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katedra

Status hmyzu na prahu 21. století: pokles diverzity, šíření invazních druhů a alternativní zdroj živin

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doktorská disertační práce

(soubor vědeckých prací s komentářem)

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Čestné prohlášení

Prohlašuji, že jsem disertační práci "Status hmyzu na prahu 21. století: pokles diverzity, šíření invazních druhů a alternativní zdroj živin" vypracoval samostatně pod vedením prof. Ing. Ivy Langrové, Ph.D. a Mgr. Vladimíra Vrabec. Ph.D., a s použitím odborné literatury a dalších informačních zdrojů, které jsou v práci řádně citovány a uvedeny v seznamu literatury. Jako autor uvedené disertační práce dále prohlašuji, že jsem v souvislosti s jejím vytvořením neporušil autorská práva třetích osob.

V Praze dne Podpis

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Obsah

1 Úvod

Hmyz je nejpočetnější skupinou živočichů obývající naši planetu, tvořící více než polovinu celkové biodiverzity. Odhaduje se, že na planetě Zemi žije až šest milionů druhů těchto členovců. Zástupci hmyzu se vyskytují téměř ve všech biotopech od tropů až téměř po polární kruh a mají nezastupitelnou funkci ve fungování terestrických i vodních ekosystémů. Mnoho z nich je tak spjato s životem člověka více, než by se na první pohled mohlo zdát.

Na začátku 21. století dochází v Evropě k postupné destigmatizaci hmyzu a částečnému uvědomění si jeho významu pro lidstvo. Nejčastějším tématem, které nyní rezonuje ve spojitosti s touto skupinou živočichů, je rapidní úbytek diverzity především endemického hmyzu. V návaznosti na toto téma pak dostává prostor detekce invazních druhů hmyzu, jakožto nepřátel endemické fauny a také využití hmyzu jako alternativního a dlouhodobě udržitelného zdroje živin, který by mohl odlehčit přetíženým zdrojům v živočišné výrobě a zpomalit či zastavit nenávratné ztráty cenných biotopů. Cílem této disertační práce je v širším kontextu informovat o těchto aspektech a na základě výsledků výzkumu referovat o konkrétních případech hodnotících ochranu stanovišť ohrožených druhů hmyzu na případu motýlů, dále na několika případech popsat invaze hmyzu do České republiky a vyhodnotit nutriční hodnoty vybraných druhů hmyzu běžně chovaných v České republice z hlediska použitelnosti v potravinářství či krmivářství včetně evaluace faktorů, které obsah živin mohou ovlivnit.

V posledních několika desetiletích se svět mění mnohem rychleji než dříve. Došlo k přechodu od tradičního zemědělství na zemědělství intenzivní, krajina je fragmentována infrastrukturou, suburbánní a urbánní aglomerace se rozrůstají na úkor lesů, polí a luk. Následkem všech těchto faktorů dochází k poklesu početnosti jedinců i celkové biodiverzity mnoha skupin živočichů, včetně hmyzu. Zatímco některé druhy z naší přírody již definitivně vymizely, další přežívají jen na několika izolovaných lokalitách a jiné, dříve zcela běžné, se pomalu stávají vzácnými. V rámci snahy o zvrácení tohoto fenoménu doby je nutné věnovat pozornost zachování biodiverzity. Prvním nástrojem v této problematice je ochrana stanovišť s výskytem ohrožených druhů. Na takových lokalitách je nutné provádět management přizpůsobený životním požadavkům těchto druhů, jehož cílem je vrátit přírodě její původní tvář, podpořit lokální populace a zabránit dalším nenávratným ztrátám biodiverzity.

Důležitým faktorem, který v důsledku globalizace a klimatických změn ohrožuje lokální faunu, je šíření nepůvodních druhů s negativním vlivem na nově kolonizované prostředí, tzv. invazních druhů. Vzhledem k absenci přirozených nepřátel, odolnosti vůči patogenům či vyšší ekologické plasticitě tyto druhy vytlačují druhy původní. Přítomnost těchto organismů může mít navíc kromě ekologických následků i následky ekonomické, kdy mohou škodit na zemědělských plodinách, dřevinách nebo uskladněných potravinách. V neposlední řadě mají některé z těchto druhů, vzhledem k synantropnímu či ektoparazitickému způsobu života, význam i z hlediska humánní či veterinární medicíny. Včasná detekce a eradikace těchto druhů je tak jedním z nástrojů, které jsou pro ochranu diverzity klíčové.

Hrozbou pro diverzitu je rovněž ztráta původních stanovišť, jejíž hlavním činitelem je intenzivní zemědělství, které ve snaze zajistit potravinový komfort nadměrně využívá zdrojů a krajina tak doznává nenávratných změn. Světová populace stále roste, navíc se mění i potravní preference. Proto je zajištění dostatku kvalitního zdroje proteinu při současném zachování cenných biotopů pro lidstvo výzvou do blízké budoucnosti. Hledání alternativních zdrojů živin je dalším horkým tématem začátku 21. století. Velmi často zmiňovanou alternativou, která z tohoto pohledu připadá v úvahu, je krmivářské či potravinářské zpracování hmyzu. Respektive, uvažuje se o možnosti využít některé druhy hmyzu z řádů brouků, švábů, rovnokřídlých, dvoukřídlých či motýlů, jež byly donedávna považovány především za obtížné škůdce zásob a vyznačují se velkou reprodukční schopností a nízkou potravní specifitou. Tyto vlastnosti jsou z pohledu produkce biomasy velice benefitní. Aby bylo možné tento potenciál využít, je nutná znalost nutriční hodnoty hmyzu, stejně jako znalost jevů, kterými ji lze ovlivňovat.

2 Literární přehled

2.1 Biodiverzita hmyzu

Biologická diverzita je termín, který byl pravděpodobně poprvé použit v roce 1968 (Dasmann 1968). Jeho definice byla pak během 80. let ustálena jako celkový počet druhů ve společenstvu (Norse & McManus 1980). Zkrácenou verzi „biodiverzita“ použil poprvé v roce 1988 Edward Osbourne Wilson (Wilson 1988). Od té doby se termín ustálil a společně se zvýšeným zájmem o tuto problematiku se stal na začátku 21. století pojmem všeobecně známým a frekventovaným. Dokládá to i počet vyhledávání klíčového slova „biodiverzita“ na Google, který se se v roce 2008 zvýšil během tří měsíců o 5 milionů vyhledávání z 12 na 17 milionů (Foottit & Adler 2017). Přestože podle služby Google trends po začátku století počet vyhledávání tohoto hesla lehce poklesl, v současnosti při vyhledávání klíčového slova „biodiversity“ zobrazí Google 78,9 milionu odkazů.

V roce 2018 bylo podle Catalogue of Life popsáno celkem 1,225,402 druhů hmyzu (ITIS 2019). Skutečný počet druhů obývající planetu však bude vyšší. Přestože se odhady globální biodiverzity během posledních několika desetiletí nedaří sjednotit a řádově se liší až v milionech (Caley et al. 2014), za zřejmě nejpřesnější odhad druhové diverzity eukaryotních organismů je považováno necelých 9 milionů druhů (Mora et al. 2011; Sweetlove 2011), z čehož přibližně 5,5 milionů druhů patří do třídy hmyzu (Stork 2018). Nejvyšší druhová koncentrace hmyzu, tvořící až 90 % se vyskytuje v tropickém pásu, kdy například jeden hektar Amazonského pralesa poskytuje stanoviště až pro 85 000 druhů hmyzu (Foottit & Adler 2017). Hmyz je velice různorodou skupinou čítající celkem 28 řádů, ale více než 80 % druhů hmyzu pochází pouze ze řádů čtyř, a to Coleoptera, Diptera, Hemiptera a Lepidoptera (Cardoso 2012).

Zdokonalování molekulárních metod umožňuje odhalovat a popisovat každým rokem vyšší množství nových druhů (Costello et al. 2012), celkově se však diverzita povážlivě snižuje. Za poslední století byla u dobře zmapovaných skupin živočichů zaznamenána o $10^{3\pm1}$ vyšší míra extinkce než v průběhu předchozí půl miliardy let (May 2011). Kromě toho se vlivem klimatických změn a antropogenních vlivů často snižuje i početnost jednotlivých populací a existence mnoha druhů je dnes bezprostředně ohrožena a mnoho druhů se pohybuje na pokraji vyhynutí. Podle IUCN (2018a) je sestupný populační trend znám u 19 258 sledovaných druhů, zatímco stoupající tendence jich má aktuálně pouze 1191. Bez patřičných opatření tudíž hrozí, že tento dramatický pokles se bude dále zrychlovat i ve 21. století. Vzhledem k tomu, že hmyz má často nenahraditelnou roli ve fungování celého ekosystému, je nutné tento trend zvrátit.

2.1.1 Deštníkové druhy a ochrana stanovišť ohrožených druhů

Klíčovým faktorem pro ochranu ohrožených živočichů je zachování lokalit, na kterých se přirozeně vyskytují. Zavedení podpůrného managementu vycházejícího ze znalosti životních požadavků cílových organismů je pro udržení těchto druhů i pro příští generace žádoucí. Ochranou deštníkových druhů se rozumí ochrana zastřešujícíi další druhy a společenstva, která stanoviště s deštníkovými druhy sdílí a mají podobné ekologické nároky, ale z různých důvodů na ochranný status nedosáhly (Roberge & Angelstam 2004; Stuber & Fontaine 2018). Na druhou stranu je třeba podotknout, že ne všechny chráněné druhy různých biotopů lze považovat za vhodné druhy deštníkové a neúspěšné využití vlajkových druhů pro tento účel je z minulosti velmi dobře známé (Roth & Weber 2007; Cabeza et al. 2007; Martín-López et al. 2007). Při výběru je proto nutné zvážit všechny potenciální důsledky (Cardoso 2012). Přestože jsou za vhodné deštníkové druhy nejčastěji využíváni obratlovci a ptáci (Roberge & Angelstam 2004), volba hmyzu za tímto účelem je též známá. Například u modrásků *Phengaris arion* (Spitzer et al. 2009) či *Phengaris nausithous,* kteří jsou deštníkovými druhy pro vlhké, extenzivně obhospodařované louky (Kempe et al. 2016).

