasonal occurrence of individual prey species. Differences in seasonal and spatial distribution of foraging activity are the adaptive outcomes of interspecific competition, since overlap is significantly lower between pairs of species that are either closely related or of similar size. Nocturnal and diurnal spiders, web-building or hunting species surely exploit different prey resources; degrees of prey specialization appear to vary widely at the species level (Nyffeler and Benz 2009). Generally, mechanisms of species coexistence are multidimensional (Enders 1976, Uetz 1977).

We analysed circadian activity rhythm of the ground living spiders of floodplain forest and surrounding clearcut area in spring and autumn. We compared patterns of activity of abundant spiders expecting differences enabling coexistence of these species. Differences in activity pattern of spiders were evaluated depending upon their body size and sex.

2. STUDY AREA

The study was conducted within the Litovelské Pomoraví Protected Landscape Area (Czech Republic, Central Europe), which protects a close to natural landscape with meandering and braided channel of the Morava River and the adjacent floodplain covered by floodplain forests and meadows. The study sites were situated in an old floodplain forest (*Querceto-Ulmetum*) and the adjacent deforested open area (49°65´N, 17°20´E; altitude 210 m a.s.l.). The mean litter biomass (dry weight) in autumn was 622 g m^{-2} (data from November 1998). The alluvial soil was loamy-sandy to loamy at the locality, with pH 4.8–5.0. The annual precipitation was around 520 mm and mean annual temperature was 9.1°C (Tuf *et al.* 2006). The herbal layer of the floodplain forest consisted predominantly of *Anemone nemorosa*, *Polygonatum* spp., *Lathyrus vernus*, and *Maianthenum bifolium*. The dominant moss was *Eurhynchium hians*. One part of this forest had been clear cut in November 2002 and replanted in March 2003 with oak, elm and lime tree plants (ratio 8:1:1). Before planting, the remaining wood residue had been chipped and scattered throughout the clearcut.

3. MATERIAL AND METHODS

The investigation was carried out in late spring (20/V–7/VI, duration 18 days) and early autumn (23/IX–18/X, duration 25 days) 2004. Spiders were collected using pitfall traps without preservative solution. At each site the traps were set in a line with 3 m distances between the traps. Sixty traps were arranged in the forest and forty in the clearcut. Traps were checked every tree hours, eight times a day (i.e. at 03:00, 06:00, 09:00, 12:00, 15:00, 18:00, 21:00 and 00:00 hours Central European Summer Time). Duration of photophase in spring was between 15 h 40 min and 16 h 10 min, in autumn between 12 h 10 min and 10 h 38 min. Surface temperature was measured at both sites using temperature data-loggers (Minikin TH). Soil surface temperature regime at the forest varied during 24 hours, ranging from 10.8°C to 11.9°C in spring and from 10.1°C to 11.5°C in autumn. Soil surface temperature regime in the clearcut ranged during 24 hours from 10.1°C and 17.3°C in spring, and from 7.5°C to 16.2°C in autumn (Tuf *et al.* 2006).

Circular data of the diurnal activity of abundant spider species (together males and females in total number higher than 45 individuals) were evaluated according to Oriana software (in Kovach 2009), including the circular mean, length of mean vector (μ), circular standard deviation and 95 and 99% confidence limits. Correspondence analysis (CA) was conducted using the software Past (Hammer *et al.* 2001), in which the data were normalized. The used spider nomenclature follows Platnick (2012). Body size data of spiders are based on previous knowledge (Miller 1971, Heimer and Nentwig 1991) or on our own measurements.

4. RESULTS

Almost 12 000 specimens of ground dwelling arthropods were trapped in this study. A total of 5 817 spiders were collected and identified. In the spring 3 766 specimens (65% of all

Spiders) were collected in the forest and 1 282 in the clearcut. In autumn 540 individuals were collected in the forest and 229 in the clearcut. Spiders belonged to 102 species from 20 families. Only a few of all the collected males were in the mating phase, the main activity of most trapped males had been foraging for food.

Approximately 83% of all specimens collected belonged to the 13 most abundant species whose diurnal activities were analysed. They represent the families Linyphiidae, Lycosidae, Thomisidae, Agelenidae, and Pisauridae. The analysed species were divided into four groups based on body size and sexual differences in body size:

- group 1: small spiders (≤ 4 mm) with low (≤ 0.3 mm) sexual differences in body size,
- group 2: small spiders (≤ 4 mm) with high (≥ 0.6 mm) sexual differences in body size,
- group 3: larger spiders (7–9 mm), and
- group 4: big spiders (≥ 10 mm).

In the group 3 and 4, differences in body size of males and females were slight. Most of the species were placed into the first two groups.

Group 1 – small spiders with low sexual differences in body size – showed a bimodal pattern of circadian activity. Females were active nocturnally; males were active in the morning (Fig. 1, Table 1). The correlation of the activity of males and females was significant (0.65). This group included the linyphiid species *Abacoproeces saltuum* (male 1.9 mm, female 2.0 mm), *Micrargus herbigradus* (male 2.0 mm, female 2.0 mm), *Ceratinella brevipes* (male 1.4 mm, female 1.7 mm), *Walckenaeria dysderoides* (male 1.8 mm, female 1.9 mm) and *Diplocephalus picinus* (male 1.3 mm, female 1.6 mm). Spider *A. saltuum* was highly abundant in the forest. Sex ratio was 1:1 (Fig. 1, Table 1). Both sexes were active from sunset throughout the night. Species *M. herbigradus* was regularly found at both habitats. Males predominated in the proportion 3:1. In the clearcut, the circadian activity was determined only by the males, with their maximum of activity before 12:00. In the forest, males were active in the early morning, whereas the females were active in the afternoon. Linyphiid *C. brevipes* was found at both habitats in both seasons. Activity of 142 individuals trapped in spring was analysed (Fig. 1, Table 1). Females were active from the afternoon into the night; males were active in the morning. Species *W. dysderoides* was found in spring at both study sites. In the forest, males were active in early morning. On the other hand, females were active in the afternoon with a maximum at 18:00. Nocturnal activity was not observed. Spider *D. picinus* was found in the forest in spring, activity of 46 individuals were analysed (Fig. 1, Table1). The proportion of males to females was 3:1. Females were active in the morning, males in the evening. A second activity phase of males occurred before midnight.

Group 2 – small spiders with high sexual differences in body size – was characterised by a bimodal pattern of circadian activity. Females were active in the late evening; males from midday to afternoon (Fig. 2). Correlation of male and female activity was low (0.51). The group included the species *Walckenaeria obtusa* (male 3.0 mm, female 3.8 mm), *Ozyptila praticola* (male 2.7 mm, female 3.5 mm) and *Panamomops mengei* (male 1.2 mm, female 1.8 mm). Linyphiid *W. obtusa* occurred both in the forest and in the clearcut in spring. At both study sites, males were active in the morning; females were active in the evening. We did not notice any nocturnal activity. Thomisid *O. praticola* was trapped in the forest in both seasons, 78 individuals were examined. The activity of *O. praticola* was divided into two day-phases. In spring, the activity of males started in the morning and reached its maximum at 12:00 (Fig. 2). Females were active in the night. Linyphiid *P. mengei* was found in the forest in spring. Males prevailed (5:1) among the 214 trapped individuals (Fig. 2, Table 1). Females were active in the evening, while the activity of males increased gradually during the afternoon.

Group 3 – larger spiders with similar body size of males and females have shown a typical circadian activity pattern (Fig. 3). Females, males as well as their juveniles were active in the afternoon. The correlation of male and female activity was really high (0.99). This group included species of the genus *Pardosa*. Lycosid *Pardosa lugubris* (male 7.1 mm, female 7.5 mm) was dominant in the spider assemblages of both habitats throughout the year (Table 1). Altogether 847 individuals were analysed. Both sexes were active in the

Table 1. Total number of trapped spiders (individuals) of abundant species during late spring and early autumn in two habitats (forest and clearcut) in the Litovelské Pomoraví PLA collected in three-hour intervals (F – females, M – males). Sixty traps were arranged in the forest and forty in the clearcut, duration of the traps exposition was 18 days in spring and 25 days in autumn.

Group and species	Sex	Season	Habitat	Activity phase (hour)							
				0:00	3:00	6:00	9:00	12:00	15:00	18:00	21:00
Group 1 – small spiders (≤ 4 mm); low (≤ 0.3 mm) sexual differences in body size											
Abacoproeces saltuum (L. Koch, 1872)	F	spring	forest	114	91	113	127	87	42	40	106
	M	spring	forest	175	308	254	303	242	137	141	220
	F	spring	clearcut	4	5	3	2	1	4	4	
	M	spring	clearcut	1	3	5	9	2	2	2	2
Micrargus herbigradus (Blackwall, 1854)	F	spring	forest	3				3	4	2	2
	M	spring	forest	4		4	1	9	11	5	7
	F	spring	clearcut	1							
	M	spring	clearcut				3	4		1	
Ceratinella brevipes (Westring, 1851)	F	spring	forest	7	4	5	15	10	4	12	8
	M	spring	forest	4	1	1	2	4	8	6	6
	F	spring	clearcut	3	1	2	3	4	3	4	
	M	spring	clearcut		1	1	4	8	4	4	3
Walckenaeria dysderoides (Wider, 1834)	F	spring	forest		2	1		5	7	10	2
	M	spring	forest		3	1	5	3	2	1	
	F	spring	clearcut				1	6			
	M	spring	clearcut		1	1	1	1	1		1
Diplocephalus picinus (Blackwall, 1841)	F	spring	forest	2			1	5	2	2	1
	M	spring	forest	5	1	1	2	6	7	9	2
Group 2 – small spiders (≤ 4 mm); high (≥ 0.6 mm) sexual differences in body size											
Walckenaeria obtusa Blackwall, 1836	F	spring	forest		1			3	4	2	3
	M	spring	forest		5	7	8	9		1	1
	F	spring	clearcut			1	1				
	M	spring	clearcut			4	3	4	1		
Ozyptila praticola (C.L. Koch, 1837)	F	spring	forest	1	2			2	1	3	3
	M	spring	forest	9	4	4	3	11	3	2	10
	F	autumn	forest	1	3			3	1	1	3
	M	autumn	forest		1			2	2		3
Panamomops mengei Simon, 1926	F	spring	forest	3	4	2	4	8	5	6	3
	M	spring	forest	7	7	21	19	42	18	38	23

Group and species	Sex	Season	Habitat	Activity phase (hour)							
				0:00	3:00	6:00	9:00	12:00	15:00	18:00	21:00
Group 3 – larger spiders (7–9 mm)											
Pardosa lugubris (Walckenaer, 1802)	F	spring	forest	2	1	1	5	32	39	33	10
	M	spring	forest			1	14	47	54	51	22
	F	spring	clearcut	15	2	4	42	79	63	40	24
	M	spring	clearcut	5		1	10	53	64	18	13
	F	autumn	forest				1	4	23	6	1
	M	autumn	forest					1	19	7	1
	F	autumn	clearcut	1			1	2	13	5	1
	M	autumn	clearcut				2	4	9	1	
Pardosa amentata (Clerck, 1757)	F	spring	forest						1		
	M	spring	forest				1		7	6	
	F	spring	clearcut			1	2	22	15	6	1
	M	spring	clearcut				2	27	16	7	2
Pardosa prativaga (L. Koch, 1870)	F	spring	forest					1	1	2	
	M	spring	forest				1	2	7	1	
	F	spring	clearcut	1		1	9	26	25	15	2
	M	spring	clearcut	10	1		9	42	28	18	4
	F	autumn	forest					3	2	6	1
	M	autumn	forest	2	1		1	2	1	2	
	F	autumn	clearcut	1			1	6	4	7	1
	M	autumn	clearcut					2	11	1	
Group 4 – big spiders (≥ 10 mm)											
Coelotes terrestris (Wider, 1834)	F	spring	forest	16	5	3		1	2		1
	M	spring	forest	3				1			
	F	autumn	forest	1	1	1					
	M	autumn	forest	2	2						2
	juv.	autumn	forest	8	12	7	25	26	31	37	34
	juv.	spring	forest	22	9	6		2	4	10	13
Pisaura mirabilis (Clerck, 1757)	F	spring	clearcut	8	10	9		3	1		1
	M	spring	clearcut	12	17	4	1		1		4
	F	spring	forest	3		1					
	M	spring	forest	1		2	1			1	1
	juv.	autumn	clearcut			1	2	1			
	juv.	autumn	forest	2		1	2		1		

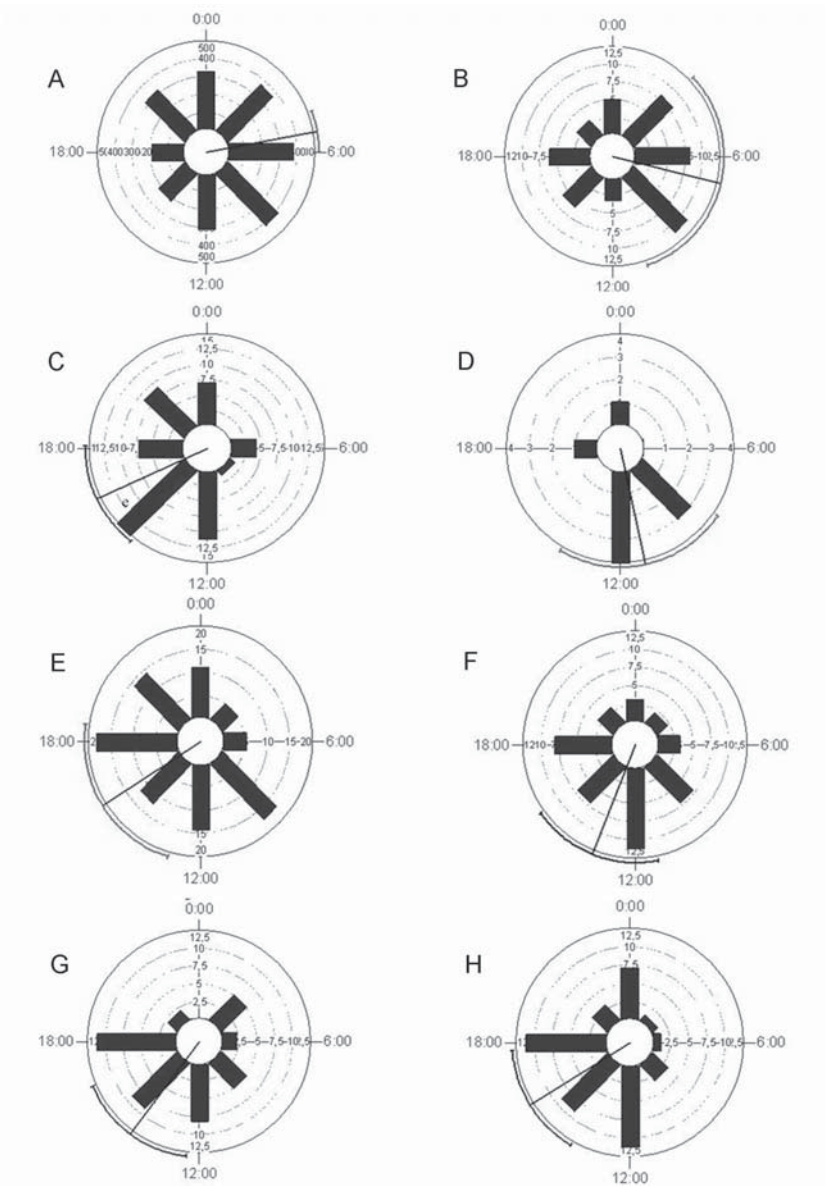

Fig. 1. Circadian epigeic activity of small spiders of group 1 (with low (≤ 0.3 mm) differences in body size): (A) *Abacoproeces saltuum*, spring, forest, (B) *A. saltuum*, spring, clearcut, (C) *Micrargus herbigradus*, spring, forest, (D) *M. herbigradus*, spring, clearcut, (E) *Ceratinela brevipes*, spring, forest, (F) *C. brevipes*, spring, clearcut, (G) *Walckenaeria dysderoides*, spring, forest, (H) *Diplocephalus picinus*, spring-forest. Black line running from the centre of the diagram to the outer edge marks mean time of activity and the arcs extending to either side represent the 95% confidence limits.

afternoon (from 12:00 to 18:00), with maximal activity at 15:00 (Fig. 3, Table 1). At the clearcut, females achieved maximum activity three hours earlier than in the forest. Both sexes (1:1) and juveniles had identical activity pattern. *Pardosa amentata* (male 8.0 mm, female 7.9 mm) was found at both study sites in spring (116 individuals; sex ratio 7:5). The activity pattern of females and males was identical. In the forest the activity maximum of both sexes was at 15:00, at clearcut the peak of activity was earlier (Fig. 3, Table 1). Altogether 261 individuals of *Pardosa prativaga* (male 7.3 mm, female 7.5 mm) were considered. Both sexes (4:3) and juveniles were active in the afternoon (from 12:00 to 18:00) (Fig. 3, Table 1). The activity of species started at clearcut three hours earlier than in forest. Juveniles of the genus *Pardosa* were found at both study sites and in both seasons. The activity of the juveniles of the genus *Pardosa* was very similar to the adults (maximum at 12:00 in spring, and at 15:00 in autumn).