2.1.2 Vliv intenzivního zemědělství na biodiverzitu

Intenzifikace zemědělství sice prozatím umožňuje lidstvu vyrovnat se s růstem celkové populace, ale nese s sebou též mnoho nežádoucích jevů. Činnosti spojené se zemědělstvím emitují 25 – 33 % skleníkových plynů (Tubiello et al. 2014), zabírají přes 40 % zemského povrchu (FAO 2016) a 70 % sladkovodních ploch (Molden et al. 2013). Jsou příčinou odlesňování, homogenizace a fragmentace krajiny (Ramankutty & Foley 1999; Batáry et al. 2017) a proto jsou také jednou z hlavních příčin poklesu světové biodiverzity (Habel & Schmitt 2018; Lanz et al. 2018; Bommarco et al. 2018).

Změna zemědělské politiky spočívá hlavně ve zvýšeném používání hnojiv, pesticidních přípravků, zavlažování, odvodňování či ve využívání těžké techniky (Kleijn et al. 2009; Cizek et al. 2012). To vede k tomu, že půdy jsou v současnosti buď intenzivně využívány, nebo naopak opouštěny, což má též negativní dopad na biodiverzitu (Loos et al. 2014; Uchida & Ushimaru 2015; Antonini & Argilés-Bosch 2017). Přestože většinou je změna diverzity demonstrována zejména na obratlovcích, velké změny probíhají v abundanci i diverzitě členovců včetně hmyzu. Vzhledem ke krátkému životnímu cyklu jsou změny v početnosti hmyzu jedním z prvních ukazatelů narušení ekosystému a dají se tak použít jako explicitní modelové ukazatele či včasná varování pro pokles biodiverzity. Hallmann et al. (2017) zaznamenali, že masa létajícího hmyzu zachycená do Malaiseho pastí se v různých regionech v Německu během posledních 27 let snížila o 75 %. Fenomén poklesu početnosti byl potvrzen i u vážek (Clausnitzer et al. 2009), střevlíků (Brooks et al. 2012) a nevyhnul se ani motýlům, kdy početnost evropských populací v letech 1990-2000 poklesla až o 60-70 % (Fox 2013; Habel et al. 2016)

2.1.3 Ochrana biodiverzity motýlů v Evropské unii

Přestože byla pro záchranu ohrožených druhů motýlů Evropskou unií vyhlášena řada přírodních rezervací či národních parků a byly vydány směrnice na vytvoření sítě lokalit NATURA 2000, početnost i druhová diverzita motýlů nadále klesá (Henle et al. 2008; Warren & Bourn 2011). Základem veškeré podpory pro uchování biologické rozmanitosti motýlů je propojení zemědělské politiky s prací biologů a ochranářů se zaměřením na prioritní oblasti, komplexní monitoring a aplikaci vhodných managementových intervencí v klíčových oblastech (EEA 2011; Warren & Bourn 2011; Diffendorfer et al. 2014). Jedním z nejdůležitějších nástrojů EU pro snížení negativních dopadů zemědělství jsou agroenvironmentální programy (AES), které pomocí dotací podporují zemědělce v tradičním způsobu využívání krajiny a aplikaci vhodného managementu na vybraných lokalitách (Konvička et al. 2005; Wätzold et al. 2008; Wrbka et al. 2008). Společná zemědělská politika „Common Agricultural Policy“ (CAM) se v současnosti zaměřuje na optimální soulad mezi produkcí a podporou biologické rozmanitosti (Henle et al. 2008; Wrbka et al. 2008; EEA, 2011). Další nařízení upravující ochranu přírody jsou například evropské směrnice pro ochranu přírody „Convention on biodiversity“ (CBD) nebo dokument „The EU Biodiversity Strategy to 2020“, shrnující 6 cílů zaměřených na rozvoj biodiverzity, kterých by mělo být dosaženo do roku 2020 (Schmeller et al. 2009; EEA, 2011; Maes et al. 2013).

2.1.4 Vztah mezi managementem a populacemi motýlů

Snižující se biodiverzita je hlavním důvodem pro znepokojení ochranářů a zároveň aktivaci ochranných opatření (Groom et al. 2006). Aktivní ochrana motýlů spočívá hlavně v pochopení požadavků definovaných přítomností a rozložením zdrojů (Dennis et al., 2006; Jansen, 2012). S přihlédnutím na životní cyklus a požadavky motýlů lze obecně aplikované managementy dělit na vyhovující a nevyhovující. Mezi vhodné způsoby hospodaření, které podporují populační rozvoj motýlů, řadíme rotační seč (Farruggia et al. 2012), extenzivní pastvu (Pöyry et al. 2006), sešlap (Kruess & Tscharntke 2002), občasný výpal (McIver & Macke 2014), dočasné vynechání managementu na pozemku (Schirmel &Fartmann 2014) a údržbu řídkých lesních porostů, keřů a pasek u lesních druhů motýlů (Slámová et al., 2013). Naopak odlesňování (Augenstein et al. 2012), meliorace luk (Kati et al. 2012), intenzivní management pozemků (Slámová et al., 2013), či naopak úplné opuštění managementu, které vede k sukcesi (Hůla et al., 2004; Skórka 2007), patří mezi managementy, které jsou pro většinu motýlů nevhodné. Způsob managementu na lokalitách je jedním z hlavních faktorů, které mají zásadní vliv na prospívání lokálních populací motýlů (Wallis de Vries et al., 2007; D´Anielo et al., 2011).

2.1.5 Bionomie a ochrana modrásků Phengaris nausithous a Phengaris teleius

Modrásci *Phengaris nausithous* (Bergsträsser, 1779) a *P. teleius* (Bergsträsser, 1779) jsou monofágní druhy motýlů, přičemž jejich živnou rostlinou je krvavec toten (*Sanguisorba officinalis*) (Thomas, 1984). Přestože imaga obou těchto modrásků mohou příležitostně sát nektar i z dalších rostlin jako například vikve ptačí (*Vicia cracca*), bukvice lékařské (*Betonica officinalis*), pcháče rolního (*Cirsium arvense*) a rozrazilu dlouholistého (*Pseudolysimachion maritimum*), může kladení a následný vývoj housenek proběhnout jedině na květenství krvavce (Sielezniew & Stankiewicz-Fiedurek 2013; Nowicki et al., 2014).

Obrázek 1: Páření modrásků *Phengaris nausithous* (Autor snímku: Karolína Hamzová)

Vzhledem k tomu, že jsou oba druhy studovaných modrásků pevně vázány na svoji živnou rostlinu, jsou jejich hlavním biotopem vlhké a podmáčené louky či pastviny, kde se hojně vyskytuje krvavec toten. Tento typ biotopu je v současné době v Evropě vážně ohrožen. Zatímco na přelomu století byly největší hrozbou pro tyto lokality lidské zásahy typu rekultivací vodních toků, meliorací luk, nevhodné doby seče či použití herbicidů (Wärner et al., 2014), nyní jsou v kontextu klimatických změn ohrožovány navíc také suchem a vysokými teplotami (Romo et al., 2015) či parazitickou houbou *Podosphaera ferruginea* napadající ve velkém měřítku jejich živné rostliny (Śliwińska et al. 2017).

Co se týče životní strategie, oba druhy modrásků *P. nausithous* i *P. teleius,* jsou poloparazitičtí a obligátně myrmekofilní, což znamená, že k dokončení vývoje potřebují hostitelské mravence rodu *Myrmica*, v jejichž hnízdech larvy určitou dobu žijí a kde parazitují, resp. predují potomstvo mravenců (Thomas et al., 1989). Po dobu prvních tří instarů žijí housenky v květenství krvavce, kde se živí rostlinným pletivem (Pech et al., 2007), ve čtvrtém instaru pak rostlinu opouští a jsou adoptovány dělnicemi hostitelských mravenců (Thomas et al., 1998a). Úspěšná adopce housenky modráska je možná díky sekretům vylučovaných z epidermálních žláz, přenosu živin z housenky na mravence i manipulativní komunikaci prostřednictvím chemických signálů (Elmes & Thomas 1991; Fiedler et al., 1996; Solazzo et al., 2015). Po přijetí hostitelskými mravenci zůstávají housenky v mraveništích po dobu 10 až 22 měsíců (Hochberg et al., 1992; Thomas et al., 1998b; Sliwinska et al., 2006). V mraveništi se housenky také kuklí (Elmes & Thomas, 1991). Po vykuklení již mravenci dokáží motýla detekovat a útočí na něj, včasné opuštění mravenčího hnízda je tak dalším krizovým momentem během života těchto modrásků (Beneš et al., 2002). Proto líhnutí probíhá většinou mezi sedmou a devátou hodinou ranní, tedy v době snížené aktivity hostitelských mravenců (Čechmánek & Hrabák, 2006).