Group 4 – big spiders with similar body size of males and females – showed a typical nocturnal activity. Females and males of large spiders were active in the night (Fig. 4). The correlation of male and female activity was high (0.86). *Coelotes terrestris* (male 10.0 mm, female 12.0 mm) and *Pisaura mirabilis* (male 13.0 mm, female 15.0 mm) belong to this group. In total, we found 287 individuals of *C. terrestris* in the forest; only 13 individuals were trapped at the clearcut (Table 1). Adults and subadults achieved identical nocturnal activity (Fig. 4) with maximal peak at midnight. In autumn, the majority of population of *C. terrestris* consists of small juveniles (95%), which were active in day with maximum 18:00 (Fig. 4, Table 1). This result corresponds with the activity of larger spiders from the group 3. Species *P. mirabilis* was trapped at both study sites in both seasons (86 individuals). None of the trapped *P. mirabilis* specimens was in the mating phase; therefore it is likely that their main activity was foraging for food. Nocturnal activity of both sexes was identical. Males and females were active from dusk; in the deforested site they reached their activity maximum at 03:00, in the forest at 01:46. However, small juveniles were active throughout the day. This result corresponded with the diurnal activity of larger spiders from the group 3 (Fig. 4, Table 1).

Time-patterns of activity of all groups and both sexes were evaluated by correspondence analysis (Fig. 5). The cumulative percentage

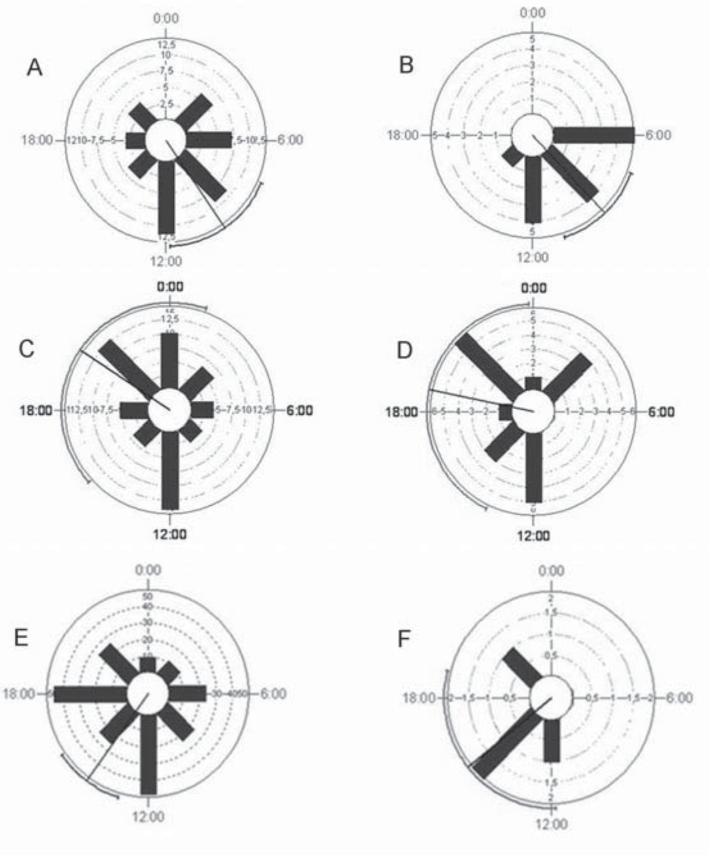

Fig. 2. Circadian epigeic activity of small spiders of group 2 (with high (≤ 0.6 mm) differences in body size): (A) *Walckenaeria obtusa*, spring, forest, (B) *W. obtusa*, spring, clearcut, (C) *Ozyptila praticola*, spring, forest, (D) *O. praticola*, autumn, forest, (E) *Panamomops mengei*, spring, forest, (F) *P. mengei*, spring, clearcut. Black line running from the centre of the diagram to the outer edge marks mean time of activity and the arcs extending to either side represent the 95% confidence limits.

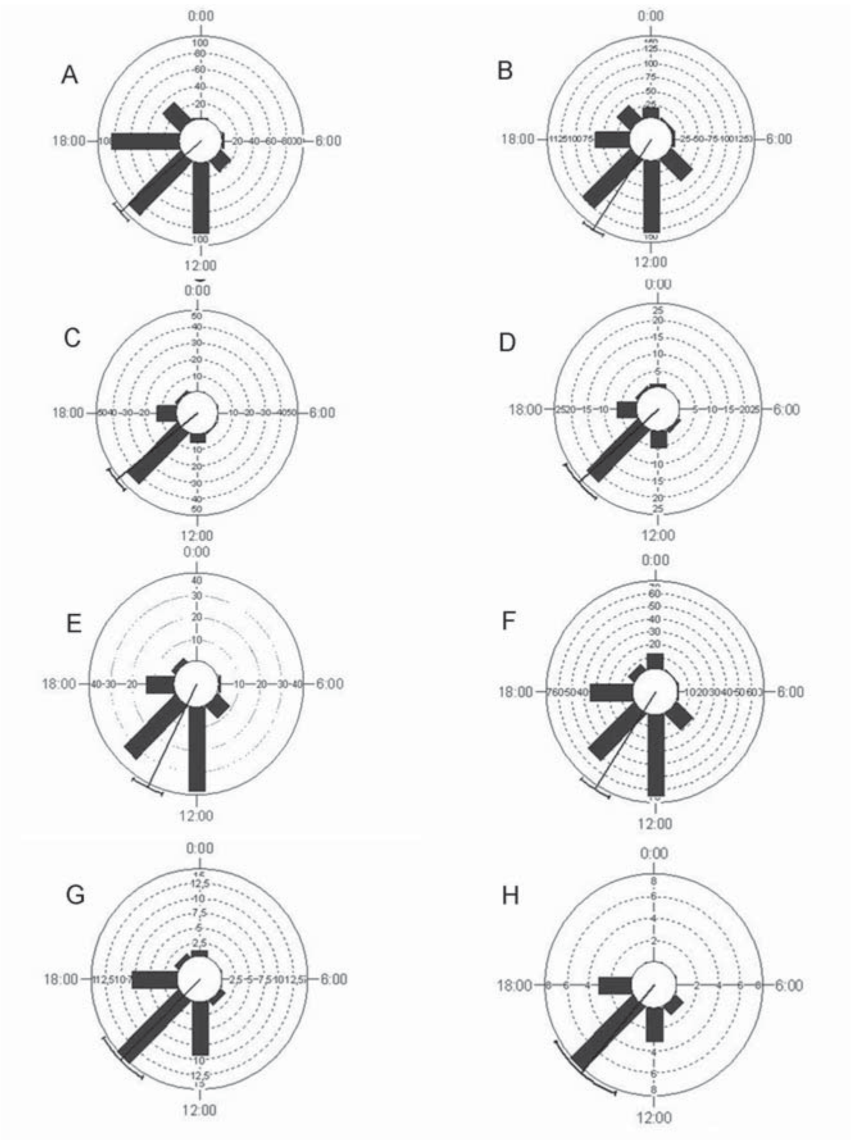

Fig. 3. Circadian epigeic activity of larger spiders of group 3 (7–9 mm): (A) *Pardosa lugubris*, spring, forest, (B) *P. lugubris*, spring, clearcut, (C) *P. lugubris*, autumn, forest, (D) *P. lugubris*, autumn, clearcut, (E) *P. amenata* spring, clearcut, (F) *P. prativaga*, spring, clearcut, (G) *P. prativaga*, autumn, clearcut, (H) *P. prativaga*, spring, forest. Black line running from the centre of the diagram to the outer edge marks mean time of activity and the arcs extending to either side represent the 95% confidence limits.

of the species variance was 89.5%. One-way ANOVA (F = 23.2, df = 22.5, $P < 0.0001$) confirmed a statistically significant differences among the variances, spiders of both sexes from group 1 were active during morning and evening, species from group 3 were active afternoon and species from group 4 during late night. Activity pattern of males from group 2 was similar to activity of group 1 and whereas activity of females of group 2 was similar to activity of spiders from group 3. Generally, despite sex and species, we found the most contrasting difference between activity of spiders bigger and smaller than 5 mm (Fig. 6A); their activities were affected by soil surface temperature (Fig. 6B). Spiders bigger than 5 mm increased their activity with increase of temperature; whereas spiders smaller than 5 mm decreased activity during warmer parts of days.

5. DISCUSSION

Based on the analysis of activity of a high number of individuals (males and females separately) in two habitats of the inundation area of the Morava River we found out the reason for the observed biphasic activity of general pattern: the different circadian rhythm of epigeic activity of smaller and bigger spiders. Early morning activity of small spiders can be caused not only with foraging for food, but by presence of water in the form of dewy on webs as well. We reached an agreement on Ward and Lubin (1992) results that the activity of progressively large spiders finished exactly in the morning. This general pattern of activity of small spiders is caused by *A. saltuum* mainly (90% of all specimens from Group 1); some species seems to be active during afternoon (*cf* Fig. 1). This shift can be a mode of suppression of interspecific competition. Similar shift in activity of two species of similar size was described for woodlice (Tuf and Jeřábková 2008). General activity pattern of small spiders is in accordance with the strategies of other predatory invertebrates as centipedes (Tuf *et al*. 2006).

Platen (1988) found out that spiders had diurnal activity in spring (May–June) and late summer (September–October), while during winter (December) they were mainly nocturnal. The present study yielded different results. We found both diurnal as well as nocturnal activity in ground living spiders and this activity pattern did not depend on the seasons (spring *vs* autumn). Flatz (1987) observed that the circadian rhythm of activity of spiders was asymmetrical; lycosid spiders were active mainly during the day, whereas linyphiids during the night or in the predawn. Granström (1977) found that the activity of *P. lugubris* and *P. amentata* under natural conditions was diurnal and that temperature seems to modify the pattern – prevention of temperature regime in laboratory condition led to irregular pattern. In our study we found relationship between activity and temperature of soil surface. Spiders of genus *Pardosa* representing 76% of all spiders bigger than 5 mm, were active at afternoon. They affected general pattern of increase of activity of bigger spiders with increasing temperature. Wolf spiders (i.e. lycosids) are excellent hunters. Many are active wandering

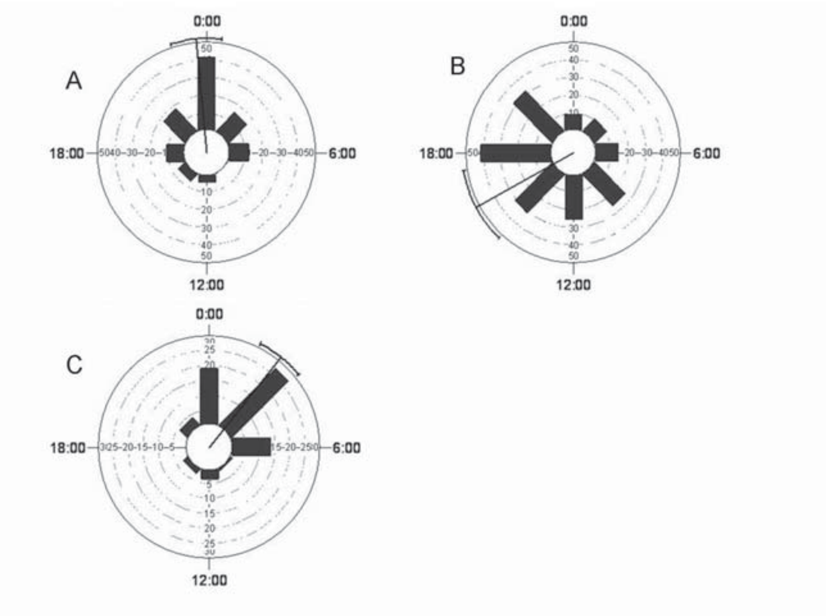

Fig. 4. Circadian epigeic activity of big spiders of group 4 (≥ 10 mm): (A) *Coelotes terrestris*, spring, forest, (B) small juveniles of *C. terrestris*, autumn, forest, (C) *Pisaura mirabilis*, spring, clearcut. Black line running from the centre of the diagram to the outer edge marks mean time of activity and the arcs extending to either side represent the 95% confidence limits.

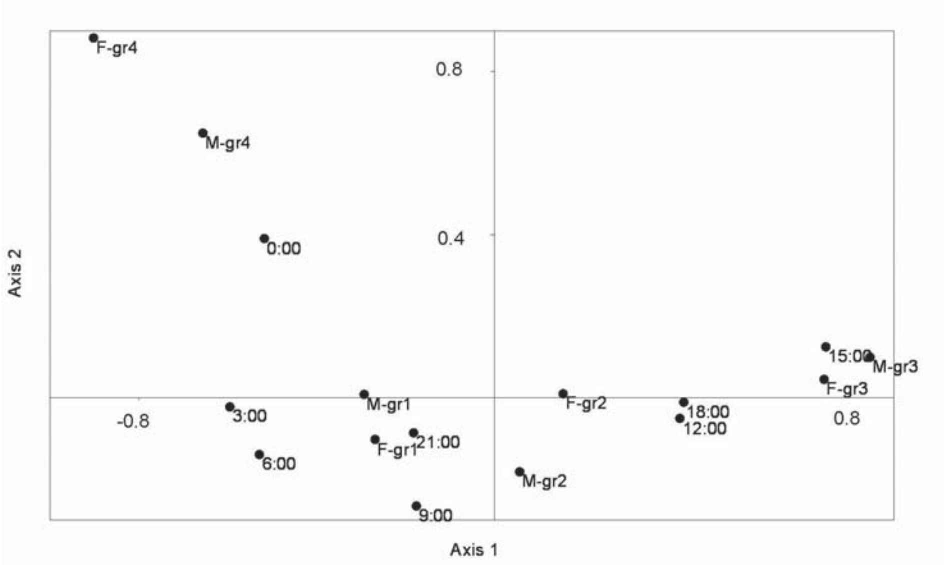

Fig. 5. Correspondence analysis of circadian activity used the syntopic epigeic spider groups (in dependence of their body size) (F – females, M – males, gr – groups, see fig. 1–4, Table 1).

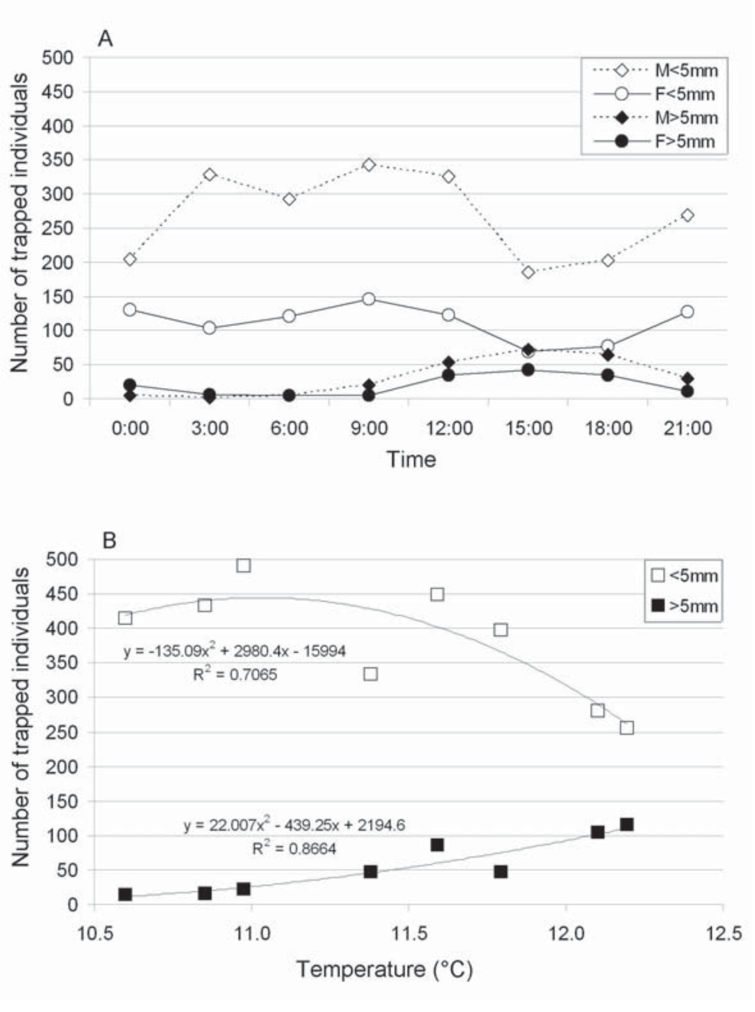

Fig. 6. Activity of spiders of different body size: (A) time-pattern of activity, (B) relation between activity and soil surface temperature.

hunters on the ground and in the vegetation during the day. On the other hand, the activity of really big spider (*P. mirabilis* ≥ 12 mm) was strictly nocturnal; mainly by about midnight. It is a typical dweller of the herb layer of various open dry habitats, including ruderal ones (Buchar and Růžička 2002); so it probably stays during day at warmer place and hunt there diurnal-active insects too. *C. terrestris* had a nocturnal activity pattern too; both sexes and its subadults were active after full darkness with the maximum at midnight and over 90% of their activity took place in darkness. It is therefore likely, that this nocturnal behaviour may be more related to biotic than to physical factors of the environment (Cloudsley-Thompson 1957, Casas *et al.* 2008).

Ward and Lubin (1992) and Lubin *et al.* (2001) noticed that spiders had two periods of activity: in the evening (at dusk) and in the pre-dawn (so called crepuscular activity). Morning-active spiders had larger webs and larger clutches than evening-active spiders. Chapman and Armstrong (1997) found linyphiid spiders being nocturnal and crepuscular, but the authors did not discriminate between the activity of males and females. According to our study, spider species, in which females are larger than males, showed an activity pattern with two peaks; females were active in the late evening or night; males were active from midday to afternoon. We found out those small spiders with similar body sizes of males and females showed diurnal activity with a single considerable phase in the morning. Ward and Lubin (1992) observed that small spiders built their webs early in the evening and progressively larger spiders set their webs through the night. This activity pattern corresponded to the increasing size of prey (nocturnal insects) becoming active in the course of the night.

Trophic interactions between epigeic spiders and their prey allow us to better understand their circadian rhythm of epigeic activity. Small-bodied arthropods such as the Collembola are in part diurnally active; the large-sized species are exclusively nocturnal (Mann *et al.* 1980, Tuf *et al.* 2006). Romero and Harwood (2010) summarised that spiders were selectively constructing webs where prey was relatively more abundant.

The data presented here support the opinion that mechanisms of species circadian activity involve habitat, seasonal occurrence and as well as the body size of spiders. Different general pattern of activity of big and small spiders can cause not only suppression of interspecific competition but also suppression of predation of bigger species upon smaller ones. Circadian rhythm of activity of spiders plays the great importance role to occupy the ecological niches.

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DOES THE DIURNAL ACTIVITY PATTERN OF CARABID BEETLES DEPEND ON SEASON, GROUND TEMPERATURE AND HABITAT?

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Abstract - The influence of season, ground temperature and habitat on diurnal epigeic activity of ground beetles (Coleoptera: Carabidae) in floodplain forest and neighboring clear-cut area was studied in late spring and early autumn by pitfall trapping. Among the material collected were 35 species of ground beetles. We recorded a significant influence of daytime factors on epigeic activity in 16 species. The epigeic activity of 13 species was co-affected by the temperature of the soil surface. The activity of some species differed, depending on season and locality.

Key words: Carabidae, diel activity, circadian activity, seasonality, floodplain forest, Moravia, Czech Republic

INTRODUCTION

Based on their diurnal activity, carabid beetles are usually divided into three categories – strictly diurnal species, species with nocturnal activity, flexible species with activity modified by microclimatic and geographic conditions (Thiele, 1977, Novák, 1977). However, we can find differences in activity among not only species and populations of the same species, but even within a single population (Thiele and Weber, 1968). Species can also show various preferences to light intensity in different phases of ontogenetic development (differing periods of development of larva and imago – *Carabus problematicus* Herbst) or changes in periodicity of reproduction (*Carabus cancellatus* Illiger).

Annual rhythms are also closely connected to the circadian rhythm – 90% of species reproducing in autumn are active at night, while among species reproducing in spring the proportion of nocturnal activity is substantially lower, about 33% (Thiele and Weber, 1968).

Diurnal activity is primarily regulated by endogenous physiological factors, so-called timers, but the length of its duration could be modified by environmental conditions. Light is known to be the most important factor influencing carabid beetle activity (Thiele, 1977), nevertheless temperature and moisture levels can also play an important role in relation to the type of habitat (Thiele, 1977, Novák, 1980, Kegel, 1990).

Studies dealing with diurnal activity can be conducted under natural or laboratory conditions. In laboratory studies, direct observations or instruments recording activity on the weight basis used to be employed (Park, 1935). Actographs are the instruments used to record activity on the basis of electric impulses, infra-red light, or sound (Park, 1935; Backlund and Ekeroot, 1950). Novák (1978) designed a special trap with automatic sampling for laboratory research. In natural conditions, two basic tools are commonly used: 1) simple pitfall traps in various modifications (Williams, 1958, Loreau, 1986), or 2) automatic traps that separate catch by individual periods (Novák, 1979, 1980).

MATERIALS AND METHODS

The study area was located in Litovelské Pomoraví Protected Landscape Area (Central Moravia, Czech Republic) with floodplain forest and meadows along the Morava River. We compared two localities – old floodplain forest (*Querco-Ulmetum*) and neighboring clear-cut (49°65´N, 17°20´E, altitude 210 m a.s.l.).

The herbal layer of the floodplain forest consists of *Anemone nemorosa, Polygonatum* spp., *Lathyrus vernus* and *Maianthenum bifolium*; the dominant moss was *Eurhynchium hians*. In November 1998, litter biomass (dry weight) was 622 g/m^2. The alluvial soil is loamy-sandy to loamy at the locality, with pH 4.8-5. Annual precipitation was about 520 mm; the mean annual temperature was 9.1°C. Part of the forest was cut in November 2002 and replanted in March 2003 by oak, elm and lime tree (ratio 8:1:1) using heavy forestry machines. Before replanting, the remaining wood was chipped and scattered throughout the whole area.

Epigeic invertebrates were caught by pitfall traps (plastic pots) without preservative solution. In total, 60 traps were set in the forest and 40 traps were set at the clear-cut, in line 3 m apart.

Experiments were carried out in late spring (May 20th – June 7th, 18 days) and early autumn (September 23rd – November 18th, 25 days) of 2005. The traps were checked every three hours during the research periods (i.e. at 03:00, 06:00, 09:00, noon, 15:00, 18:00, 21:00, and midnight).

In order to obtain comparable values, all the data were recounted for one trap. The biotope preferences (forest *vs.* open-habitat species) and reproduction biology (spring *vs.* autumn breeders) of each species were taken from Larsson (1939), Lindroth (1949), Thiele (1977), Novák (1979, 1980) and Hůrka (1996). The temperature of the soil surface was recorded using data-loggers Minikin TH (Environmental Measuring Systems Brno, www.emsbrno.cz). Canonical Correspondence Analysis and Generalized Additive Models for evaluating of results were created in the program CANOCO for Windows 4.5© (ter Braak and Šmilauer. 1998); graphs were created in CanoDraw for Windows 4.0 and Microsoft Excel.

RESULTS

During our research, 820 specimens of carabids belonging to 35 species were captured (Table I). The clear-cut was more abundant in both species (32) and the overall number of beetles (471, D = 57%); 349 specimens representing 25 species of carabid beetles were collected in the forest. Most specimens were collected in the spring (664 specimens, 29 species), while only 156 specimens of 21 species were collected in the autumn. The dominant species in the forest was *Abax parallelus* (D = 42%); *Bembidion lampros* dominated in the clear-cut (D = 25%). These two species together formed nearly 40% of the catch. The similarity between the forest and clear-cut communities was low (Jaccard index = 51%). Such a low similarity of populations is caused by a significant presence of forest species in the woodland (11 species, 44%) and open habitat specialists in the clear-cut (15 species, 47%).

The epigeic activity of carabids in the forest was concentrated to evening and nighttime hours, with a peak period between 21:00 and midnight; in the clear-cut, activity had a distinctive daytime character, with peaks in the afternoon hours.

Spring epigeic activity was more or less evenly spaced throughout the entire day, declining late at night and in the early morning hours. Autumn was characterized by significant afternoon peaks of activity in a relatively narrow span, between 12:00 and 15:00 h, and a gradual decline during evening hours (Fig. 1).

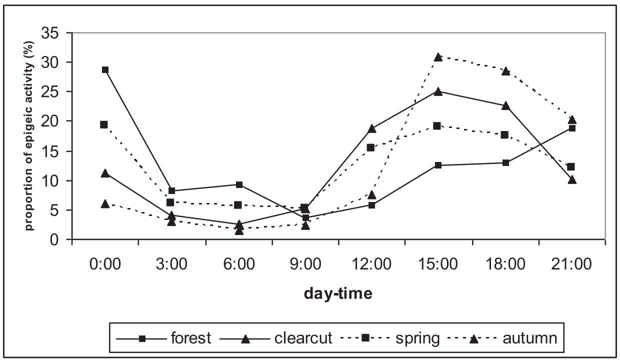


Fig. 1. Influence of daytime temperatures on the level of epigeic activities of carabid beetles in different seasons and at different localities.

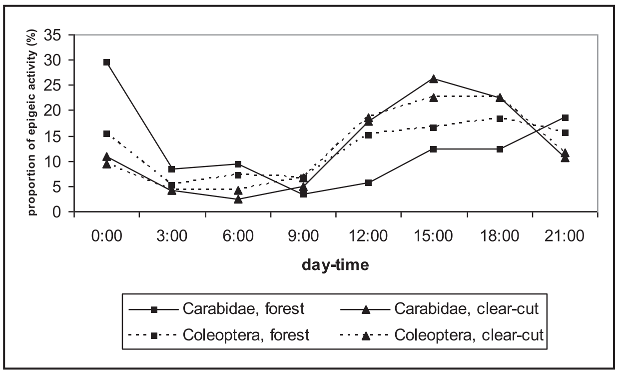
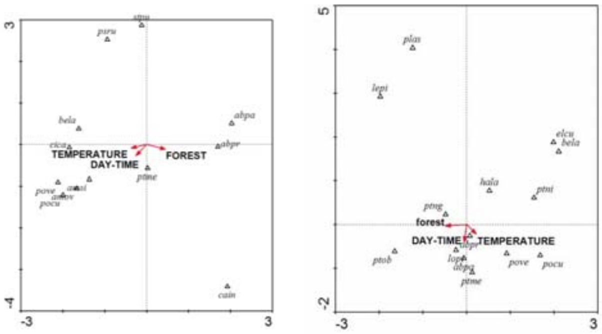


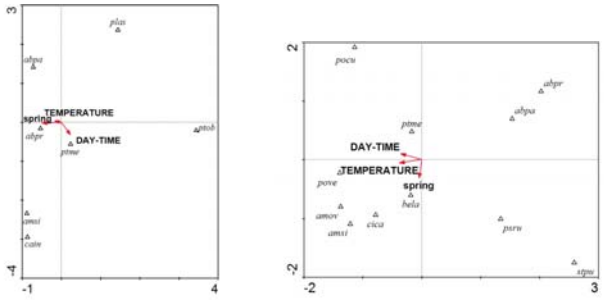
Fig. 2. Comparison of diurnal activities of carabid beetles and its dependence on the locality.



a) b)

Fig. 3. CCA analysis for spring (a) and autumn season (b). Model for spring season explained 87 % of variability (forest: F = 29.74, 57.1 %; time: F = 9.60, 17.7 %; temperature: F = 6.73, 12 %), model for autumn 90 % of variability (forest: F = 4.20, 40.4 %; time: F = 2.99, 28.1 %; temperature: F = 2.31, 21.4 %).

A separate analysis of material collected in the spring and in the autumn showed that the factor *locality* had greater significance in the spring. On the other hand, the factors of the *daytime* and *temperature* of the ground surface played a more significant role in the autumn (Fig. 3).



a) b)

Fig. 4. CCA analysis for forest (a) and clear-cut (b). Model for forest gave 66% variability (spring: F = 18.25, 44.2 %; time: F = 6.84, 15.9 %; temperature: F = 2.37, 5.4 %), model for clear-cut 49 % of variability (temperature: F = 8.57, 26 %; time: F = 5.71, 14.8 %; spring: F = 4.47, 8.2 %).

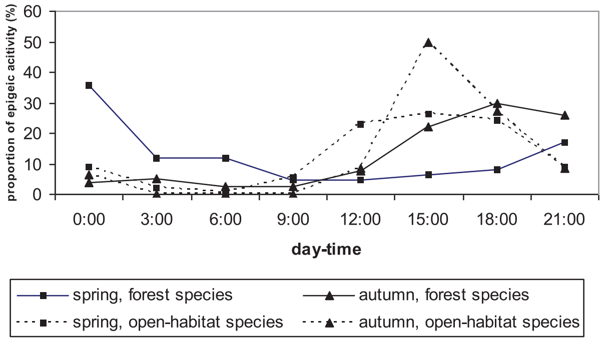


Fig. 5. Comparison of the epigeic activities of forest species and open-habitat species of carabid beetles and its seasonal dependence.

Samples collected in the forest growth were significantly influenced by *season*, while the influence of *temperature* was significantly greater in the clear-cut (Fig. 4). Both localities differed in temperature regime, with temperatures more significantly fluctuating in the clear-cut than in the forest. While spring temperatures in the clear-cut fluctuated between 7 and 21°C, forest temperatures only ranged from 7 to 15°C. In the autumn, temperatures fluctuated between 1 and 21°C in the clear-cut, in the forest from 5 to 13°C. The amount of light at both localities was approximately the same (Fig. 4).