Obrázek 2: Samice *Phengaris nausithous* na živné rostlině. (Autor snímku: Karolína Hamzová)

Phengaris nausithous a *P. teleius* jsou univoltinní a letová sezóna trvá dle klimatických podmínek většinou od začátku července do poloviny srpna (Thomas et al., 1998a). Přestože oba druhy žijí většinou sympatricky (Popović et al., 2017), jejich bionomie je v některých aspektech rozdílná. Samice *P. nausithous* klade až pět vajíček na zralou květní hlávku, zatímco *P. teleius* klade separovaně po jednom, a především na mladá květenství (Figurny et Woyciechowski, 1998; Sielezniew et al., 2013), která jsou ostatních snůšek prosté (van Dyck et al., 2000). Samice *P. teleius* dále kladou vajíčka na rostliny v blízkosti hnízd mravenců *Myrmica*, zatímco u *P. nausithous* z hlediska tohoto faktoru nebyla zjištěna žádná preference (Wynhoff & van Langevelde 2017). Dále bylo také prokázáno, že *P. nausithous* se vyskytuje spíše na stanovištích u zalesněných okrajů luk (Kőrösi et al., 2012), zatímco *P. teleius* upřednostňuje hlavně vnitřní oblasti louky (Batáry et al., 2009). Z hlediska přežívání je *P. nausithous* v porovnání s *P. teleius* druhem odolnějším. Za prvé dokáže dlouhodobě přežívat na izolované lokalitě, zatímco *P. teleius* vyžaduje systém lokalit propojených průletovými koridory (Nowicki et al., 2007). Je také známo, že *P. teleius* je náchylnější k poklesu hustoty mravenišť (Figurny et al., 2000; Bereczki et al., 2015) a na základě výzkumu, který se zabýval reintrodukcí obou druhů v Nizozemsku je také zřetelné, že modrásci druhu *P. teleius* mají nižší schopnost osidlování nových stanovišť (Wynhoff, 1998).

Oba studované druhy modrásků, *P. nausithous* a *P. teleius,* jsou v současnosti uznávány jako vlajkové, a jak už bylo zmíněno výše, také jako deštníkové druhy pro ochranu biodiverzity travních porostů v Evropě (Thomas & Settele, 2004; van Swaay et al., 2012). Jejich ochrana je ukotvena v rámci několika směrnic a nařízení EEA 2011, NATURA 2000, CBD nebo „The EU Biodiversity Strategy to 2020“ (Schmeller et al. 2009; Maes et al. 2013). V rámci světového českého i evropského červeného seznamu jsou shodně řazeni do kategorie téměř ohrožený – near threatened pro *P. nausithous* a zranitelný – vulnerable pro *P. teleius* (Hejda et al., 2017; van Swaay et al., 2010) „International Union for Conservation of Nature“ (IUCN) řadí na svém seznamu oba druhy do kategorie téměř ohrožený („Near threatened“) (IUCN, 2018).

2.1.6 Vhodný management pro modrásky

Vzhledem k tomu, že studované druhy modrásků jsou monofágní, univoltinní, myrmekofilní a žijí většinou v sympatrickém metapopulačním systému, je zapotřebí nastavit pro ně ochranný management tak, aby bylo vyhověno všem těmto požadavkům. Důležitým faktorem je zapojení sítě lučních porostů, ale také okrajových části cest či pozemků (Nowicki et al., 2013), což by mělo vést ke snížení fragmentace krajiny a podpoře metapopulačního systému (Nowicki et al., 2014). Naprosto klíčovým faktorem na stanovištích těchto modrásků je správné provedení a načasovaní seče. Nejvhodnějším managementem je z tohoto pohledu seč jednou ročně, případně minimálně jednou za dva až tři roky (Johst et al., 2006; Vrabec et al., 2008). Seč ale nesmí proběhnout během letové sezóny, aby nedošlo ke ztrátě živných rostlin a hnízd hostitelských mravenců. Z hlediska nízké disperzní schopnosti studovaných druhů modrásků pro ně opakovaná seč v nevhodném termínu může mít fatální následky (Johst et al., 2006; Dover 2010). Optimálním termínem pro kosení lokalit je začátek května nebo září (Beneš at al., 2002; Konvička et al., 2005; Kőrösi et al., 2014). Je ale nutné míti na paměti, že pokud je seč provedena před začátkem letové sezony, nemusí vlivem různých stochastických jevů dojít k obnově rostlin a květenství krvavce totenu. Vhodnějším datem pro seč se proto zdá být podzimní termín. Kromě toho, že nedojde k ohrožení živných rostlin, má podzimní termín seče pozitivní vliv i na hostitelské mravence (Grill et al., 2008). Důležité je také technické hledisko provedení seče. Z tohoto pohledu se za nejlepší řešení považuje seč mozaiková (Beneš et al., 2002; Konvička et al., 2005), která napodobuje tradiční způsob hospodaření na loukách (Pöyry, 2007). Princip seče tkví v ponechání části louky nekosené, tak aby zde byly v jednom okamžiku porosty různé délky (Morris 2000; Saarinen & Jantunen, 2005; Farruggia et al., 2012). Nevhodné je také ponechání luk na dlouhou dobu bez managementu. Dochází k nástupu sukcese, tedy k zarůstání pozemků vysokou trávou, keři a následně stromy (Provoost et al., 2009; Schirmel & Fartmann, 2014).

2.2 Biologické invaze

Na začátku 21. století dochází v rámci globalizace k intenzivnímu obchodu a pohybu osob po celém světě. Nechtěné zavlečení cizích organismů je tak za této situace víceméně nevyhnutelné. Biologické invaze jsou po fragmentaci a úbytku stanovišť považovány za třetí největší hrozbu pro globální biodiverzitu (MA 2005) a dále jsou také jedním z nejvýznamnějších důvodů vedoucích k extinkci vzácných druhů zvířat (Clavero & García-Berthou 2005). Za biologickou invazi je považováno šíření organismu mimo místo svého původního výskytu, velmi často spojené s lidskou činností, při které dojde k záměrnému či neúmyslnému zavlečení organismu (Hulme 2015). Vzhledem k absenci přirozených nepřátel, potravní kompetici, predaci, přenosu patogenů, hybridizaci a působení změn na stanovištích, působí invazní druhy negativně na druhovou pestrost a celkové fungování ekosystémů (Levine 2008; Simberloff et al. 2013).

Nejčastější formou šíření nepůvodních či invazních druhů je jejich transport ve formě kontaminantů zboží nebo „černých pasažérů“ v různých dopravních prostředcích (Hulme 2017). Dále není výjimkou zavlečení organismu jakožto předmětu obchodu se zbožím určeným pro prodej do hobby chovů akvarijního či terarijního charakteru, odkud jsou následně z takových chovů buď vypuštěni do volné přírody, nebo se jim podaří uniknout (McFadden et al. 2017; Patoka et al. 2017, 2018; da Rosa et al. 2018). Dalším případem šíření invazních živočichů může být i nevhodně použitá biologická kontrola (Roy & Wajnberg 2008), pěstování či import okrasných rostlin (Lake & Leishman 2004). Většina takto uniklých jedinců v novém prostředí nepřežije, nebo se jim nepodaří vytvořit perzistentní populaci. Nejčastěji je za limitující faktor považována teplota, nicméně v kontextu globálních změn klimatu se riziko invazí zvyšuje i pro teplomilné živočichy (Hulme 2017). Průběžné vyhodnocování rizika invazí pro jednotlivé regiony je společně s cíleným monitoringem jedním z klíčových nástrojů pro boj s tímto jevem (Haugom et al. 2002). Dalším faktorem, který hraje roli v procesu aklimatizace druhů, je jejich původ. Ve střední Evropě je 40 %

nepůvodních druhů hmyzu spojeno s komoditami z Asie, 32 % z jiných částí Evropy a pouze 2 % se Severní Amerikou (Lis et al. 2001).

Protože pro politiku rozvojových tropických zemí je téma biologických invazí nedůležité, je složité odhadovat celkový počet invazních druhů v celosvětovém měřítku. Je ale jisté, že jejich množství jde minimálně do řádu desetitisíců. Co se týče rostlin, (van Kleunen et al. 2015) spočítali celkové množství invazních cévnatých rostlin na 13 168, což je 3,9 % celkového množství těchto rostlin na planetě. V Evropě se biologické invaze staly na začátku 21. století tématem velice diskutovaným a dostává se mu i legislativních opatření („EU Regulation 1143/2014 on Invasive Alien Species“; „Target 5 of the EU 2020 Biodiversity Strategy“). Podle Mezinárodního svazu ochrany přírody se v současnosti vyskytuje v Evropě více než 12 tisíc nepůvodních druhů, z čehož až 15 % je považováno za organismy invazní (IUCN 2018b). Kromě vlivu ekologického je neméně významný i impakt ekonomický. S biologickými invazemi jsou spojeny také poměrně vysoké náklady v důsledku působení na veřejné zdraví, zničené infrastruktury či ztrát v zemědělství. Ty například ve Spojených státech amerických dosahují ročně okolo 100 miliard dolarů (Levine 2008), v Evropské unii je to pak více než 12 miliard euro (IUCN 2018b).

2.2.1 Invazní hmyz v České republice

Přestože hmyz tvoří pouze 1-2 % z celkového množství invazních druhů, někteří zástupci z této třídy jsou velice významnými škůdci z hlediska ekonomického či představují hrozbu pro veřejné zdraví. V České republice bylo zaznamenáno téměř 400 nepůvodních druhů, z nichž většina pochází z řádu Homoptera (30,3 %), Coleoptera (28,7 %) a Lepidoptera (9,7 %). Za invazní druhy v pravém slova smyslu lze pak považovat 18,0 % z nich (Šefrová 2005).