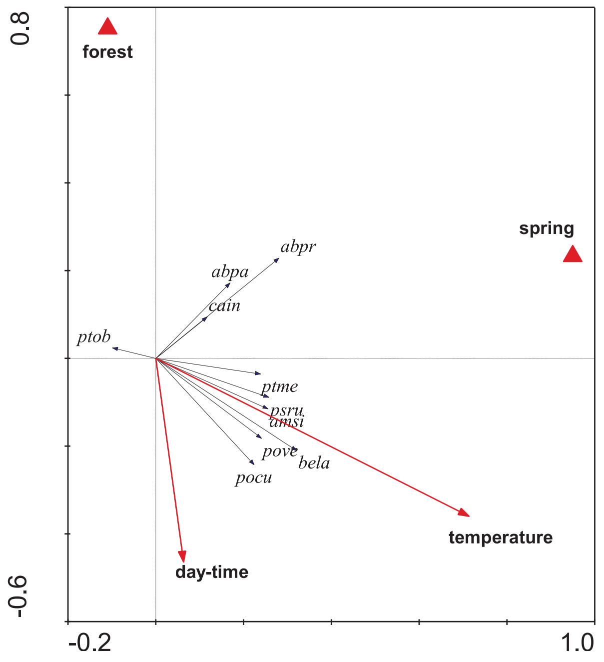


Fig. 6. Comparison of the epigeic activities of spring breeders and autumn breeders and its dependence on locality.

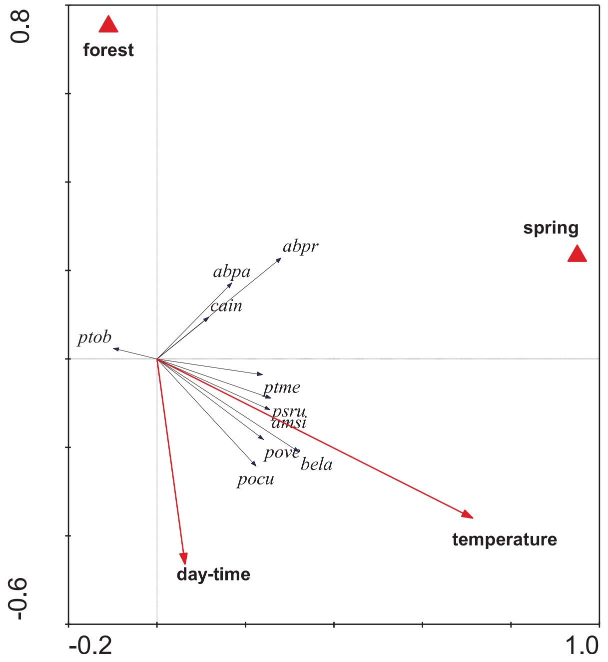
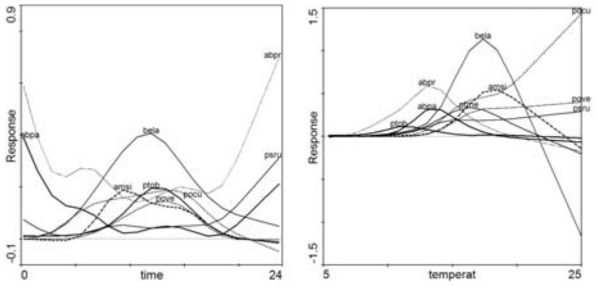
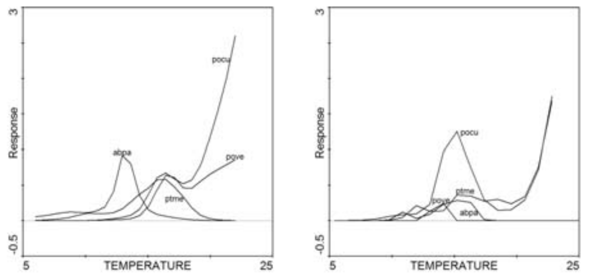


Fig. 7. RDA ordination illustrating distribution of the most numerous carabid species (dominancy more than 1.5%) in relation to environmental factors. Significance of factors: spring – F 40.85, forest – F 21.42, temperature – F 12.70, time – F 7.35, p for all factors lower than 0.01.



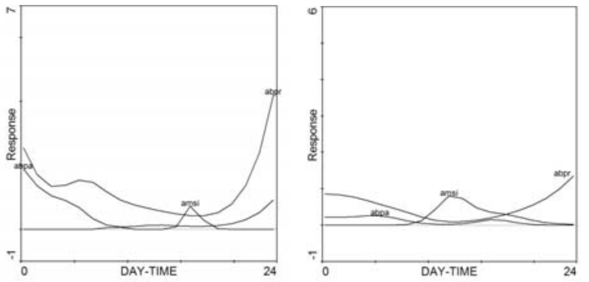
a) b)

Fig. 8. Response of epigeic activity of carabid species to (a) time and (b) temperature. For significance of response see Table II.



a) b)

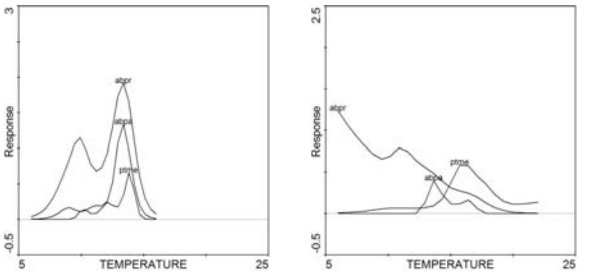
Fig. 9. Response of epigeic activity of carabid species to temperature in (a) spring and (b) autumn. For significance of response see Table III.



a) b)

Fig. 10. Response of epigeic activity of carabid species to *daytime* (a) in forest and (b) in clear-cut. For significance of response see Table III.

Captured species were compared by their biotope preferences. The activity of forest species fluctuated during the year; they were active from evening to midnight in spring, and in the afternoon in autumn, respectively. Species in the clear-cut had the same pattern of activity, with afternoon peaks in both seasons; however, in the autumn, the peak was narrower



a) b)

Fig. 11. Response of epigeic activity of carabid species to *temperature* (a) in forest and (b) in clear-cut. For significance of response see Table III.

– 50% of all specimens were collected in the period 12:00 – 15:00 (Fig. 5).

For the second comparison, the carabids were sorted by reproduction biology. So-called spring breeders became activate after dusk in the forest, and during daylight in the clear-cut. Autumn breeders exhibited an elevated proportion of nocturnal activity; in the forest nocturnal activity clearly dominated, while in the clear-cut a second peak appeared – in addition to the afternoon maximum there was also increased activity in the early nighttime hours (Fig. 6).

Epigeic activity vs. exogenous factors

The influence of *temperature* and the *daytime* was tested on all species collected. The activity of 16 species was significantly affected by *daytime*, while 13 species were influenced by *temperature* (Table III).

In RDA analysis, with *season*, *locality*, *temperature* and *daytime* as the variables (Fig. 7), the majority of species were positively influenced by spring and the clear-cut environment. A bond to the forest environment was found in *A. parallelipipedus*, *A. parallelus*, *Calosoma inquisitor* and *P. oblongopunctatus*, which was the only species with higher autumnal activity. Only the dominant species significantly responding to at least one of the factors were used for the analysis. A total of 8 species responded significantly to *daytime*, while 9 species responded to *temperature* (Table II).

With the aid of generalized additive models (GAM), the influence of *daytime* and *temperature* abiotic factors on the epigeic activity of the dominant species were tested. Both *Abax* species and *P. rufipes* exhibited significant nocturnal activity, while typical clear-cut species *A. similata*, *P. versicolor*, *P. cupreus*, *B. lampros* together with *P. oblongopunctatus* (the only representative of forest specialist), showed striking diurnal activity (Figs. 8a, 8b).

Increasing temperature of the soil surface positively influenced both *Poecilus* species as well as the other species with clear-cut preference: *B. lampros*, *A. similata* and *P. melanarius.* The forest species *A. parallelus*, *A. parallelipipedus* and *P. oblongopunctatus* preferred lower temperatures.

Diurnal activity vs. season

We studied the differences in epigeic activity during spring and autumn. Only *P. cupreus* provided a significant response to *daytime* in both periods, being active during the day, although this activity finished earlier in the autumn due to light deficiency. Four species – *A. parallelipipedus*, *P. melanarius*, *P. versicolor,* and *P. cupreus* – showed a significant connection between epigeic activity and *temperature* in both seasons (Figs. 9a and 9b). *P. melanarius* and *A. parallelipipedus* preferred higher temperatures in the autumn compared to the spring. *P. cupreus* and *P. versicolor* were more active in autumn when the temperatures were even lower than in spring.

Diurnal activity vs. habitat

Three species (*A. parallelipipedus*, *A. parallelus* and *A. similata*) responded significantly to the *daytime* factor in both habitats (Figs. 10a and 10b). Both species of *Abax* had a longer period of nocturnal activity in the forest than in the clear-cut. *A. similata* showed one significant peak at 15:00 in the forest and was active in a relatively narrow range of conditions, while in the clear-cut, the activity peak occured much sooner (around noon) and the range of activity was broader. The *temperature* factor was significant for *A. parallelipipedus*, *A. parallelus* and *P. melanarius*

Table I: The carabids caught during the spring and autumn periods in forest and clear-cut sites (number of animals trapped in individual daytime period in 18 days by 60 traps). O – open-habitat species, F – forest species, E – eurytopic species, S – spring breeders, A – autumn breeders, ? – unknown preference or biology. Time periods: 3, 6, 9, 12, 15, 18, 21 and 0; 3 means period from 0:00 to 3:00.

		FOREST								**CLEAR-CUT**								**FOREST**								**CLEAR-CUT**						
	type	3	6	9	12	15	18	21	0	3	6	9	12	15	18	21	24	3	6	9	12	15	18	21	0	3	6	9	12	15	18	21
Abax parallelipipedus (Piller et Mitt.)	F/A	10	7	-	2	1	-	6	34	2	2	1	-	-	2	-	3	-	-	-	-	1	2	1	-	-	-	-	-	3	-	-
Abax parallelus (Duftschmid)	F/S	12	19	7	7	9	6	25	47	7	4	4	-	1	3	8	11		1	1	1	1	3	6	-	-	-	-	2	2	6	2
Amara aulica (Panzer)	O/A	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Amara eurynota (Panzer)	O/S	-	-	-	-	1	2	-	-	-	-	-	2	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amara ovata (Fabricius)	E/?	-	-	-	-	-	1	-	-	-	-	-	-	7	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amara similata (Gyllenhal)	O/S	-	-	1	-	5	1	-	-	-	-	1	17	10	6	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Badister bullatus (Schrank)	?/?	-	-	-	-	2	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Badister lacertosus Sturm	?/?	-	-	-	-	2	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bembidion lampros Herbst	O/S	-	-	-	-	-	-	1	-	1	1	11	26	36	22	11	6	-	-	-	-	-	-	-	-	-	-	-	1	1	1	-
Carabus coriaceus Linnaeus	F/A	-	1	-	1	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1
Carabus granulatus Linnaeus	O/S	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Calosoma inquisitor (Linnaeus)	F/S	1	-	-	1	4	3	6	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carabus scheidleri Panzer	F/A	-	-	-	-	1	-	2	-	-	-	-	1	2	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carabus ullrichi Germar	E/S	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cicindela campestris Linnaeus	O/?	-	-	-	-	-	-	-	-	-	-	1	4	2	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clivina fossor (Linnaeus)	O/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Elaphrus cupreus Duftschmid	?/?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2
Epaphius secalis (Paykull)	E/?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Harpalus latus (Linnaeus)	O/A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	1	1	2
Harpalus luteicornis (Duftschmid)	O/S	-	-	-	-	1	-	-	-	-	-	-	2	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Leistus ferrugineus (Linnaeus)	O/A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Leistus piceus Froelich	F/?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2	-	-	-	-	1	-	-	-	-	-	-	-	-
Loricera pilicornis (Fabricius)	F/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	1	-	-	-	-	-	-	2	-
Notiophilus palustris (Duftschmid)	O/S	-	-	-	-	-	-	-	-	-	-	1	2	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Platynus assimilis (Paykull)	F/S	-	1	-	1	-	-	-	2	-	-	-	-	-	-	-	1	1	-	-	-	-	-	1	2	1	-	-	-	-	-	-
Poecilus cupreus (Linnaeus)	O/S	-	-	-	-	2	-	-	-	-	-	1	6	14	14	5	-	-	-	-	-	2	1	-	-	-	-	-	2	10	5	-
Poecilus versicolor (Sturm)	O/S	-	-	-	-	-	-	-	-	-	-	-	4	12	11	1	-	-	-	-	-	1	-	-	-	-	-	-	-	1	1	-
Pseudoophonus rufipes (De Geer)	O/A	1	-	-	-	-	-	-	1	6	-	-	5	3	5	5	16	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
Pterostichus anthracinus (Illiger)	E/S	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pterostichus melanarius (Illiger)	O/A	-	-	1	2	1	6	3	7	-	1	3	7	1	9	2	2	-	-	-	-	2	3	1	-	-	-	-	1	4	-	-
Pterostichus niger (Schaller)	F/A	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2	-	-	-	1	1	-	-	-	-	-	-	-	1	-	-
Pterostichus nigrita (Paykull)	F/S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	2	-	2
Pterostichus oblongopunctatus (Fab.)	F/S	-	-	1	1	-	2	-	1	-	-	-	-	-	-	-	-	-	-	1	1	6	17	10	-	-	-	-	-	1	-	1
Pterostichus vernalis (Panzer)	E/S	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stomis pumicatus (Panzer)	E/S	1	-	-	-	-	-	1	-	1	2	1	-	-	1	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table II: Epigeic activities of the dominant species of carabid beetles and its dependence to predictor (*daytime, temperature*) (n.s. – non-significant, * p lower than 0.05, ** p lower than 0.01).

predictor	epigeic activity of	F	AIC
day-time	*A. parallelipipedus*	4.21**	299.59
	A. parallelus	4.33**	473.996
	A. similata	4.55**	95.63
	B. lampros	4.01**	376.997
	P. cupreus	3.6**	121.119
	P. versicolor	4.92**	50.49
	P. rufipes	2.81*	72.907
	P. melanarius	n.s.	-
	P. oblongopunctatus	6.07**	65.352
temperature	*A. parallelipipedus*	5.44**	296.552
	A. parallelus	8.6**	457.631
	A. similata	19.64**	84.925
	B. lampros	25.03**	320.46
	P. cupreus	21.8**	105.02
	P. versicolor	16.83**	45.937
	P. rufipes	6.23**	70.855
	P. melanarius	11.82**	73.52
	P. oblongopunctatus	2.51*	67.334

in both localities; however, while *A. parallelipipedus* showed a peak of activity under similar temperatures at both localities, *A. parallelus* was active in the clear-cut in lower temperatures than in the forest and *P. melanarius* was active in the clear-cut in higher temperatures than in the forest (Figs. 11a and 11b).

DISCUSSION

According to Park (1941), during their evolution animal populations tend to converge towards symmetry of activity in the framework of a 24-hour cycle. In our study, the carabid population in a two-year old clear-cut represented the early stage of evolution, while the forest population represented the advanced (climax) stage. Thus, with the aid of Park's hypothesis we can predict that the activity in the open habitats will be probably asymmetric, while the activity in the forest would approach symmetry. If we include all the collected beetles in the analysis (beside the carabids, the most abundant were members of Staphylinidae and Geotrupidae), activity in the forest growth approached real symmetry (Fig. 2). In the case of carabid beetles, the clear-cut was significantly asymmetrical in diurnal activity, and the forest environment activity had a nocturnal character. Thus, our results do not correspond with those of Williams (1959), who confirmed the validity of Park's hypothesis for carabid beetles, but rather agree with Loreau (1986), who found predominately nocturnal activity in all dominant species of carabid beetles in a beech forest.