Příkladem invazních živočichů, kteří úspěšně osídlili území našeho státu, je slunéčko východní *Harmonia axyridis* (Pallas, 1773), invazní druh původem z Asie. Dospělí jedinci měří 8 milimetrů, základní zbarvení krovek je červené s až 19 symetricky rozloženými černými skvrnami. Tento druh byl zavlečen lidmi do nových oblastí v rámci biologického boje proti škůdcům. Pro tento účel se slunéčko východní jevilo jako ideální organismus, zejména díky nízké potravní specializaci, ekologické plasticitě a vysoké predační schopnosti – jedno slunéčko dokáže za život zkonzumovat až 5000 mšic (Michaud 2002). Přestože se ve Spojených státech amerických k výše uvedenému účelu tento druh slunéček používal již od roku 1916 (Gordon 1985), první nalezené etablované populace mimo místa záměrné introdukce byly potvrzeny v Lousianě v roce 1988 (Chapin & Brou 1991). Během konce 20. a začátku 21. století byl tento druh shledán jako dominantní zástupce slunéček v USA (Colunga-Garcia & Gage 1998; Hesler et al. 2001). V Evropě se slunéčka *H. axyridis* začaly v biologickém boji proti mšicím poprvé využívat v Belgii v roce 1997, přičemž první invazní populace byly zachyceny na území stejného státu v roce 2001 (Adriaens et al. 2003). Invaze druhu následně pokračovala do sousedních zemí a v roce 2007 již byly populace slunéček etablovány ve 13 evropských státech včetně České republiky (Brown et al. 2007). Co se týče škodlivosti tohoto organismu pro nové prostředí, můžeme pozorovat hned několik negativních vlivů. Za prvé je hlavně díky úspěšnosti v potravní kompetici, ale i predaci, schopna vytlačovat původní druhy slunéček, což platí pro evropské i americké území (Brown & Miller 1998; Colunga-Garcia & Gage 1998; Michaud 2002; Roy et al. 2012). Dále se též předpokládá, že je predátorem jiných druhů hmyzu na lokalitě výskytu, tento jev byl potvrzen například pro motýla monarchu stěhovavého (*Danaus plexippus)* (Koch et al. 1991) a nakonec je třeba také zmínit přímý vliv na lidskou populaci. Slunéčko východní totiž na podzim často vyhledává lidská obydlí jako svá zimoviště. Zde pak může při své obraně uvolnit tzv. „reflexní krev“, jež zanechává skvrny na nábytku či stěnách (Majerus et al. 2006). Při poklesu populací mšic vyhledává *H. axyridis* nové zdroje potravy. Během tohoto chování může dojít i k pokousání člověka, na které byly zaznamenány i alergické reakce (Yarbrough et al. 1999; Magnan et al. 2002).

Mezi nápadné invazní druhy hmyzu, které se na začátku 21. století dostaly do Evropy, potažmo České republiky patří i vroubenka americká - *Leptoglossus occidentalis* Heidemann, 1910. Tato 9-18 mm dlouhá ploštice je škůdcem jehličnatých stromů původem ze Severní Ameriky. V Evropě byly její perzistentní populace objeveny poprvé v Itálii (Bernardinelli & Zandigiacomo 2001). V následujících letech se pak vroubenka rozšířila do dalších evropských zemí (Foldessy 2006; Gapon 2013; Petrakis 2017) včetně České republiky (Beránek 2007). Kromě škod, které způsobuje na stromech, může znepříjemnit život i obyvatelům, jejichž příbytek si kolonie vroubenek zvolí za své zimoviště (Gall 1992).

Na území České republiky se na začátku 21. století dostali i významní škůdci ovocných plodů. Tmavka švestková - *Eurytoma schreineri* Schreiner, 1908 je fytofágní chalcidka původem z Ruska a Ukrajiny parazitující v larválním stádiu v endospermu švestek a mandlí (Zerova & Fursov 1991). Na hostitelském ovoci může způsobit ztrátu plodů až 80 % (Růžička 2014). V České republice byla poprvé objevena v roce 2012 (Pultar 2014). Zdrojem nákazy byl zřejmě import infikovaných plodů (Růžička 2014). Druhým takovým škůdcem je octomilka *Drosophila suzuki* (Matsumura, 1931), původem z jihovýchodní Asie (Ometto et al. 2013), která se v Evropě a Severní Americe začala šířit shodně v roce 2008 (Hauser 2011; Calabria et al. 2012; Rota-Stabelli et al. 2013; Saguez et al. 2013; Cini et al. 2014). Od té doby se druh usídlil ve 30 evropských zemích (Ørsted & Ørsted 2018), kde je díky své invazivitě, vysoké toleranci ke klimatickým podmínkám a vysoké reprodukční schopnosti vnímán jako hrozba, proti které je snahou se bránit za pomocí konvenčních pesticidů i alternativních metod jako například použití sterilních samců (Cini et al. 2002; Nikolouli et al. 2018).

Další skupinou hmyzu, jehož invaze byly v nedávné době zaznamenány v České republice i zbytku Evropy jsou zástupci hmyzu z čeledi dvoukřídlých, jejichž larvální vývoj probíhá v organicky znečištěné vodě a z původních přírodních biotopů se druhotně adaptovaly na antropogenní prostředí. Jako první příklad lze uvést koutuli *Clogmia albipunctata* (Williston, 1893). Tato tropická či subtropická muška, jež původně obývala kaluže a stromové dutiny, se pasivně šíří pomocí vzdušných proudů či povrchové dopravy a stále častěji je detekována v domácnostech, kde jsou za její líhniště považovány zejména odpadní vody v koupelnách, kuchyních či na toaletách. V České republice byla přítomnost populací *C. albipunctata* poprvé ohlášena v roce 2012 (Ježek et al. 2012). Vzhledem k tomu, že často žijí ve znečištěné stojaté či pomalu tekoucí vodě, je tento druh považován za rizikový při přenosu patogenů (Faulde & Spiesberger 2013). Navíc je prokázáno, že tyto koutule mohou být zdrojem různých druhů myáz, tedy onemocnění vyvolaných larvami, které se vylíhly z vajíček nakladených do lidské tkáně (Tu et al. 2007; Gelardi et al. 2009; El-Badry et al. 2014). Z hlediska lidské či veterinární medicíny zřejmě vůbec nejvýznamnější invazní druh hmyzu, komár tygrovaný *Aedes albopictus* (Skuse, 1894) (Obrázek 3). Pochází z jihovýchodní Asie, kde využíval stromové dutiny a paždí rostlin jako líhniště, odkud se rozšířil do téměř všech částí tropického a subtropického pásu (Amerika, Austrálie, části Afriky, Indie, jižní a západní Evropa, Pacifik) (Kraemer et al. 2015). Díky své ekologické plasticitě a schopnosti pasivního transportu jej organizace „Invasive Species Specialist Group“ řadí mezi top 100 světových invazních druhů na 4. místo (ISSG 2019). V Evropě byl jeho výskyt poprvé zaznamenán v roce 1979 v Albánii, kam byl pravděpodobně zavlečen spolu s pneumatikami z Číny (Adhami & Reiter 1998).

Obrázek 3: Larva a dospělec komára tygrovaného odchyceného při monitoringu jeho výskytu v České republice (Autor: Miroslav Petrtýl).

Obchod s pneumatikami byl později určen jako primární zdroj infestace tímto komárem v celosvětovém měřítku (Reiter & Sprenger 1987). Ojeté pneumatiky, ve kterých se kumuluje voda, která je lehce organicky znečištěná, přitahují gravidní samice k ovipozici stejně jako kdysi stromové dutiny. Vajíčka kladou těsně nad hladinu vody a larvy se tak líhnou až při dalším přelití vodou, přičemž vajíčka vydrží dlouhou dobu vysychat. Pneumatika s vajíčky tak může být v této době přepravena z jednoho kontinentu na druhý. Kromě ojetých pneumatik dokáže *Ae. albopictus* využívat i další dočasné vodní plochy s malým objemem včetně konví, podkvětináčů, záchytných sudů na dešťovou vodu, plastových lahví či plechovek. Vyjma obchodu s pneumatikami se šíří též povrchovou dopravou či okrasnými rostlinami rodu *Draceana* (Madon et al. 2002; Scholte et al. 2007). Dle Evropského střediska pro prevenci a kontrolu nemocí se komár tygrovaný usídlil ve více než 20 evropských zemích, v dalších sedmi státech byl sice zachycen, ale prozatím není považován za etablovaný (ECDC 2018a). Mezi takto postižená území patří i Česká republika, kde byl komár poprvé zachycen v roce 2012 (Šebesta et al. 2012). Možnost zavlečení silniční dopravou byla prokázána opakovaně v letech 2014 - 2018 na několika lokalitách v Čechách i na Moravě (Rettich & Kulma 2018; Rudolf et al. 2018). Komár tygrovaný je v Evropě již adaptován natolik, že dokáže i přezimovat ve stádiu vajíčka (Thomas et al. 2012) a vytvořil tak početné stabilní populace až v Horním Porýní na jihovýchodě Německa (Pluskota et al. 2016; Becker et al. 2017), kde jsou na základě výpočtu softwaru „Climatch tool" klimatické podmínky srovnatelné s většinou území České republiky a z tohoto pohledu lze do budoucna předpokládat jeho usídlení i zde (Kulma & Rettich 2018). Tento komár saje na savcích včetně člověka (Faraji et al. 2014) a je kompetentním vektorem více než 25 arbovirů včetně horečky Chikungunya, Dengue nebo viru Zika (Paupy et al. 2009) či filariálních onemocnění (Medlock et al. 2012). Od začátku 21. století je s výskytem komára tygrovaného spojeno několik epidemií těchto do té doby „exotických" onemocnění v Itálii, Chorvatsku, Francii či Španělsku (Rezza et al. 2007; Calba et al. 2017; Venturi et al. 2017; ECDC 2018b).

2.2.2 Příklady dalších invazí hmyzu v Evropě

Kromě výše zmíněného komára se v Evropě v posledních letech etablovali další exotičtí komárovití z rodu *Aedes*, konkrétně *Ae. japonicus* (Theobald, 1901) (Versteirt et al. 2009; Schaffner, Chouin, & Guilloteau, 2003; Schaffner, Kaufmann, Hegglin, & Mathis, 2009) a *Ae. koreicus* (Edwards, 1907) (Ciocchetta et al. 2018) kteří jsou též potenciálními vektory patogenů. Ve Spojených státech amerických se navíc spolu s *Ae. albopictus* rychle šíří i *Ae. Aegypti* (Linnaeus, 1762) (Peper, Wilson-Fallon, Haydett, Greenberg, & Presley, 2017; Porse et al., 2015), další antropofilní druh a velice schopný vektor různých onemocnění (Kraemer et al. 2015), jehož invazi (resp. reinvazi) do Evropy lze v blízké budoucnosti též očekávat (Wint et al. 2018) a to i přesto, že v současnosti se vyskytuje pouze na ostrově Madeira, v Gruzii a v evropské části Ruska (ECDC 2018c). Výskyt žádného z výše uvedených druhů v České republice dosud nebyl potvrzen.

Nebezpečnou invazí je též zavlečení sršně asijské - *Vespa velutina nigrithorax* Buysson, 1905, agresivního druhu vosy a významného predátora včely medonosné (Tan et al. 2007) do Francie, kam byla v roce 2005 importována zřejmě ve formě hibernujících královen prostřednictvím obchodu se zahradnickými pomůckami, konkrétně s květináči, z Číny (Villemant et al. 2006). Kromě Francie byl výskyt sršně asijské zaznamenán i v okolních státech, konkrétně Španělsku, Belgii, Baleárských ostrovech,Velké Británii a Německu (Witt 2015; Arca et al. 2015; Leza et al. 2018; Takahashi et al. 2018).

Dalším druhem hmyzu dynamicky se šířícím Evropou je rybenka druhu *Ctenolepisma longicaudata* Escherich, 1905, je striktně synantropní invazní druh hmyzu z řádu šupinušek dosahující v dospělosti velikosti až 2 cm. Stejně jako endemické druhy rybenek, tato rybenka není, vyjma potenciální kontaminace uskladněných potravin, lidskému zdraví přímo nebezpečná. Na druhou stranu je díky své schopnosti trávit celulózu a škrob schopna působit významné škody na tapetách, knihách, fotoalbech, sbírkách, obrazech, archivech dokumentů (Van der Weijden et al. 2007; Smith 2017) a na hedvábných či bavlněných textiliích (Mallis et al. 1958). Co se týče životního cyklu, samice klade snůšky obsahující 2 - 20 vajíček, které se pak líhnou během 14 – 32 dní. Pohlavně dospělé jsou tyto rybenky ve 2 - 3 letech, v dospělosti se pak ještě několikrát svlékají a mohou žít až dalších 2 - 5 let (Lindsay 1940). Přestože byla *C. longicaudata* poprvé popsána v jižní Africe, původně pochází zřejmě ze Střední Ameriky (Zettel 2010), odkud se pasivním transportem spolu se zbožím rozšířila na všechny trvale obydlené světadíly.

Sežerou nás rybenky. Chraňte si hotovost a toaletní papír

22. dubna 2017, 06:00 - Lubomír Heger

Z Nizozemska se do střední Evropy šíří škůdce, kterýsi pochutnává na kartonu, tapetách, archiváliích a starých fotografiích. Nejlépe se mu vede v šanonech. Šíří se třeba poštou.

Není rybenka jako rybenka. Ty z Nizozemska žerou celé archivy

17. července 2017

Běžné domácí rybenky většinou žádnou škodu nenadělají, jejich exotický sourozenec už představuje větší nebezpečí. Alespoň pro archivy, sbírky knih, časopisů nebo fotografií, ale třeba i pro domácí tapety. Brzy se může pustit i do těch českých.

Obrázky 4 a 5: Varování před rybenkou *Ctenolepisma longicaudata* v českých médiích (idnes.cz a euro.cz).

Přestože v Evropě byl její výskyt poprvé zaznamenán ve Francii v roce 1914 (Paclt 1967), její skrytý a nenápadný způsob života zřejmě způsobil, že další zmínky o jejím výskytu v rámci Evropy pocházejí až z konce 20. a začátku 21. století. Na rozdíl od evropských rybenek není její výskyt omezen požadavky na vyšší teplotu a vlhkost, tudíž má vysoký potenciál nejen kolonizovat nová území, ale též se zde stávat dominantním synantropním druhem. Jako příklad lze uvést Nizozemsko, kde byla první populace tohoto druhu objevena v roce 1989 (Nierop & Hakbijl 2002) a nyní je *C.*

longicaudata nejrozšířenějším rybenkovitým škůdcem domácností (Schoelitsz & Brooks 2014). Kromě již výše zmíněných zemí byl výskyt této rybenky zaznamenán v Itálii (Molero-Baltanás et al. 2000), Švédsku (Pape & Wahlstedt 2002), Belgii (Lock 2007), Německu (Meineke & Menge 2014), Rakousku (Zimmermann 2016) a Anglii (Goddard et al. 2016). Vzhledem k velmi čilému obchodu mezi těmito evropskými zeměmi a Českou republikou bylo spíše jen otázkou času, kdy se tento úspěšný invazní hmyz dostane i na naše území. Na riziko invaze rybenky *C. longicaudata* proto v roce 2017 upozorňovala i některá česká on-line média (www.idnes.cz a www.euro.cz) viz obrázky 4 a 5.

2.3 Hmyz jako alternativní zdroj živin

Jak již bylo zmíněno výše, intenzivní zemědělství je jedním z nejvýznamnějších faktorů, které negativně ovlivňují biodiverzitu. Na druhou stranu je třeba, aby produkce tohoto sektoru uživila stále rostoucí světovou populaci. Zatímco v roce 1945 bylo na planetě pouze 2,5 miliardy obyvatel v současné době se světová populace přehoupla přes 7,6 miliard lidí. Rostoucí trend je předpokládán i pro další desetiletí. V roce 2050 má světová populace přesáhnout 9,8 miliard a v roce 2100 11,2 miliard obyvatel (United Nations 2017). Vlivem globalizace navíc dochází ke změně stravovacích návyků a zvýšení průměrné roční spotřeby jídla. Všechny výše zmíněné faktory způsobují enormní zvýšení požadavků na kvalitu i kvantitu živočišné produkce. Předpokládá se, že do roku 2050 bude poptávka po produktech živočišné výroby až o 50 % vyšší než v době současné, kdy živočišná výroba (včetně svých zdrojů) zabírá 30 % plochy zemského povrchu, 75 % všech zemědělsky využívaných půd a využívá 8 % světové spotřeby vody (FAO 2011). Mimo jiné je tento sektor také jedním z hlavních producentů skleníkových plynů (Foley et al. 2011; Gerber et al. 2013). Zabezpečení zdrojů kvalitních živin, které by byly dlouhodobě udržitelné, je proto do budoucna výzvou pro celé lidstvo. Jednou z alternativ, který se v této souvislosti nabízí je využití hmyzu jako potraviny nebo krmiva pro hospodářská zvířata.

Hmyz, zvláště pak druhy s krátkým životním cyklem a vysokou reprodukční schopností, dříve považované za škůdce, byl poprvé zmíněn v souvislosti s řešením světové bílkovinové krize již v roce 1975 (Meyer-Rochow 1975), nicméně komplexně a intenzivně se toto téma začalo řešit až na začátku 21. století. V porovnání s konvenčními zdroji potravy je hmyz považován za alternativní zdroj živin, šetrný k životnímu prostředí díky nízkým emisím skleníkových plynů (Oonincx et al. 2010). Navíc je možné jej v některých případech chovat na vedlejších produktech potravinářského průmyslu, potravinách s prošlým datem expirace (Finke et al. 2015; Oonincx et al. 2015) nebo dokonce na odpadních produktech organického původu (Čičková et al. 2015). Protože je hmyz organismem poikilotermním a tedy nepotřebuje energii k zachování tělesné teploty, je schopen též dosáhnout výborné konverze krmiv či prosperovat na krmivech horší kvality (Akhtar & Isman 2018). Hmyz hraje důležitou roli v potravním řetězci, je přirozenou složkou potravy mnoha živočichů a je považován za kvalitní zdroj živin.

Na základě souhrnu dostupných informací o nutriční hodnotě hmyzu je jasné, že obsah živin se liší mezi jednotlivými druhy hmyzu, ale i v rámci jednoho druhu (Van Huis et al. 2013; Sánchez-Muros et al. 2014; Vrabec et al. 2015). Faktory, které nutriční hodnotu mohou ovlivnit, je proto nutné zkoumat a podrobně definovat. Obecně lze však říct, že z pohledu nutriční hodnoty je hmyz dobrý zdroj bílkovin, jejichž kvalita vyjádřená profilem esenciálních aminokyselin je sice horší než u živočišných proteinů, ale na druhou stranu srovnatelná nebo lepší než v případě těch nejkvalitnějších rostlinných bílkovin jako je sója (Yi et al. 2013; Barroso et al. 2014). Druhou nejvíce zastoupenou živinou v hmyzu je obvykle tuk, který je v porovnání s konvenčními zdroji bílkovin u hmyzu významně vyšší a jehož kvalita je vyjádřena profilem mastných kyselin. Z dostupných zdrojů vyplývá, že dominantními kyselinami u hmyzu jsou nejčastěji kyseliny olejová, palmitová a linolenová (Finke 2002; Barroso et al. 2014; Chakravorty et al. 2014; Ghosh et al. 2017; Paul et al. 2017). Kvalita tuku je srovnatelná s konvenčními zdroji a je z velké části závislá na mastných kyselinách obsažených v potravě hmyzu (Sánchez-Muros et al. 2014). Sacharidy jsou v hmyzu zastoupeny většinou pouze ve formě chitinu, jež je pro většinu monogastrů nestravitelný (Finke 2002; Mlcek et al. 2014) a obecně tak považovaný za antinutriční faktor. Nicméně jsou známé i jeho pozitivní účinky na imunitní a zažívací systém (Halder et al. 2013; Van Huis et al. 2013). Hmyz obsahuje dostatečné hladiny minerálních látek jako je fosfor, hořčík, sodík a další stopové prvky. Z hlediska výživy obratlovců jsou ovšem i látky, které jsou obsaženy v nedostatečném množství. Typickým příkladem je vápník, jehož nízká hladina je způsobena absencí endoskeletu u členovců. V kombinaci s vysokým obsahem fosforu je pak poměr Ca:P pro obratlovce spíše nevyhovující (Finke 2002).