In our research, clear-cuts have greater species diversity than woodlands, which is again in agreement with many authors (Koivula, 2002, Magura, 2002, Ings and Hartley, 1999) who studied the influence of forest clearing on carabid beetle populations.

Carabids showed substantially greater activity in the spring (85% of the specimens), which is in agreement with Dondale *et al.* (1972), whose spring collection of Carabidae and Staphylinidae composed 85% of specimens collected per year. Novák (1980) reported a similar pattern in carabid catches depending on the season in the forest biotope.

Table III: Epigeic activities of carabid species in connection to predictor (*daytime, temperature*) and locality/season.

			specimens collected in/at							
			forest		clear-cut		spring		autumn	
predictor	epigeic activity of		F	AIC	F	AIC	F	AIC	F	AIC
day-time	*A. parallelipipedus*		7.3**	179.511	2.27*	62.21	7.97**	227.726	n.s.	-
	A. parallelus		7.87**	169.547	4.99**	106.717	10.59**	250.292	n.s.	-
	A. ovata	n.t.	n.s.	-	9.42**	42.835	13.49**	49.072	n.s.	-
	A. similata		7.84**	32.76	6.37**	122.923	12.66**	139.169	n.s.	-
	B. lampros		n.s.	-	3.02**	226.128	5.65**	297.746	n.s.	-
	C. campestris	n.t.	n.s.	-	n.s.	-	5.67**	59.038	n.s.	-
	E. cupreus	n.t.	n.s.	-	n.s.	-	n.s.	-	2.77*	36.511
	L. piceus	n.t.	n.s.	-	n.s.	-	n.s.	-	14.4**	13.57
	P. asimilis	n.t.	3.37**	40.831	n.s.	-	n.s.	-	14.21**	15.391
	P. cupreus		n.s.	-	11.26**	122.376	10.87**	138.181	5.77**	66.547
	P. versicolor		n.s.	-	11.34**	86.896	16.73**	96.558	n.s.	-
	P. rufipes		n.s.	-	7.4**	99.582	6.1**	138.378	n.s.	-
	P. melanarius		2.59*	96.865	n.s.	-	4.45**	147.678	n.s.	-
	P. niger	n.t.	n.s.	-	n.s.	-	n.s.	-	7.17**	16.199
	P. oblongopunctatus		6.27**	98.995	n.s.	-	n.s.	-	3.72**	84.971
	S. pumicatus	n.t.	n.s.	-	4.45**	37.702	2.29*	59.353	n.s.	-
temperature	*A. parallelipipedus*		3.08**	224.221	4.08**	54.438	4.86**	254.939	2.4*	37.685
	A. parallelus		2.53*	207.705	4.23**	110.417	7.96**	260.467	n.s.	-
	A. ovata	n.t.	n.s.	-	3.64**	50.834	6.29**	54.33	n.s.	-
	A. similata		n.s.	-	7.88**	110.372	9.05**	138.706	n.s.	-
	B. lampros		n.s.	-	4.31**	214.284	11.29**	250.303	n.s.	-
	C. campestris	n.t.	n.s.	-	5.79**	47.712	7.11**	51.594	n.s.	-
	E. cupreus	n.t.	n.s.	-	n.s.	-	n.s.	-	2.93**	34.2
	H. latus	n.t.	n.s.	-	n.s.	-	n.s.	-	3.35**	34.448
	P. cupreus		n.s.	-	4.26**	147.948	14.01**	115.124	6.81**	58.334
	P. versicolor		n.s.	-	3.99**	104.834	10.57**	91.354	5.12**	12.685
	P. melanarius		3.09**	93.782	3.89**	94.869	3.78**	152.011	3.31**	44.296
	P. niger	n.t.	n.s.	-	n.s.	-	n.s.	-	2.3*	35.285
	P. oblongopunctatus		n.s.	-	n.s.	-	n.s.	-	2.52*	90.062

* p less than 0.05
** p less than 0.01
n.s. - non significant
n.t. - non tested in whole material for low dominancy (≤ 2 %)

The significant influence of locality type for the catches in spring and autumn was given by the fundamental differences in the character of vegetation cover in open habitats between both seasons. In spring, the relatively thin herbal layer in the clear-cut was scattered with patches of exposed soil; in autumn the locality was covered with dense growth dominated by *Impatiens glandulifera.* Areas with dense vegetation have a higher moisture level and therefore populations of invertebrates significantly differ from those in spring conditions (Haysom et al., 2003).

In spring, the activity of forest species reached a peak in the evening and early night hours, while in autumn activity had a peak in the afternoon. Ilosvay (1982) reports a significant peak of activity in the night hours (22:00-4:00) in June and July. Also, Thiele (1977) showed that most of forest species are night-active. In autumn, our forest species were mostly active in the afternoon hours, probably because of extremely low temperatures in the night and early morning.

Species in the clear-cut showed a peak of activity in the afternoon in both spring and autumn; the only difference was that the carabid beetles were active in a much narrower time range in autumn. We recorded a significant decline in activity in the autumn night and early morning hours. Similarly, Dondale et al. (1972) recorded the lowest number of collected specimens in the field in the period between 04:00 and 06:00. At this time, the temperature of the soil surface is the lowest, and according to Thiele (1977), temperature with light have the greatest influence on activity of carabids preferring open habitats. Preiszner and Karsai (1990), who conducted their research on sandy grasslands, reached this same conclusion. In our study, most of species (66%) showing a significant response to temperature were those living in open habitats.

Spring breeders in the forest environment were the most active at dusk and in the early night hours, while at the clear-cut their activity had a significant diurnal character. Novák (1980) recorded a predominance of activity in the daylight phase both in the forest and in the open habitats for spring breeders; but he also noted that the behavior of spring breeders is markedly influenced by microclimate. The evening and night peak of activity in the forest samples was connected with the presence of dominant *A. parallelus*, which is typically a moisture-preferring species (Thiele, 1964), and is therefore connected with nocturnal activity. On the other hand, we found predominantly diurnal activity in clear-cut carabids, which is in agreement with Kegel (1990), who studied the activity of carabid beetles in agrocenoses.

Autumn breeders showed a significant increase in activity in the night-time hours in comparison to spring breeders. In the forest environment nocturnal activity predominated; in the clear-cut it was accompanied by activity in the afternoon hours. A predominance of nocturnal activity in autumn breeders was confirmed also by Kegel (1990) and Novák (1979, 1980), who recorded a peak of activity in the first two hours of night, which is in agreement with our results.

P. melanarius and *A. parallelipipedus* show a preference for higher temperatures in the autumn than in the spring. This fact seems to be surprising with respect to the annual course of temperatures (autumn is substantially cooler). A preference toward higher temperatures in the autumn period is apparently related to the biology of reproduction of these two species – both are autumn breeders, which means that their sexual glands mature in autumn. The influence of temperature on egg size and the speed of development was studied in *Notiophilus biguttatus* (*Ernsting and Isaaks,* 1997), *Amara* spp. and *Brachynus* spp. (Saska and Honek, 2003a, 2003b). Higher temperatures accelerated the maturation of the sexual glands; this could provide an answer as to why the activity of the two otherwise nocturnal species mentioned above shifts to a diurnal pattern in autumn. They were probably actively searching for places with higher temperatures.

Discussion on selected species

Thiele (1977) recorded *A. parallelipipedus* to be a eurytopic forest species without a pronounced relationship to temperature, but naturally preferring lower light intensities. Novák (1980) describes the peak of activity of this species in early night hours. In agreement with this, the activity of *A. parallelipipedus* was limited mainly by the daytime period (50% of specimens were collected in the interval 21:00 to 00:00), while in the autumn extremely low nighttime temperatures moved a limited activity to afternoon hours.

The activity of *Abax parallelus* showed the same pattern as in *A. parallelipipedus*. Thiele (1977) mentioned *Abax parallelus* as a stenotopic forest species; however, in our study *A. parallelus* was abundant in the material collected from the clear-cut; in autumn the numbers of specimens were equal at both localities.

Both species of *Poecilus* can be considered as typical representatives of open habitats, as they show a positive connection to the higher temperatures of the soil surface and to light. Thiele (1977) recorded them as euryphotic species. *P. cupreus* also showed noticeable diurnal activity (Novák, 1979, 1980), both in the forest and in open habitats. Our GAM showed that this species preferred the lower temperatures in the autumn than in the spring simply because the temperatures in autumn did not reach the level of the spring temperatures. *P. cupreus* in fact preferred the highest temperatures reached in both periods.

Pseudoophonus rufipes is regarded as a thermophile and xerophile field species with nocturnal activity (Thiele, 1977). According to Kegel (1990), this species in fields showed significantly higher activity during warm nights. While nocturnal activity dominated in our study, the number of specimens collected in the light phase was also relatively high (cca. 33%). GAM analysis showed a significant dependence of epigeic activity of *P. rufipes* on the daytime, while dependence on temperature was not confirmed, despite the fact that its activity was divided more or less evenly throughout the entire day, with a peak in the evening and early nighttime hours.

P. melanarius is regarded as a eurytopic and eurythermal field species preferring low light intensity (Thiele, 1977). In our research, its activity was significantly dependent on the temperature of the soil surface and it was active during periods with high temperatures. Alderweireldt and Desender (1990) also reported the diurnal activity of this species in the field with a substantial peak in the evening and early night hours. They assumed that declining light intensity increased its activity. This pattern would correspond to our results from the forest, while in the clear-cut the activity of this species was clearly diurnal. Novák (1979, 1980) recorded an opposite pattern of activity for this species – diurnal activity in the forest and nocturnal in the open habitats.

Bembidion lampros is characteristic species of open habitats with a striking preference for diurnal activity in both field (Alderweireldt and Desender, 1990, Novák, 1979) and forest environments (Novák, 1980). In our research, this species was also significantly active during the afternoon and positively correlated with temperature. Thiele (1977) and Novák (1980) reported the species as a eurytopic forest one with a strong ecological bond to temperature and light; however, Loreau (1986) regarded this species as substantially nocturnal. This species dominated in the autumn and in the forest environment where its activity was significantly diurnal (15:00 – 21:00) and was also influenced by temperature.

CONCLUSIONS

A total of 924 specimens representing 35 species of carabid beetles were collected. The carabid beetles were significantly more active in the clear-cut than in the forest and in the spring compared to autumn.

In the forest, nocturnal activity predominated, while in the clear-cut, activity had a significant diurnal character. This is contrary to Park's hypothesis on the development of populations toward symmetry of activity, which would predict symmetric activity in the forest environment representing the climax stage in the study. The time distribution of the epigeic activity of the entire beetle population better corresponds to Park's hypothesis.

In spring, activity was spread almost evenly throughout the day, while in autumn we recorded a significant afternoon peak of activity caused by low temperatures in the nighttime and early morning hours.

The daytime factor had a significant influence on 16 of the 35 species and the temperature of the soil surface significantly influenced 13 species.

CCA analysis showed a substantial increase in the explanatory potential of the factors of daytime and temperature for the activity of carabid beetles in the autumn compared to spring. CCA analysis also showed the substantial response of the activity of carabid beetles in the clear-cut to the soil surface temperature.

The activity of the forest species during the year shifted from the evening and night hours in spring to the afternoon hours in autumn, especially in the last weeks of the research when temperatures at night and in the early morning hours dropped below zero in the clear-cut. The activity of the species in the clear-cut maintained the same significantly diurnal pattern during the whole year.

The activity of spring breeders in the forest environment reached a peak at dusk, while their activity in the clear-cut was diurnal. Autumn breeders in both environments were mostly night-active.

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Příloha 10

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Diurnal epigeic activity of myriapods (Chilopoda, Diplopoda)

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Tuf, I.H., Tufová, J., Jeřábková, E. & Dedek, P. 2006. Diurnal epigeic activity of myriapods (Chilopoda, Diplopoda). Norw. J. Entomol. 53, 335-344.

Diurnal epigeic activities of myriapods in a floodplain forest and a neighbouring deforested area, was studied in late spring and early in autumn of 2004, by pitfall trapping. One hundred traps were checked every three hours. In total, 7 species of centipedes and 11 species of millipedes were trapped. Only *Lithobius mutabilis, Glomeris connexa, Unciger foetidus,* and *Unciger transsilvanicus* were dominant. The whole millipede community and all its dominant species, and the centipede *Lithobius forficatus* showed significant patterns of diurnal activity. The millipedes, both the whole community and *U. transsilvanicus* and *Polyzonium germanicum*, were significantly affected in their activity by temperature of soil surface too.

Key-words: Chilopoda, Diplopoda, diurnal activity, diel activity, circadian rhythm, floodplain forest

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INTRODUCTION

Soil is inhabited by a huge number of invertebrate species, the so called soil fauna. For some of them, the soil represents a refuge. Out of the soil the environment is less suitable; there is too much light, and the humidity and temperature are more instable. For these reasons many species of soil fauna show specific pattern of epigeic activity – they are more active during periods with more favourable conditions, i.e. during spring and autumn in annual rhythm and/or during night in diurnal rhythm.

Circadian rhythms are widespread in the animal kingdom. Rhythms of epigeic activity is not only shown by surface animals, reacting to changes in light conditions, but also by troglobiotic species from constant dark environment (Koilraj et al. 2000). Generally, myriapods show mainly epigeic activity at night with various exceptions (Lewis 1981, Hopkin & Read 1992), for example dusk (dawn & twilight) activity (Bano & Krishnamoorthy 1979). This rhythm is controlled by internal physiological factors (timers), but it can differ in length in relation to environmental conditions. For instance, the centipede *Scolopendra* sp. with strictly night activity (in natural light regime) rapidly lost this rhythm in LL (constant light) conditions (Cloudsley-Thompson & Crawford 1970). In another experiment, with varying DL (dark-light) periods, it was shown that the length of the activity cycle (from peak to peak) is correlated to the length of the light period. This cycle was shorter in DD conditions (24 hours of dark), than in 12:12 DL or LL conditions for the millipede *Syngalobolus* sp. (Koilraj et al. 1999). In comparison, millipedes showed less activity during the light periods than centipedes (Dondale et al. 1972).

Beside light stimulus, other environmental conditions like temperature or humidity were tested for their influence on diurnal rhythms of myriapods. For millipedes, Cloudsley-Thompson (1951) described the decrease of temperature

Table 1. Diurnal epigeic activity of myriapods during late spring (May-June). Catches of individuals by 60 traps during 18 days in individual time-parts of day (3:00 means that animals were catched from 0:00 to 3:00), greyed columns mark dark period.

	forest								**clear-cut**							
	3:00	**6:00**	**9:00**	**12:00**	**15:00**	**18:00**	**21:00**	**24:00**	**3:00**	**6:00**	**9:00**	**12:00**	**15:00**	**18:00**	**21:00**	**24:00**
Lithobius agilis L.Koch, 1847	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	2
Lithobius curtipes C.L.Koch, 1847	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lithobius forficatus Linnaeus, 1758	2	1	2	-	1	-	-	2	17	8	-	5	-	-	2	14
Lithobius microps Meinert, 1868	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lithobius mutabilis L.Koch, 1862	14	23	8	9	4	4	3	18	35	23	21	15	5	12	17	47
Strigamia acuminata (Leach, 1814)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Strigamia transsilvanica (Verhoeff, 1928)	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chilopoda	**17**	**25**	**10**	**9**	**5**	**4**	**3**	**20**	**53**	**30**	**21**	**20**	**5**	**12**	**18**	**62**
Glomeris connexa C.L.Koch, 1847	11	3	-	2	1	-	3	19	45	20	5	3	2	-	21	54
Brachydesmus superus Latzel, 1884	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-
Polydesmus denticulatus C.L.Koch, 1847	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Haplogona oculodistincta (Verhoeff, 1893)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Melogona voigti (Verhoeff, 1899)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ochogona caroli (Rothenbuehler, 1900)	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-
Enantiulus nanus (Latzel, 1884)	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Leptoiulus proximus (Němec, 1896)	4	3	1	2	-	1	-	9	3	-	2	2	-	-	2	-
Unciger foetidus (C.L.Koch, 1838)	2	3	7	-	1	1	1	4	3	3	3	2	2	-	2	6
Unciger transsilvanicus (Verhoeff, 1899)	2	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Polyzonium germanicum Brandt, 1831	1	2	2	3	-	2	2	2	-	-	-	-	-	-	-	-
Diplopoda	**20**	**13**	**10**	**7**	**2**	**4**	**6**	**36**	**53**	**24**	**9**	**6**	**5**	**-**	**24**	**60**

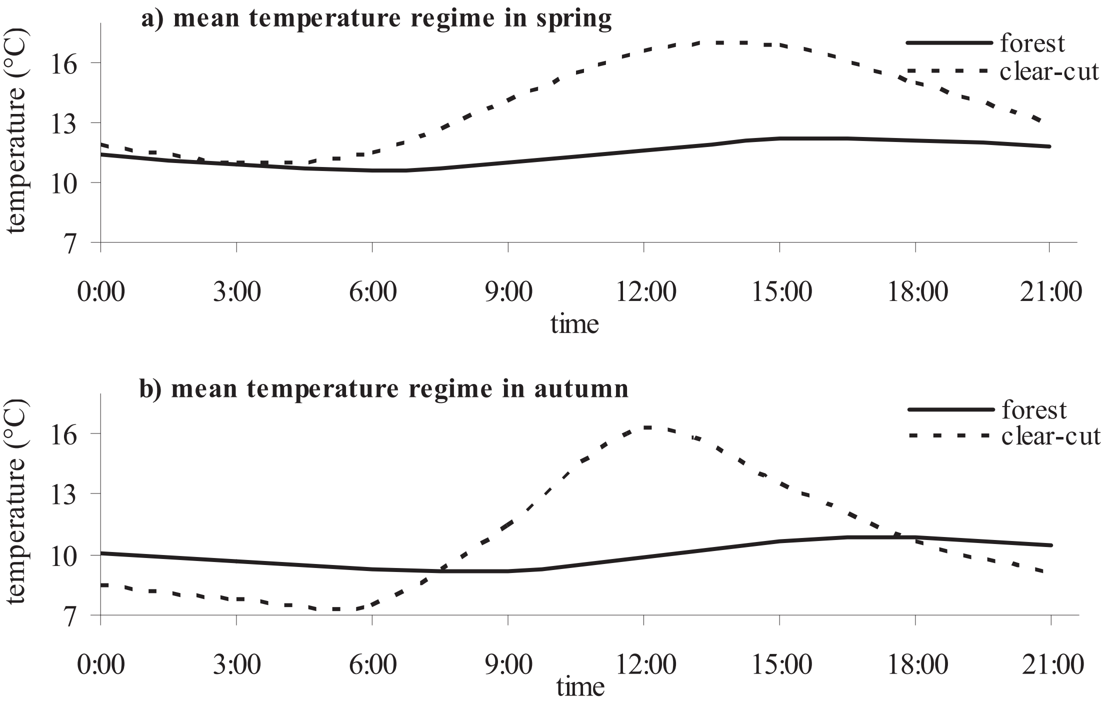

Figure 1. Mean day-temperature regime on the localities during the study period: a) in late spring (May-June 2005), b) in early autumn (September-October 2005).

in night as the starter of locomotory activity. Similarly, Banerjee (1967) found correlations (coefficient higher than 0.5) between temperature of air and activity of all studied millipedes, but the correlation of activity and humidity was relatively low.

Diurnal activity of centipedes and millipedes, as a part of an epigeic macrofauna, was studied in a floodplain forest and a nearby deforested (clear cut) area, during spring and autumn 2004.

MATERIAL AND METHODS

The study area was located in the Litovelské Pomoraví Protected Landscape Area, a natural landscape around the meandered Morava River (Central Moravia, Czech Republic) with floodplain forests and meadows. The localities under comparison were an old floodplain forest (*Querco-Ulmetum*) and a nearby deforested area (49°65´N, 17°20´E, altitude 210 m a.s.l.). The herbal layer of the floodplain forest was created by *Anemone nemorosa, Polygonatum* spp., *Lathyrus vernus* and *Maianthenum bifolium*. The dominant moss was *Eurhynchium hians*. In November 1998, the litter biomass (dry weight) was 622 g/m^2. The alluvial soil was loamy-sandy to loamy at the locality, with pH 4.8-5. The annual precipitation was around 520 mm and mean annual temperature was 9.1 °C. Part of this forest was cut in November 2002 and replanted in March 2003 with oak, elm and lime tree (ratio 8 : 1 : 1) using heavy forest machines. Before that, the remaining wood residue was chipped and scattered throughout the whole area. Epigeic invertebrates were caught using pitfall traps (plastic pots) without fixative solution. 60 traps were arranged in the forest and 40 traps in the deforested area. The traps were placed in line with a spacing of three meters.

Table 2. Diurnal epigeic activity of myriapods early in autumn (September-October). Catches of individuals by 60 traps during 18 days in individual time-parts of day (3:00 means that animals were catched from 0:00 to 3:00), greyed columns mark dark period.

	forest								**clear-cut**							
	3:00	**6:00**	**9:00**	**12:00**	**15:00**	**18:00**	**21:00**	**24:00**	**3:00**	**6:00**	**9:00**	**12:00**	**15:00**	**18:00**	**21:00**	**24:00**
Lithobius agilis L.Koch, 1847	1	1	-	-	-	-	2	-	-	-	-	1	-	-	-	-
Lithobius curtipes C.L.Koch, 1847	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Lithobius forficatus Linnaeus, 1758	3	2	1	-	-	-	3	2	6	1	1	-	-	-	2	6
Lithobius microps Meinert, 1868	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lithobius mutabilis L.Koch, 1862	9	16	15	5	3	3	20	19	6	5	6	1	-	2	6	5
Strigamia acuminata (Leach, 1814)	2	1	1	-	-	-	-	1	-	-	-	-	-	-	-	-
Strigamia transsilvanica (Verhoeff, 1928)	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Chilopoda	**14**	**20**	**17**	**5**	**3**	**3**	**27**	**21**	**11**	**6**	**7**	**2**	**-**	**2**	**8**	**10**
Glomeris connexa C.L.Koch, 1847	2	2	1	1	-	-	3	3	6	1	-	-	-	-	2	8
Brachydesmus superus Latzel, 1884	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polydesmus denticulatus C.L.Koch, 1847	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Haplogona oculodistincta (Verhoeff, 1893)	-	1	1	-	1	-	-	1	-	-	-	1	-	-	-	1
Melogona voigti (Verhoeff, 1899)	-	-	-	-	-	1	2	2	-	-	-	-	-	-	-	-
Ochogona caroli (Rothenbuehler, 1900)	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Enantiulus nanus (Latzel, 1884)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Leptoiulus proximus (Němec, 1896)	1	2	-	-	-	-	1	2	-	-	-	-	-	-	-	-
Unciger foetidus (C.L.Koch, 1838)	14	14	3	-	-	1	14	17	2	6	2	-	-	1	5	5
Unciger transsilvanicus (Verhoeff, 1899)	27	17	5	1	-	-	11	23	3	3	1	-	1	1	2	5
Polyzonium germanicum Brandt, 1831	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
Diplopoda	**44**	**35**	**10**	**2**	**1**	**2**	**32**	**48**	**11**	**10**	**3**	**3**	**1**	**2**	**9**	**18**

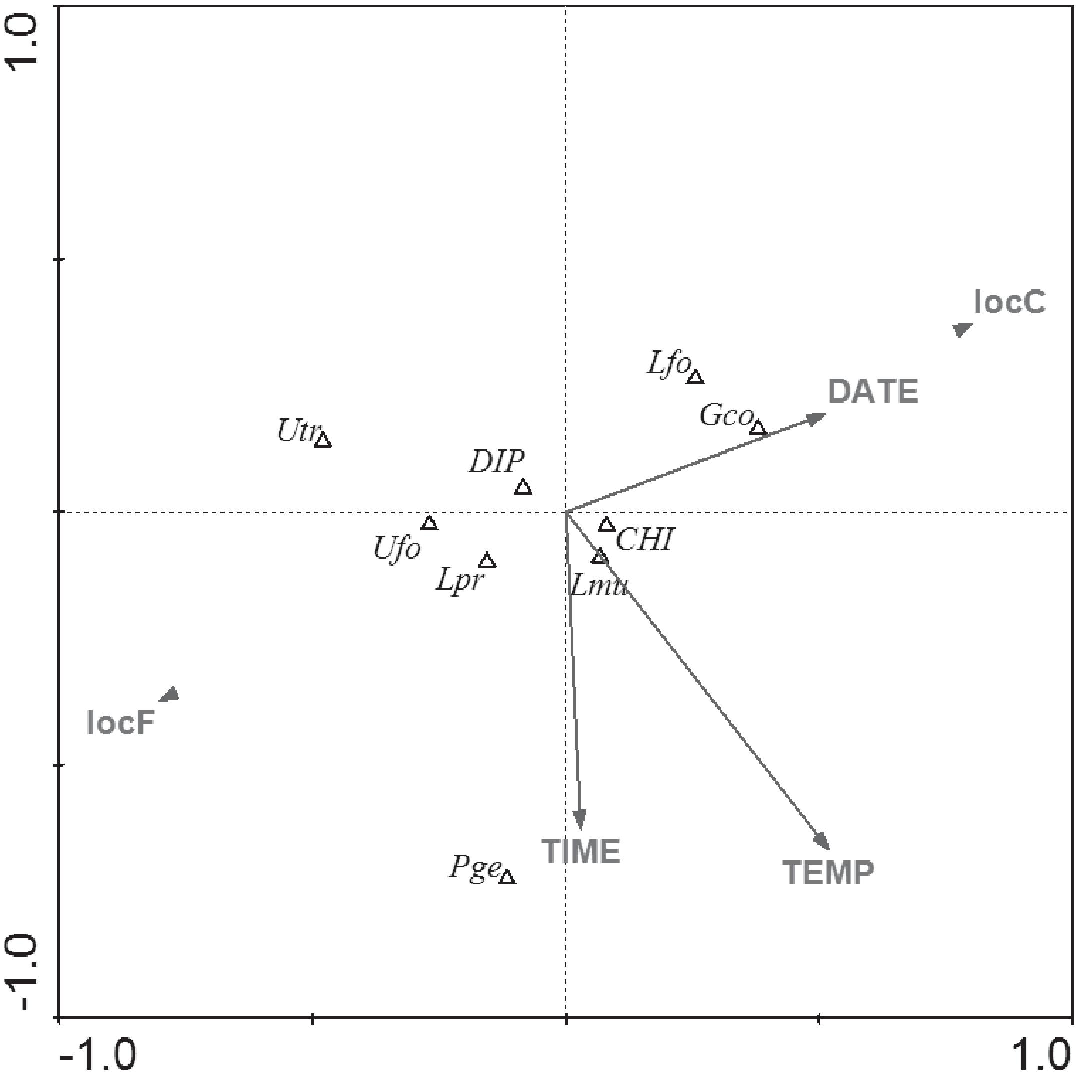

Figure 2: CCA ordination plot showing epigeic activity of dominant myriapod species (catched more than 10 specimens) in relation to locality (locF-forest, locF-clear-cut), day-time (TIME), season (DATE), and TEMPERATURE. Abbreviations: CHI – Chilopoda, Lfo – *L. forficatus*, Lmu – *L. mutabilis*, DIP – Diplopoda, Gco – *G. connexa*, Lpr – *L. proximus*, Ufo – *U. foetidus*, Utr – *U. transsilvanicus*, Pge – *P. germanicum*. (Plot: F = 2.65, p = 0.0052, number of permutation (Monte Carlo) = 5000; Conditional effects: clear-cut: F = 10.9, p = 0.000, DATE: F = 4.54, p = 0.000, TIME: F = 2.65, p = 0.005, TEMPERATURE: F = 5.35, p = 0.000).

The investigation was carried out in late spring (20 May – 7 June, 18 days) and early autumn (23 September – 18 November, 25 days) of 2004. Traps were checked every three hours (i.e. at 3, 6, 9, 12, 15, 18, 21 and 24 hours). The temperature of the soil surface was measured at collecting hours at both localities by the use of data-loggers, Minikin TH. A canonical correspondence analysis and generalised additive models for evaluating the results were created in the programme CANOCO for Windows 4.5© (ter Braak & Šmilauer 1998). Graphs were created in CanoDraw for Windows 4.0©.

The soil macrofauna of isopods, spiders, harvestmen, centipedes, millipedes, bugs and ground beetles was sorted out. Here only the myriapods are treated.

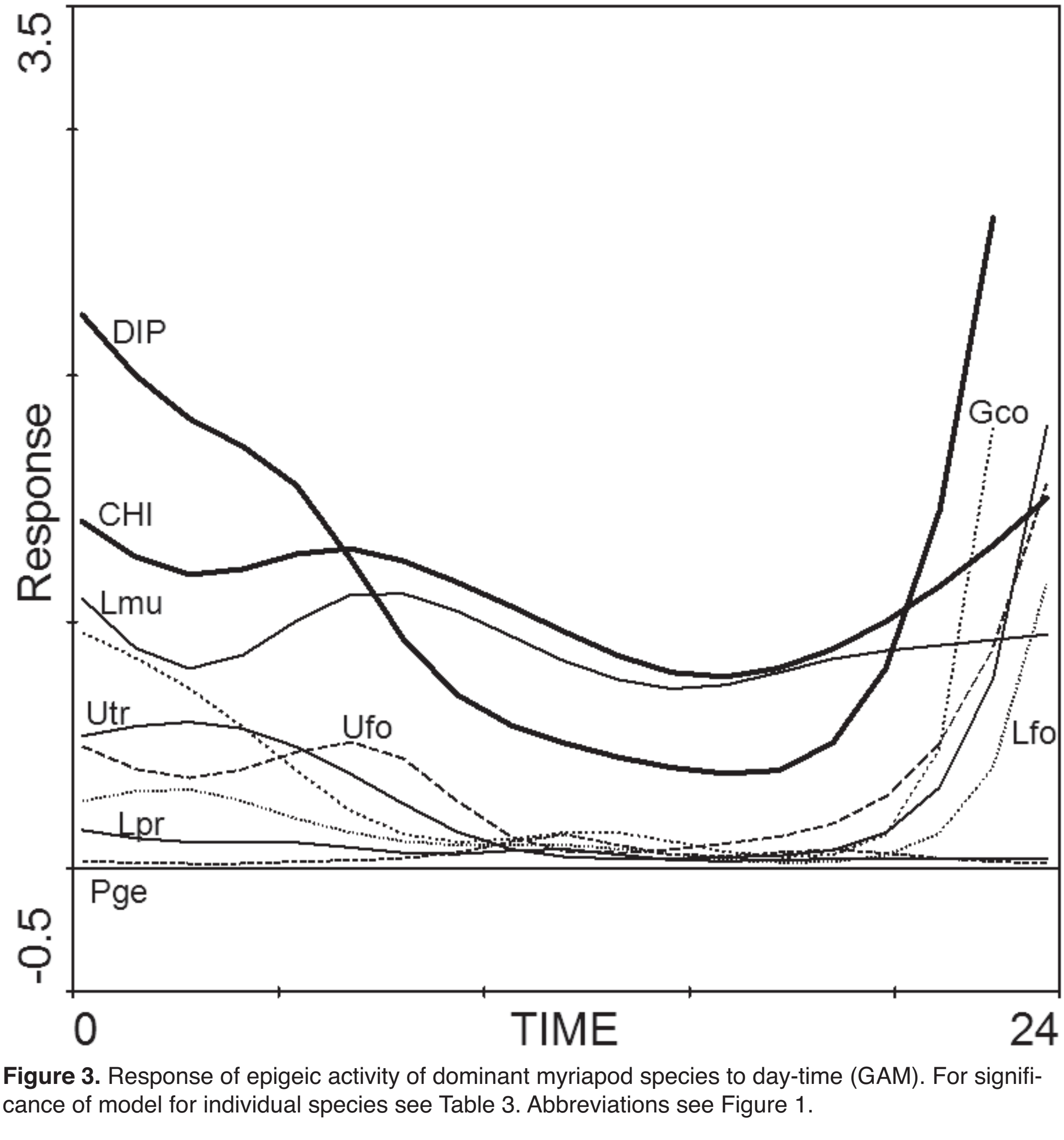

Figure 3. Response of epigeic activity of dominant myriapod species to day-time (GAM). For significance of model for individual species see Table 3. Abbreviations see Figure 1.