2.3.1 Hmyz v lidské výživě

Na celém světe bylo zdokumentováno 1745 druhů hmyzu, které jsou či v minulosti byly konzumovány lidmi v celkem 113 státech, nejčastěji položených v Asii a Oceánii, Africe a Jižní Americe (Durst et al. 2010). Západní civilizace se k hmyzu staví spíše negativně, stále jej považuje spíše za obtížné škůdce či známku nečistoty než za něco, co by mělo obohatit jídelníček (Durst et al. 2010). Na druhou stranu se v uplynulých letech zvýšila snaha hmyz z tohoto pohledu destigmatizovat a zvýšená ochota akceptovat hmyz v určité formě za vhodný ke konzumaci byla prokázána v zemích západní Evropy i Severní Ameriky (Hartmann et al. 2015; Ruby et al. 2015; House 2016). Hmyz je nejčastěji konzumován vcelku syrový či po určité kulinářské úpravě nebo namletý. Mezi nejčastější úpravy patří smažení, pečení a vaření. Tyto úpravy mají, stejně jako technologie chovu, vliv na výsledné nutriční i senzorické vlastnosti hmyzu (Akhtar & Isman 2018). Nejčastěji je chuť hmyzu přirovnávána k ořechům, jablkám, pečivu či rybám (Tan et al. 2015). Chitin způsobuje křupavost hmyzu vedoucí ke srovnání s pochutinami typu sušenek či preclíků (Ramos-Elorduy & Menzel 1998). Trend zvyšující se poptávky po této alternativní potravině reflektovala i Evropská unie, která na začátku roku 2018 zařadila hmyz do skupiny potravin nového typu.

2.3.2 Hmyz ve výživě zvířat

Aby bylo možné dosáhnout naplno potenciálu produkce hospodářských zvířat a zajistit tak maximalizaci výnosů živočišné produkce, je důležité zajistit zvířatům přísun kvalitního bílkovinného krmiva. Po zákazu zkrmování masokostních mouček v EU pro hospodářská zvířata kvůli obavám z prionů, podezřelých ze vzniku bovinní spongiformní encefalopatie (Tvrzník & Zeman 2004) jsou v krmivářství využívány hlavně sójové šroty a rybí moučka. Sója je považována za nejkvalitnější rostlinný zdroj bílkovin (Banaszkiewicz 2011; Heuzé et al. 2015), který ale může obsahovat významné antinutriční látky jako je například trypsin inhibitor, hemaglutinin či antivitaminy (Tacon 1993). Mezi další potíže spojené s vysokou konzumací sóji patří přítomnost vysokého obsahu fytoestrogenů, hlavně isoflavonů a kumestanů, které při vstřebávání mohou konkurovat pohlavním hormonům a mohou tak mít nepříznivý vliv na působení na hypotalamo–hypofýzo-gonadární osu a periferní reprodukční orgány (Zhao & Mu 2010). Popsány byly také negativní účinky genisteinu na aktivitu respektive motilitu spermií (Eustache et al. 2009; Martinez-Soto et al. 2010) nebo na vývoj oocytů (Jung et al. 1993; Van Cauwenberge & Alexandre 2004; Vodková et al. 2008). Nelze opomenout také environmentální dopady způsobené pěstováním sóji. Kvůli zvýšení produkce sóji dochází k další nevratné devastaci unikátních biotopů v jihoamerickém deštném pralese (Laurance et al. 2004; Verburg et al. 2014). Využíváni hnojiv a pesticidů je navíc zesilujícím negativním vlivem na okolí plantáží (Steward 2007). Většina světové produkce sóji je geneticky modifikovaného původu (Rakouský & Hraška 2007), jenž může též mít negativní účinky (Malatesta et al. 2008; Sakamoto et al. 2008). Jedinou živočišnou moučkou, která je dnes častěji komerčně využívána ke krmení, je moučka rybí. Ta je nepochybně zdrojem velice kvalitní živočišné bílkoviny (Zelenka et al. 2007), která je navíc velmi hodnotná i z energetického hlediska (Shepherd & Jackson 2013). Nevýhodou je ovšem její cena, která je v srovnání s rostlinnými krmnými komponenty mnohonásobně vyšší. Kvůli omezení rybolovu a znečištění vodního prostředí došlo v letech 2005-2010 k útlumu této produkce a následnému zvýšení ceny produktu téměř o dvojnásobek. Cena této komodity má od této chvíle pouze vzrůstající trend (International Monetary Fund 2010). Navíc typický rybí pach může negativně ovlivnit senzorickou kvalitu produktu a těžké kovy kumulované v tkáni ryb pak mohou představovat zdravotní riziko (Brooks & Rumsey 1974). Při hledání nových zdrojů bílkovinné složky krmiv je tedy hmyz, vzhledem k výše uvedeným skutečnostem, často zmiňovanou alternativou a recentně byl testován pro krmení různých hospodářských zvířat.

Ryby

Hmyz je přirozenou složkou potravy ryb a recentně byl po celém světě proveden intenzivní výzkum ohledně jeho implementace do krmných směsí pro různé druhy ryb. V roce 2017 navíc Evropská komise vydala nařízení EU 2017/893, které autorizovalo využití hmyzu jako bílkovinného komponentu pro akvakulturu a výzkum v této oblasti výrazně nabyl na intenzitě. (Elia et al. 2018) testovali využití moučky z pestřenky *Hermetia illucens* (Linnaeus, 1758)), a přestože nebyly pozorovány vedlejší efekty na produkci pstruha duhového až do 50 % nahrazení komerční moučky, při vyšší inkluzi hmyzí moučky docházelo ke zvýšení biomarkerů oxidativního stresu, a proto autoři nedoporučují přidávat více než 20 % této komponenty. Pestřenka *H. illucens* byla také experimentálně testována pro okrasnou rybku klauna očkatého, kdy Vargas-Abúndez et al. (2019) nezjistili negativní vliv na růst ani délku života těchto ryb. Madu & Ufodike (2003) zjistili, že použitím larev mouchy domácí - *Musca domestica* Linnaeus, 1758 do krmné směsi v chovu keříčkovců *Clarias anguillaris* došlo ke zvýšení přírůstků hmotnosti, ale při úplném nahrazení ostatních bílkovinných komponent nebyl tento efekt tak vysoký jako při užití larev ve směsi s komerčně vyrobeným krmivem pro ryby. Hmyzí moučku získanou z larev mouchy domácí úspěšně otestoval také Ogunji et al. (2008) při chovu tilápií. (Ng et al. 2001) otestoval larvy potemníka moučného - *Tenebrio molitor* Linnaeus, 1758 jako potenciální náhradu rybí moučky pro keříčkovce *Clarias gariepinus* a výsledky testované směsi s nahrazením rybí moučky do 40 % se v tomto případě nijak nelišily od kontrolních skupin. Dobrý přírůstek i nízká konverze krmiva pak byla zaznamenána až do nahrazení 80 % rybí moučky. Nicméně, vyšší množství tuku obsažené v hmyzu se promítlo také na vyšší protučnělosti poražených ryb. Stejný druh hmyzu zařadili do krmiva pro mořana zlatého *(Sparus aurata)* také Piccolo et al. (2014, 2017), v jejichž pokusech se po zařazení moučných červů v množství 25 % neprojevil žádný negativní dopad na produkt, ale při nahrazení 50 % rybí moučky v krmivu se významně snížila konverze krmiva a zaznamenali také signifikantní pokles stravitelnosti proteinu. Snížení příjmu krmiva a tím i výsledné hmotnosti při použití většího množství larev tohoto brouka v krmné směsi (50 %) zmiňuje i Gasco et al. (2014b) v chovu mořčáků evropských (*Dicentrarchus labrax*). Naopak v chovu pstruha duhového (*Oncorhynchus mykiss*) (Gasco et al. 2014a) nezaznamenali žádný negativní vliv na kvalitu a zdraví ryb, a to i při nahrazení 50 % rybí moučky v krmné dávce. V chovu sumečků *Ameiurus melas* lze využít hmyzí moučky z moučných červů i jako jediného bílkovinného komponentu (Roncarati et al. 2015). Dále bylo popsáno zařazení moučných červů do potravy paokouna Scherzerova (*Siniperca scherzeri*) do 30 % bez vedlejšího efektu přírůstky a využití živin

(Sankian et al. 2018). Jako alternativní zdroj bílkovin pro ryby byly testovány také některé druhy hmyzu z řádu rovnokřídlých. Možná náhrada části bílkovinné komponenty krmiva byla bez vedlejších účinků otestována v chovu keříčkovců (*C. gariepinus*) a to v koncentraci do 25 % pro moučky vyrobené ze sarančete druhu *Schistocerca gregaria* (Forskål, 1775) (Balogun et al. 2016), tak *Zonocerus variegatus* Linnaeus, 1758 (Alegbeleye et al. 2012). Stejně velkou část rybí moučky lze podle Abbakinda, 2012 citován v (Makkar et al. 2014) nahradit moučkou ze sarančete *Locusta migratoria* (Linnaeus, 1758) u tilápie nilské (*Oreochromis niloticus*). Cvrček domácí - *Acheta domestica* (Linnaeus, 1758) sice doposud nebyl v chovu ryb testován, nicméně – vzhledem ke známé a velice příznivé nutriční hodnotě – má v budoucnu potenciál využití v této oblasti (Henry et al. 2015).