RESULTS

Almost 12.000 specimens of the epigeic macrofauna were trapped. Myriapoda represented 8 % of the total catch. Seven species of centipedes (Chilopoda) and 11 species of millipedes (Diplopoda) were recorded totally. Dominant species were the centipede *Lithobius mutabilis,* and the millipedes *Glomeris connexa, Unciger foetidus,* and *Unciger transsilvanicus*.

Although the epigeic macrofauna as a total did not show differences between night and day time, the highest epigeic activity of the myriapods was during night, from 18 to 6 o'clock approximately. This pattern of diurnal activity was the same for centipedes as well as millipedes, for the forest as well as the clear-cut area and for spring as well as autumn (compare Tables 1 and 2). Almost all species showed this pattern except *Polyzonium germanicum*, which was active during the whole day without any evident peak in activity (Table 1). In spring, *Leptoiulus proximus* and *U. foetidus* showed tendency to be active during the light-period too.

Beside this main pattern, there were observed differences between localities and seasons. Higher epigeic activity was evident during spring on the clear-cut area, while higher epigeic activity was showed during autumn in the forest. This difference was probably due to different temperature regime. During late spring, the temperature was higher in the clear-cut area than in the forest during whole day. On the other hand the nights were warmer in forest in the autumn (Figure 1). This aroused high epigeic activity of *L. mutabilis, Lithobius forficatus,* and *G. connexa* in the clear-cut area during spring. In the Canonical Correspond Analysis, all tested factors (locality, day-time, date and temperature) were significant. A similar effect of season (DATE) and locality (clear-cut area) is evident from CCA too (Figure 2). Models for individual, dominant species showed that day-time is an important factor influencing epigeic activity of *L. forcifatus, G. connexa,* both species of the genus *Unciger*, and the entire millipede community (Figure 3, Table 3). *L. forficatus* and *U. transsilvanicus* were the most active species at 3 A.M., whereas *U. foetidus* had a peak of activity later, at 6.30 A.M. *G. connexa* and the entire millipede community were most active around midnight.

Beside this, another model showed that knowledge of temperature is a useful predicting tool for epigeic activity of *U. transsilvanicus, P. germanicum* and the entire millipede community (Figure 4, Table 4). From that model it is evident that *P. germanicum* was more active

Table 3. Significance of day-time factor to prediction of epigeic activity of dominant myriapod species (GAM).

Species/Group	Deviance	F	p
L. forficatus	75.86	2.64	**0.016819**
L. mutabilis	452.48	1.04	0.398784
Chilopoda	618.28	1.17	0.320182
G. connexa	383.22	5.89	**0.000008**
L. proximus	40.41	1.07	0.380031
U. foetidus	169.49	3.03	**0.007005**
U. transsilvanicus	224.35	3.84	**0.001097**
P. germanicum	16.78	1.82	0.095785
Diplopoda	744.03	11.68	< **1.0e-6**

in higher temperature and *U. transsilvanicus* in lower temperatures. Other species showed peak of epigeic activity somewhere between these species. This model created unimodal curves for several species. Only *L. mutabilis* and *G. connexa* had a peak around 14 °C. *U. foetidus* was the most active at temperatures of 5 °C or lower.

DISCUSSION

Almost all the studied species showed epigeic activity during night and the dusk period, several species also showed a low activity during full light (*L. mutabilis, G. connexa, Haplogona oculodistincta, U. foetidus* and *L. proximus* in late spring) and one species was active during whole day without evident peak (*P. germanicum*). The higher activity of myriapods during dark period

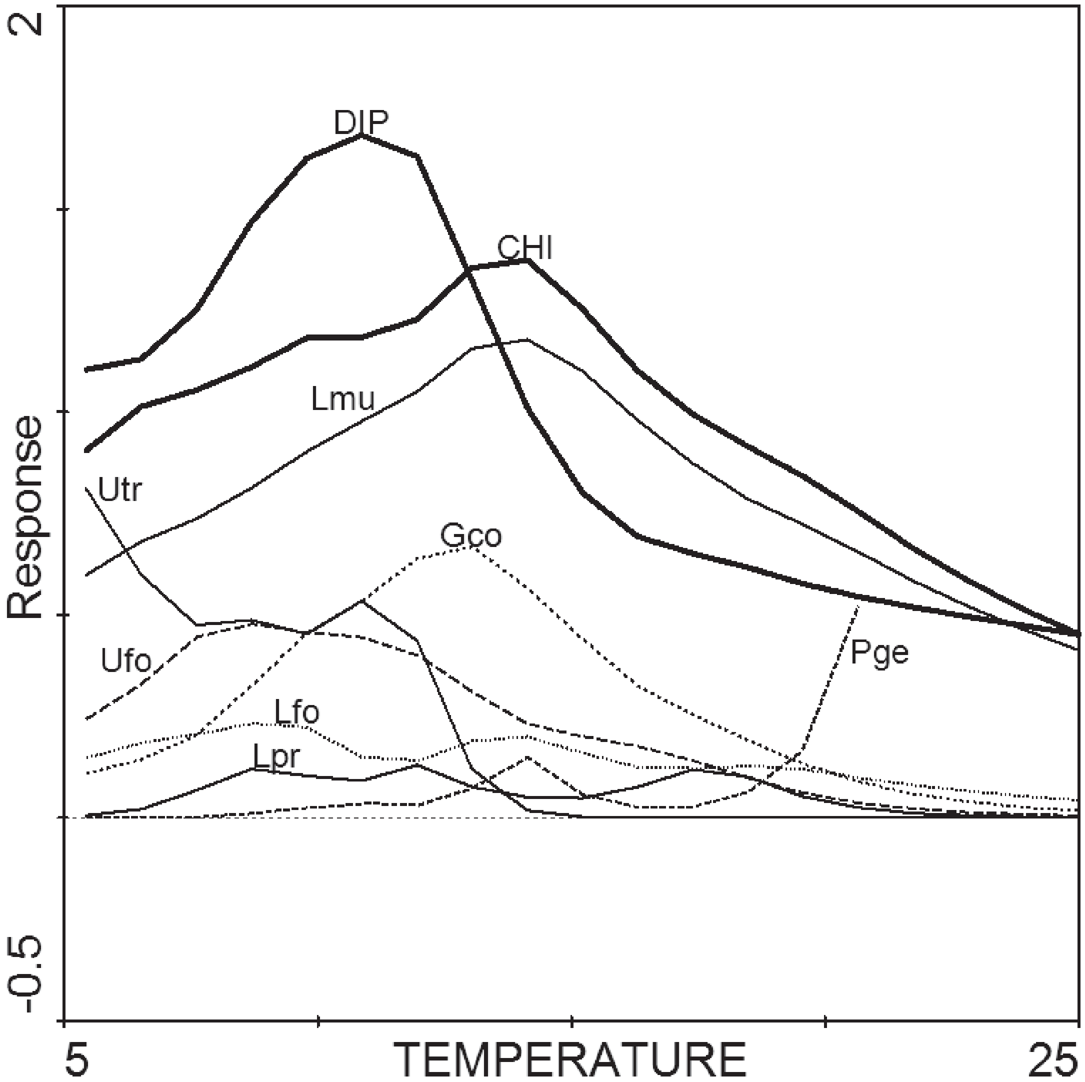

Figure 4. Response of epigeic activity of dominant myriapod species to temperature (GAM). For significance of model for individual species see Table 4. Abbreviations see Figure 1.

has been found by many researchers (Cloudsley-Thompson 1951, Banerjee 1969, Dondale et al. 1972, Koilraj et al. 1999, 2000). Dondale et al. (1972) showed that millipedes are stricter in their night activity than centipedes. Similarly, in our observation, time was a significant predictor of activity for the millipede community only. Time was a significant predictor for the three most abundant species of millipedes. As to the centipedes, the pattern of activity for *L. forficatus* was significantly affected by day-time. It wasn't possible find this relation for the most dominant species, *L. mutabilis*.

Prolongation of period of activity of some millipedes in summer has been described by several authors (Banerjee 1969, Dondale et al. 1972), and is probably caused by their higher resistance to desiccation in the summer months (Perttunen 1953). This can be the mechanism that enabled an activity of *G. connexa, H. oculodostincta, U. foetidus* and *L. proximus* in late spring 2004 with relatively warm weather. This is in accordance with our experiences from the field. We repeatedly found several specimens of *Glomeris* on paths or similar exposed surfaces in the direct sunlight on hot summer days. We also found a significant correlation between temperature and epigeic activity of *U. transsilvanicus, P. germanicum* and the total millipede community. Both these species of millipedes had unimodal curve of dependence of temperature and activity with a peak on the edge of the temperature span. *U. transsilvanicus* seems to be more active at lower temperatures whereas *P. germanicum* shows activities at higher temperatures (Figure 4). Although Banerjee (1967) found a relatively good correlation between temperature and activity in millipedes, this can be caused by differences in development. *P. germanicum* is spring active (from end of March), with egg laying during May-June (Lokschina 1969). *U. transsilvanicus*, has its main peak of epigeic activity in September, and a smaller one in April (Ožanová 2001). Since our project only covered parts of the year the patterns of activity for these species here shown, can be biased.

Generally, the millipedes showed a higher affinity to the dark phase of the day than did centipedes. This is in accordance with previous researches in this field. For some species, temperature can influence epigeic activity too.

Acknowledgement. This observation was done with permission of Authority of Litovelské Pomoraví Protected Landscape Area. We are very grateful to colleagues, friends and students from our department for their help in field; without their help it could not have been possible for us to check traps every three hours for such a long

Table 4. Significance of temperature factor to prediction of epigeic activity of dominant myriapod species (GAM).

Species/Group	Deviance	F	p
L. forficatus	78.13	0.834	0.457669
L. mutabilis	450.80	1.26	0.273524
Chilopoda	622.46	0.766	0.404730
G. connexa	410.38	1.56	0.157943
L. proximus	40.58	0.820	0.446732
U. foetidus	173.79	1.49	0.183428
U. transsilvanicus	222.55	4.34	**0.000330**
P. germanicum	16.12	4.31	**0.000360**
Diplopoda	854.30	2.46	**0.024764**

time. We would also like to acknowledge our colleagues Adam Véle and Emil Tkadlec for their help with the statistical analyses.

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Diurnal epigeic activity of terrestrial isopods (Isopoda: Oniscidea)

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Abstract

Diurnal epigeic activity of woodlice in a floodplain forest and nearby clear-cut areas was studied during late spring and early autumn of 2005 by pitfall trapping technique. A total of 100 traps was being checked every three hours. In total, 6 species of isopods were trapped, but only *Trachelipus rathkii* and *Protracheoniscus politus* were found being dominant. The species *P. politus* and *Porcellium conspersum* showed specific significant patterns of their epigeic activity depending on day-time.

Keywords – circadian rhythm, diel activity, floodplain forest, woodlice

Introduction

Soil is inhabited by a huge number of invertebrate species. For these species, the soil fauna, the soil represents a refugium. Out of the soil, the environment is less suitable for them: there is too much light, and humidity and temperature are rather unstable. For these reasons, many species of the soil fauna show specific patterns of epigeic activity – they are more active during periods of time with more favourable conditions, i.e. during spring and autumn in an annual rhythm and/or during night in a diurnal rhythm.

Circadian rhythms of epigeic activity have been recognised not only for surface-dwelling animals that adjust their activity to the prevailing light conditions, but also for troglobiotic species that live in a constantly dark environment (Koilraj *et al.*, 2000). Generally, woodlice mainly show epigeic activity during the night (Cloudsley-Thompson, 1959) with various exceptions, for example twilight activity (Ammar & Morgan, 2005). According to Cloudsley-Thompson (1952), the diurnal epigeic activity is primarily correlated with light-dark changes, but not with other conditions (humidity, temperature) that may change over the course of the day, although there are intrinsic physiological mechanisms (a kind of timer) for the maintenance of a rhythm in permanent darkness, too (Cloudsley-Thompson, 1956a). This intrinsic timer is apparent in behaviour of *Hemilepistus reaumurii* (Milne-Edwards 1840), but not of *Oniscus asellus* Linné 1758 (Cloudsley-Thompson, 1952). An endogenous timer was found to control activity in *Armadillidium vulgare* (Latreille 1804) (Smith & Larimer, 1979), and its development during ontogenesis was described for *Tylos granuliferus* Budde-Lund 1885 (Ondö, 1954).

Besides light, other environmental stimuli have been investigated for their ability to control diurnal rhythms of woodlice. The most important of them for starting locomotory activity are humidity (Paris, 1963; Ilosvay, 1982) and temperature (Cole, 1946). Air temperature has been recognised as important for epigeic activity of myriapods, too (Banerjee, 1967; Tuf *et al.*, 2006). Probably the most interesting trigger for activity has been studied by Moore (1983), who found that *A. vulgare* infected by an acanthocephalan parasite is more active, runs farther and rests less time than non-infected ones.

Diurnal epigeic activity of terrestrial isopods, an ecologically significant element of the epigeic macrofauna, was studied in a floodplain forest and nearby clear-cut areas during spring and autumn.

Material and Methods

The locality is situated in the Litovelské Pomoraví Protected Landscape Area, a natural landscape surrounding the meandering Morava river (Central Moravia, Czech Republic) by a

Table 1. Diurnal epigeic activity of terrestrial isopods during late spring (May-June) and early autumn (September-October). Catches of individuals by 60 traps during 18 days at different times of the day (3:00 means that animals were caught from 0:00 to 3:00), grey columns mark night time.

Spring	Forest							
	3:00	6:00	9:00	12:00	15:00	18:00	21:00	24:00
Ligidium hypnorum (Cuvier, 1792)	-	-	-	-	-	-	-	1
Hyloniscus riparius (C.L. Koch, 1838)	1	-	1	1	-	-	-	-
Trichoniscus pusillus Brandt, 1833	-	-	-	-	-	-	-	-
Porcellium conspersum (C.L. Koch, 1841)	-	-	8	7	7	2	1	1
Protracheoniscus politus (C.L. Koch, 1841)	7	14	14	8	1	-	3	5
Trachelipus rathkii (Brandt, 1833)	1	1	1	-	-	-	-	1

Autumn	Forest							
	3:00	6:00	9:00	12:00	15:00	18:00	21:00	24:00
Ligidium hypnorum (Cuvier, 1792)	0.7	1.4	1.4	0.7	-	0.7	-	2.2
Hyloniscus riparius (C.L. Koch, 1838)	-	-	-	-	-	-	-	-
Trichoniscus pusillus Brandt, 1833	-	-	-	-	-	-	-	-
Porcellium conspersum (C.L. Koch, 1841)	-	-	-	-	-	-	-	-
Protracheoniscus politus (C.L. Koch, 1841)	1.4	1.4	2.2	-	-	-	3.6	0.7
Trachelipus rathkii (Brandt, 1833)	7.9	7.9	5.8	2.2	2.9	0.7	8.6	9.4

Spring	Clear-cut							
	3:00	6:00	9:00	12:00	15:00	18:00	21:00	24:00
Ligidium hypnorum (Cuvier, 1792)	7.5	1.5	3	-	-	-	3	3
Hyloniscus riparius (C.L. Koch, 1838)	-	3	-	-	-	-	-	-
Trichoniscus pusillus Brandt, 1833	3	-	-	-	-	-	-	-
Porcellium conspersum (C.L. Koch, 1841)	-	1.5	-	-	-	-	-	-
Protracheoniscus politus (C.L. Koch, 1841)	-	-	1.5	-	-	-	-	-
Trachelipus rathkii (Brandt, 1833)	4.5	4.5	3	-	-	1.5	-	4.5

Autumn	Clear-cut							
	3:00	6:00	9:00	12:00	15:00	18:00	21:00	24:00
Ligidium hypnorum (Cuvier, 1792)	1.1	-	1.1	-	-	-	-	-
Hyloniscus riparius (C.L. Koch, 1838)	-	-	-	-	-	-	-	-
Trichoniscus pusillus Brandt, 1833	-	1.1	-	-	-	-	-	-
Porcellium conspersum (C.L. Koch, 1841)	-	-	-	-	-	-	-	-
Protracheoniscus politus (C.L. Koch, 1841)	-	-	-	-	-	-	-	-
Trachelipus rathkii (Brandt, 1833)	2.2	4.3	6.5	4.3	3.2	-	3.2	4.3

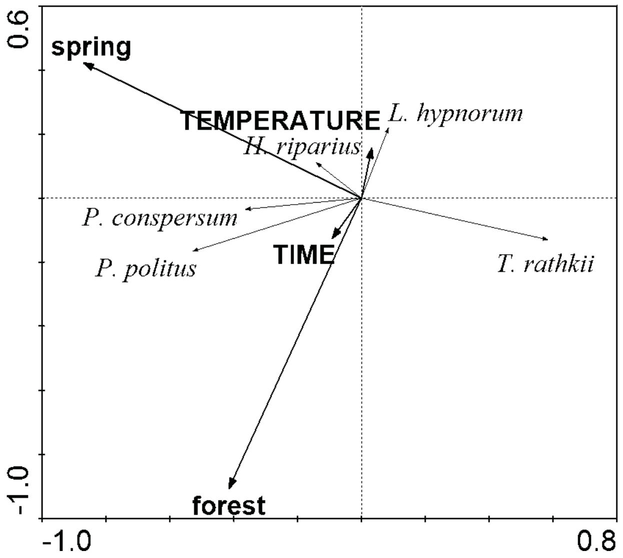

Figure 1. RDA ordination plot, showing epigeic activity of woodlice species in relation to habitat (*forest vs. clear-cut*), time of the day (*time*), season (*spring vs. autumn*), and *temperature*. For description of model and significance of environmental variables see Tabs 2 and 3.

Table 2. Summary of the explained variability of RDA plot

Axis	**1**	**2**	**3**	**4**
Eigenvalues	0.242	0.020	0.009	0.000
Species–environment correlations	0.692	0.302	0.220	0.080
Cumulative percentage variance of species data	24.2	26.2	27.1	27.1
Cumulative percentage variance of species–environment relation	89.2	96.7	99.9	100.0

Sixty traps were spread over the forest habitat, whereas 40 traps were placed in a clear-cut area in transect lines with three meters distance spacing. The experiment was carried out in late spring (May 20th to June 7th, 18 days) and early autumn (September 23rd to October 18th, 25 days) 2004. The traps were checked every three hours during these seasons (i.e. at 3.00, 6.00, 9.00, 12.00, 15.00, 18.00, 21.00, and 24.00). Soil surface temperature was measured in both localities using data-loggers Minikin TH (Environmental Measuring Systems Brno, www.emsbrno.cz). Redundancy Analysis and Generalised Additive Models for the evaluation of results were created using the software CANOCO for Windows 4.5© (ter Braak & Šmilauer, 1998), the illustrations were created in CanoDraw for Windows 4.0©. The model was evaluated using Monte-Carlo permutation tests (4999 permutations).

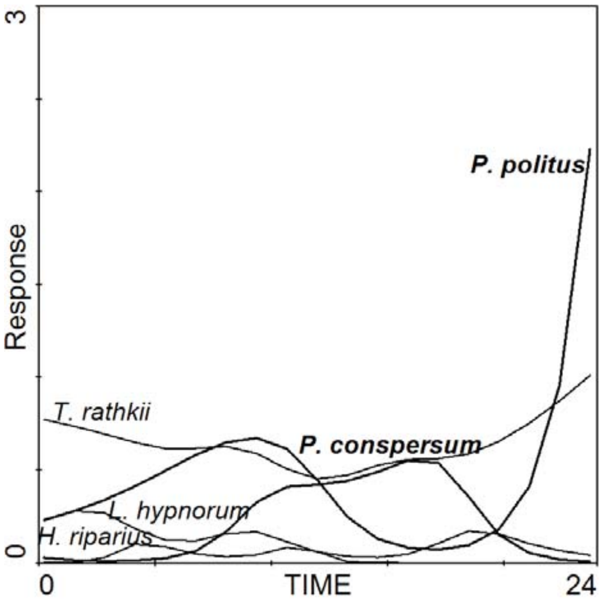

Figure 2. Epigeic activity (Response) of terrestrial isopod species in relation to time of the day. For significance of model for individual species see Tab. 4.

Results

In total, 231 individuals of six species of terrestrial isopods were trapped. Dominant species were *Trachelipus rathkii* and *Protracheoniscus politus*.

Terrestrial isopods were mostly active throughout the day at both seasons and localities. However, in the clear-cut area during spring, woodlice were not active from 9.00 to 15.00 (Tab. 1). Individual species showed this pattern of behaviour generally too, although there were several exceptions. *Ligidium hypnorum* and *T. rathkii* were active during night and twilight in spring, *P. politus* was active during twilight mainly, and *Porcellium conspersum* was active mainly during the day in spring. A comparison of activity at different sites shows that *P. politus* and *P. conspersum* were active mainly in the forest and *L. hypnorum* and *Trichoniscus pusillus* were active mainly in the clear-cut area. During spring, higher numbers of isopods were caught than in autumn (131 vs. 93 ind. caught into 60 traps during 18 days), and this pattern was most evident in the catches of *P. conspersum* that was not found in autumn. The dominant species, *T. rathkii*, was more active in autumn.

RDA (Fig. 1, Tab. 2) was significant (first axis F = 50.457, p = 0.0002; all axes F = 14.705, p = 0.0002) and explained 27 % of species data variability. The most important environmental variables were the nominal variables season (*spring*) and locality (*forest*). *Time* was significant but less important, and *temperature* was not significant, albeit only marginally not so (Tab. 3). Similar results were obtained in other analyses, when excluding the variables *temperature* or *time*; in the latter case, *temperature* proved significant.

Table 4. Significance of time of the day for epigeic activity of terrestrial isopod species.

species	Deviance	F	P	AIC
L. hypnorum	91.32	1.35	0.239966	105.165
H. riparius	29.63	1.85	0.110987	36.470
P. conspersum	91.04	5.00	0.000131	106.328
P. politus	145.24	2.72	0.017544	158.257
T. rathkii	159.53	0.532	0.221699	172.308

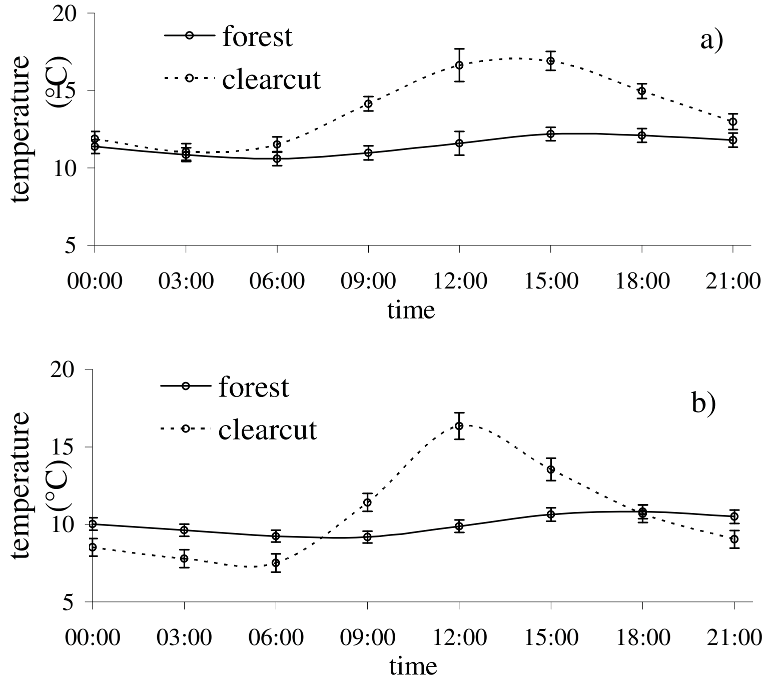

Figure 3. Mean temperature regime during the study period: a) late spring (May-June 2005), b) early autumn (September-October 2005) (mean ± SE).

As the models for individual species (*T. pusillus* was excluded for low number of individuals) shows, time of the day is an important factor to influence epigeic activity of *P. politus* and *P. conspersum* (Fig. 2, Tab. 4). *P. politus* was most active before dusk and after dawn, whereas, oppositely, *P. conspersum* was most active during the day. The activity of *P. politus* was highest in the forest in spring; from 6.00 to 12.00, the temperature was slowly increasing (Fig. 3). The major activity of *P. conspersum* was measured in the forest in spring from 10.00 to 17.00, i.e., during increasing temperature, too.

Table 3. Significance of environmental variables in RDA model to prediction of activity of woodlice

Variable	Var.N	LambdaA	P	F
spring	3	0.19	0.000	37.02
forest	4	0.06	0.000	13.10
time	2	0.01	0.049	2.48
temperature	1	0.01	0.053	2.44

We observed –albeit non-significant– differences between sexes for *T. rathkii* and *P. politus*. Thus, females of *P. politus* were active at a mean temperature 11.2 ± 2.0 °C, whereas males were active at a mean temperature of 10.5 ± 2.2 °C; *T. rathkii* females at 10.8 ± 2.3 °C and males at 10.4 ± 3.0 °C. A bigger difference (non-significant though) was evident between the activities of gravid and non-gravid females of *P. politus* (gravid at 11.9 ± 2.1 °C vs non-gravid at 10.8 ± 1.9 °C).

Discussion

Terrestrial isopods lose water by transpiration through their entire integument (Gunn, 1937), and their responses to humidity (hygrokinesis) are controlled by the intensity of dessication (Waloff, 1941). To avoiding dessication, they stay in damp and humid environment under stones, fallen leaves, logs or bark or in crevices in soil during daytime. They leave these habitats during night when temperature falls and relative humidity increases (Cloudsley-Thompson, 1959). The most desiccating species are the most photonegative ones, with strict nocturnal activity. Thus, among e investigated species were likely of *Philoscia muscorum* (Scopoli 1763), *O. asellus*, *Porcellio scaber* Latreille 1804 and *A. vulgare*, *P. muscorum* is the most nocturnal one and *A. vulgare* the least (Cloudsley-Thompson, 1956b).

The typical duration of activity is about one hour for *P. scaber* (den Boer, 1961). In shelter, woodlice absorb water from air humidity through their cuticle, that is lost again after leaving shelter. Within about one hour, they lose a critical amount of water (Waloff, 1941; Edney, 1951) and they must hide in shelter with higher relative air humidity again.

The majority of the recorded variability in activity of woodlice was explained by habitat type (forest *vs* clear-cutt) and season (spring *vs* autumn). An influence of these factors is not surprising, differences between densities of woodlice in different biotopes are well known (e.g. Farkas *et al.*, 1999), and their changes in abundances and activity during year are well known, too (e.g. Hornung & Warburg, 1995; Zimmer & Brauckmann, 1997). Nevertheless, beside these factors, time of the day is significant, too. Thus, *P. politus* was mainly active in twilights and in the morning. The activity of this has also been studied in Hungarian beech forests in summer (Ilosvay, 1982). Although several specimens were caught during daytime, the peaks of activity were at dusk and at midnight at a humidity of 75-80 %. Based on big catches in forests during winter, Ilosvay (1982) supposed that temperature is not an important factor for their activity. According to our results, too, time of the day was more important than temperature for the activity pattern of this species. Its activity before dusk and in the morning could be related to higher air humidity on the soil surface due to dew, as its activity started with increasing of temperature. *Porcellio scaber* was active after dusk and before dawn, i.e. at times of highest relative air humidity (lowest saturation deficit respectively), too (den Boer, 1961).

The other species with a significant diurnal pattern of activity was *P. conspersum* with strict daylight activity. According to the argumentation that *A. vulgare* is more resistant to desiccation than other species (Edney, 1951) and that it is active in the morning hours (Cloudsley-Thompson, 1951), we conclude that *P. conspersum* is most resistant to dessication of the species found. This species shows a primitive level of volvation. Its activity occurred at temperatures of ca 15 °C, recorded during the warmest spring days in forest, similar to midday wandering of *H. reaumurii* during winter months, when temperature and humidity are suitable at noon (Ammar & Morgan, 2005). Similarly, a positive correlation between temperature and horizontal activity was found for *P. scaber* (den Boer, 1961).

Temperature and locomotory activity relation can be a clue for an explanation of lower catches in autumn than in spring. Paris (1965) studied locomotory activity of *A. vulgare* in summer and winter in California. He found that woodlice were more active in summer (they walked 13 m per 12 hours) than in winter (10 m per 6 days), probably due to differences in temperature; the lowest temperatures in both seasons in California were comparable with temperatures measured by us. Occasional prolongation of this species' activity from night to morning was caused by high humidity during nights (Paris, 1963).

Low catches of some species were probably caused by their generally low epigeic activity, inhabiting upper soil layers (family Trichoniscidae). Other species can be sedentary. For example, Brereton (1957) re-caught tagged specimen of *P. scaber* on the same tree after six months. Another interesting observation related to low numbers of isopods is the evidence for that individual woodlice are not active every night (or day, respectively), as documented by den Boer (1961) through marking of *P. scaber* specimens.

The females of dominant species were active at higher temperatures than males, and gravid females of *P. politus* were more active at the higher temperatures that those non-gravid. Although these differences were not significant, they seem to be plausible, because the developmental time of the brood is affected by temperature. Dangerfield & Hassall (1994) found female-biased sex ratios under a cryptozoic board (with higher temperature than in soil) for *A. vulgare* and *P. scaber*. The activity of gravid females of *P. politus* in our study can be a result of their tendency to search actively for sites with more favourable conditions for brood development.

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