Drůbež

Hmyz patří mezi přirozenou složku potravy divoce žijící hrabavé drůbeže, a proto je drůbež považována z hlediska inkluze hmyzího proteinu do krmných směsí za skupinu velice kompatibilní. Schiavone et al. (2017) nahradili sójový olej v krmné směsi pro kuřecí brojlery tukem extrahovaným z larev dvoukřídlých bráněnek *Hermetia illucens*. Na základě dosažených výsledků pak zjistili, že v průběhu výkrmu nedošlo k negativní změně v přírůstcích konverzi, kvalitě ani palatabilitě produktu. Předkládané krmivo ovlivnilo poměr mastných kyselin, kdy se zvýšilo množství nasycených na úkor polynenasycených mastných kyselin v prsní svalovině. Hladina mononenasycených kyselin pak zůstala stejná jako u kontrolní skupiny inkluze hmyzu. Extrahovanou moučku ze stejného druhu bráněnek využili ke svému experimentu ve výkrmu křepelčích brojlerů také Cullere et al. (2016), kteří po přidání této moučky sledovali senzorické změny na výsledném produktu, nicméně při nahrazení sójového šrotu do 15 % považují autoři tuto moučku za možnou plnohodnotnou alternativu. Sójový šrot nahradili z 12 resp. 24 % moučkou z bráněnek také Maurer et al. (2016), kteří se zaměřili na výživu nosnic Leghorn a též nenašli statisticky významný rozdíl mezi testovanou a kontrolní směsí v příjmu krmiva a nosnosti. Nicméně zvýšený výskyt průjmů v pokusných skupinách zvířat indikoval potenciál vedlejších účinků a dosud neznámých rizik dlouhodobého využití těchto směsí ve výživě zvířat. Dalším zástupcem dvoukřídlého hmyzu jehož efekt jakožto krmného komponentu byl v minulosti testován je moucha domácí (*Musca domestica*). Pozitivní efekt přidání larev tohoto druhu jako doplňku krmiva je popsán u slepic z domácího volného chovu, kde po jeho zahrnutí do krmné dávky došlo ke zvýšení přírůstků a také k dřívější snůšce (Dankwa et al. 2002). Dále byla krmná moučka z larev mouchy domácí testována ve výživě kuřecích brojlerů. Hwangbo et al. (2009) dosáhli využitím krmné směsi s hmyzí moučkou zvýšení přírůstků brojlerů ROSS 308 a to za stejné spotřeby krmiva, bez vlivu na posuzované parametry jatečně upravených těl. Na základě vyhodnocení výsledků považují Hwangbo et al. (2009) za nejvhodnější použití hmyzí moučky v rozmezí 10-15 %.

Đorđević et al. (2008) testovali záměnu 50 % rybí moučky za sušené, ale i živé larvy mouchy domácí, předkládané ve speciálně připravených krmítkách, a právě larvy mouchy domácí vykázaly nejlepší výsledky v přírůstku a konverzi krmiva. V porovnání s kontrolní skupinou se v této práci lepšími sledovanými znaky prezentovala směs s obsahem sušené hmyzí moučky a nejhorší charakteristiky pak paradoxně vyšly u kontrolních skupin, krmených směsí obsahující rybí moučku.

Dalším druhem hmyzu, který byl testován v této souvislosti, je brouk potemník moučný (*Tenebrio molitor*), respektive jeho larvy, a to v krmných směsích pro drůbež. Biasato et al. (2016) nahradili kukuřičnou složku krmiva za tento druh hmyzu, kdy konkrétně přidali do 1 kg krmné dávky slepic chovaných ve volném výběhu 75 g sušených larev. Zařazení hmyzu do krmiva ani v tomto případě nijak neovlivnilo přírůstky a porážkovou hmotnost, a na základě histologického i morfometrického profilu ani zdraví zvířat v pokusné skupině. Larvy potemníka moučného považuje za adekvátní náhradu sójové bílkoviny také Bovera et al. (2015), který navíc zjistil, že díky zvýšené koncentraci globulinů, způsobené pravděpodobně probiotickým účinkem chitinu, pozitivně působí na imunitní systém zvířat. Probiotický efekt moučky z potemníka moučného a jeho možné využití jako alternativy k běžným antibiotikům využívaných v intenzivních chovech drůbeže proti infekcím způsobených bakterií rodu *Salmonella* či *Eimeria* uvádějí též Islam & Yang (2017). Moučkou z potemníka moučného lze bez vedlejších efektů na životní charakteristiky, welfare vykrmovaných kuřecích brojlerů a kvalitu produktu nahradit až 10 % startérové výživy také dle (Ramos-Elorduy et al. 2002). Larvy dalšího zástupce čeledi potemníkovitých brouků, potemníka stájového - *Alphitobinus diaperinus* (Panzer, 1797) byly testovány jako možná kompletní náhrada bílkovinné složky ve startérové směsi. Bohužel, životní charakteristiky takto vykrmovaných kuřat byly v porovnání s běžným výkrmem horší a pokusná kuřata navíc dosáhla i nižší jatečné hmotnosti. Nicméně pokud byly larvy stejného druhu využity během startérové výživy jenom jako doplněk, efekt byl naopak pozitivní a po ukončení výkrmu byla hmotnost kuřat z testovací skupiny v porovnání s kontrolou vyšší (Despins & Axtell 1995). Ve výkrmu brojlerů byly dále experimentálně uplatněny také moučky z rovnokřídlého hmyzu. Adeyemo et al. (2008) ve svém pokusu nahradil 50 % rybí moučky v krmné směsi pro brojlery moučkou ze sarančete *Schistocerca gregaria* a zaznamenal zvýšení jatečné hmotnosti, přírůstků i snížení konverze krmiva. Rybí moučku zkoušeli z 20 % a 40 % nahradit sarančetem *Acrida cinerea* (Thunberg, 1815) také Liu & Lian (2003) a dosáhli s touto směsí totožných výsledků jako v kontrolní skupině za použití komerčních krmných směsí. Nakagaki et al. (1987) nahradili sójový šrot v krmné směsi moučkou z cvrčka domácího a takto krmení kuřecí brojleři dosáhli vyšší jatečné hmotnosti než při použití referenční směsi, a navíc při nižší spotřebě krmiva. Cvrček polní - *Gryllus testaceus* Walker, 1869 nebyl sice podroben přímému pokusu *in-vivo*, nicméně na základě rozboru nutriční hodnoty a srovnání s nutričními požadavky kuřecích brojlerů lze předpokládat, že by pro tento účel byl rovněž využitelný (Wang et al. 2005). Nahrazení rybí moučky drcenými larvami bource morušového - *Bombyx mori* (Linnaeus, 1758) do směsi pro závěrečnou fázi výkrmu brojlerů a také do směsi pro nosnice zkoušel Wijayasinghe (1977). V obou případech zaznamenali autoři lepší užitkovost v porovnání s kontrolní skupinou. Na základě vesměs pozitivních výsledků výzkumu Evropská komise naznačila, že by v roce 2019 měla povolit využití hmyzu v chovu drůbeže.

Prasata

Krmná moučka z larev bráněnek *Hermetia illucens* byla použita pro odstavená selata jako náhrada sušené plazmy (Newton et al. 1977), zatímco při nahrazení 50 % plazmy v pokusné skupině došlo k lepším přírůstkům a zlepšené konverzi krmiva, při kompletním nahrazení došlo ke snížení přírůstku až o 13 %. Dalším zástupcem ze skupiny dvoukřídlého hmyzu, který se podařilo experimentálně implementovat do výživy prasat, jsou larvy mouchy domácí (*Musca domestica*), které byly bez vedlejších efektů na celkovou konstituci a kondici zvířat použity jako náhrada rybí moučky a doplněk sójového šrotu (Viroje & Malin 1989) či jako náhrada pšeničného šrotu u odstavených selat (Adeniji 2008). Negativní vliv na přírůstek či zdraví odstavených selat nezjistili ani Jin et al. (2016), kteří naopak konstatují, že při dávce do 6 % dochází ke zlepšení celkové konstituce a to bez statisticky významných vlivů na imunitní systém. Coll et al. (1992) ve svém pokusu kompletně nahradili sójový šrot moučkou vyrobenou z kukel bource morušového na posledních 10 dní výkrmu. Došlo sice k poklesu příjmu potravy, ten však byl kompenzován lepším využitím živin a ve výsledku tak nedošlo k žádnému škodlivému vlivu na zdraví zvířat ani závadnosti výsledného produktu. Pozitivní výsledek, co se týče přírůstků ve stejné fázi výkrmu i kvality masa, zaznamenal zařazením sušených housenek bource morušového také Medhi (2011).

Přežvýkavci

Dosud nejméně informací je dostupných o potenciálu využití hmyzu při krmení přežvýkavců. Jediným druhem, který byl testován v souvislosti s krmením těchto zvířat, je bourec morušový, nicméně většina zdrojů hovoří o využití spíše v teoretické rovině na bázi znalosti nutriční hodnoty a potřeb zvířat (Ioselevich et al. 2004), případně in-vitro testech stravitelnosti (Chandrasekharaiah et al. 2004; Trivedy et al. 2008). Patrně jediným publikovaným pokusem in-vivo tak zůstává přidání sušených larev bource do krmné dávky kříženců merino landrace a skotské černohlavé ovce, kdy Ioselevich et al. (2004) dosáhli lepších výsledků než za využití kontrolní směsí, ale také upozorňují, že limitem použití tohoto hmyzu je vysoký obsah tuku. Doporučují před krmením larvy nejen zbavit hedvábných žláz, ale také odtučnit.

Zájmové chovy

Po celém světě je v lidské péči chováno velké množství insektivorních či omnivorních zvířat, bezobratlých i obratlovců, pro které je hmyz hlavní složkou potravy nebo zpestřením. Na rozdíl od hospodářských zvířat, nutriční požadavky těchto zvířat nejsou většinou příliš známé a dají se spíše odvozovat na základě determinace nutriční hodnoty předkládaného hmyzu (Barker et al. 1998). Na druhou stranu je většina druhů krmného hmyzu, který je za tímto účelem množen, je v chovech udržována dlouhodobě a znalosti metodiky chovu se mohou stát základem pro jejich produkci v průmyslovém měřítku. Nejčastěji zmiňovanými druhy v této souvislosti jsou pak brouci z čeledi potemníkovití, cvrčci domácí, sarančata (vesměs druhů zmíněných výše) a některé druhy švábů (Finke 2002; Oonincx et al. 2010; Finke & Oonincx 2013). Pro krmné účely se ovšem dají využít i další druhy hmyzu, jejichž nutriční hodnota je známa, jako jsou zavíječi voskoví (*Galleria mellonela*), octomilky (*Drosophoila melanogaster*), komáři rodu *Aedes* či pakomáři rodu *Chironomus* (Bernard et al. 1997).

3 Cíle práce

Cílem práce je popsat status hmyzu na počátku 21. století z hlediska změn biodiverzity a definovat účinná opatření, která by mohla změny zmírnit či zastavit.

Dílčí cíle:

- Hodnocení parametrů, které ovlivňují ohrožení motýlů.
- Definice postupů údržby vhodných pro ochranu stanovišť motýlů na základě jejich životního cyklu.
- Sledování a hodnocení invazních druhů hmyzu na území České republiky za účelem jejich včasné detekce a eradikace.
- Stanovení nutriční hodnoty různých druhů hmyzu a faktorů, které ji ovlivňují
- Zhodnocení potenciálu hmyzu stát se alternativním zdrojem živin pro lidskou populaci i hospodářská zvířata.

4 Publikované články

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[Vliv krajinného managementu na ochranu evropských motýlů: literární přehled]
Změny ve využití krajiny způsobené přechodem k intenzivnímu zemědělství a lesnictví během 20. století se negativně projevily na diverzitě motýlů v Evropě. Aby bylo možné tento negativní trend zvrátit, je nutné stanoviště ohrožených motýlů účinně chránit. Cílem této studie bylo podat ucelený souhrn dostupných informací o vlivu managementu lokality na evropské druhy motýlů a poskytnout tak doporučení pro vhodný management k podpoře lokálních populací. Hlavní hrozbou pro motýly je vegetační sukcese. Nejúčinnější hospodářské postupy, které tento jev potlačují, jsou pastva a mozaiková seč, tedy údržba, která při nižší intenzitě napodobuje tradiční zemědělství. Ty byly shledány jako vhodné pro nejvíce druhů motýlů. Potřebných narušení stanovišť lze dosáhnout také cíleným sešlapem (pastva, turistika, cyklistika, jízda na koni) nebo lokálním vypálením. Pro druhy vázané na biotopy řídkých lesů je pak vhodným opatřením kácení lesních pasek. Naopak odlesňování a meliorace luk byly shledány jako aktivity s nejvíce negativním vlivem na evropské populace motýlů a na stanovištích s motýly se jim proto doporučujeme vyhnout.

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REVIEW PAPER

Land management impacts on European butterflies of conservation concern: a review

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Abstract Recent land use changes, namely the intensification of agriculture and forestry as well as the abandonment of traditional grassland management methods, have resulted in the decline of butterfly diversity in Europe. Appropriate management of butterfly habitats is thus required in order to reverse this negative trend. The aim of our study was to review the available literary information concerning the effects of various types of management on European butterflies of conservation concern, and to provide practical recommendations for the management of butterfly habitats. Since vegetation succession is a major threat to butterfly populations, there is a need for activities to suppress this process. Extensive grazing and rotational mowing, which imitate the traditional way of meadow use, appear to be the most suitable management in this respect. Both grazing and mowing should optimally be of low intensity and follow a mosaic design, with different land fragments being successively used at different times. Habitat disturbance through trampling, either associated with grazing or various sporting activities (hiking, biking, horse riding), or through occasional small-area burning, also prove to be beneficial for many butterflies. In the case of woodland species, maintaining open habitats within forests (glades, clearings, wide road verges) and thinning forest stands is recommended. Among the unfavourable management activities identified, the most harmful are afforestation of open lands and drainage works. Therefore, such activities must be stopped at butterfly sites in order to ensure the effective conservation of species of conservation concern.

Keywords Agriculture · Biodiversity · Forestry · Habitat management · Land use · Species conservation

Introduction

European landscapes and their use by agriculture and forestry have been undergoing considerable changes in recent decades (Reidsma et al. 2006). These changes have typically led to the cessation of the traditional use of semi-natural habitats, causing either the complete abandonment of land or the introduction of intensive agriculture and forestry (Balmer and Erhardt 2000; Young et al. 2005). Both processes are considered prominent threats to biodiversity in Europe (Morris 2000; Benton et al. 2003; Saarinen and Jantunen 2005; Young et al. 2005). They have led to the destruction of many habitats as well as to the deterioration of the quality of the remaining habitat fragments (Begon et al. 2006). They have also affected the spatial structure of habitats, usually increasing their fragmentation, which threatens the survival of numerous species (Krauss et al. 2005; Pöyry 2007). One of the main groups of organisms negatively affected by these processes are butterflies (Öckinger and Smith 2006; Wenzel et al. 2006; Kőrösi et al. 2012).

On the other hand, through proper conservation-oriented land management we are able to enhance the chances of butterfly survival even in severely altered and fragmented landscapes. Management activities may improve the quality of habitat patches of individual species (Kruess and

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Tscharntke 2002; Sawchik et al. 2003; Wenzel et al. 2006). This is particularly true for many endangered butterflies, which have suffered from long-term land abandonment leading to meadow succession and subsequent penetration by shrubs and trees (Morris 2000; Hula et al. 2004; Pöyry et al. 2006).

However, high quality habitat patches do not always foster species occurrence. Even if a patch fulfils all of the species requirements, individuals will not occur there as long as the patch is too isolated and far beyond their ability to disperse (Schtickzelle et al. 2006). Therefore, it is important to maintain well-connected networks of habitat patches (Hanski et al. 1994, 1995; Thomas et al. 2001; Schtickzelle and Baguette 2003; Öckinger and Smith 2007). Again, proper land management may help in this respect by facilitating butterfly dispersal and thus reducing the impact of fragmentation of their habitat patches. This can be achieved either directly through creating corridors and stepping stone habitats (Haddad 1999; Skórka et al. 2013) or indirectly through promoting dispersal behaviour in butterflies (Begon et al. 2006). Dispersal distance and individual willingness to emigrate are key traits for the persistence of populations in fragmented landscapes (Schtickzelle et al. 2005; Fric et al. 2010; Hambäck et al. 2010; Zimmermann et al. 2011).

In our paper we have compiled and reviewed available information regarding the effects of different small-scale land management practices on endangered European butterfly species. By doing so, we aim to drawing general conclusions about their relative role, either positive or negative, in shaping butterfly communities. We also attempt to provide conservation recommendations based on the outcome of our review.

We focused on management activities applicable at the small-scale of nature reserves or Natura 2000 sites, because we believe that such a scale is the most relevant for successful butterfly conservation. First of all, populations of most European butterflies typically exist in relatively small local populations expanding over a few to few tens of hectares (Warren 1992). Apart from this, small-scale conservation actions, following the principle of "think globally, act locally", proved to be more effective for butterflies (cf. Thomas et al. 2011), although obviously their applicability (or preventing in the case of unfavourable management) is affected by large-scale environmental policies.

Review approach

We conducted a comprehensive search for research papers dealing with the effects of various types of land management on butterflies in Europe, using the Web-of-Knowledge (http://apps.webofknowledge.com/) and Scopus (http://www.scopus.com/) databases. Wherever possible, we additionally supplemented the information gathered in this way with the material from relevant 'grey literature' known to us.

We focused our review primarily on species of conservation concern that are listed in the European Red List of Butterflies, including those classified as Near Threatened (van Swaay et al. 2010). There is a clear discrepancy in the scientific literature dealing with the conservation of European butterflies, with many papers from Northern and Western Europe, and much lower numbers of those from other parts of the continent. We partly mitigated this problem by including a large bulk of local literature or even unpublished reports, mostly from Central and Eastern Europe. Nevertheless, as many local publications are not easily accessible and/or published in national languages unknown to us, some biases in geographical coverage of our review still remain. In particular, the amount of information we have managed to collect for butterflies with distribution ranges restricted to the Alpine and Mediterranean regions is not as large as that available for species from other regions. However, we strongly believe that the material we have gathered is extensive and comprehensive enough (>100 papers representing almost all European countries) to allow drawing general conclusions about the impacts of various types of habitat management.

We classified the management types into two categories: favourable or unfavourable for butterflies. Their impacts on butterflies of conservation concern have been summarised in Tables 1 and 2. In turn, Fig. 1 presents the relative importance of different management types for butterflies. Below, we discuss their effects in a systematic way.

Favourable management

Rotational mowing

One of the most effective ways for the conservation for endangered butterflies through meadow management is rotational mosaic mowing, usually complemented by extensive grazing as described below (Saarinen and Jantunen 2005; Farruggia et al. 2012). Rotational mosaic mowing implies successive mowing of different meadow fragments (Morris and Rispin 1987; Saarinen and Jantunen 2005; Novák et al. 2007; Gaisler et al. 2011). This mowing method resembles traditional meadow management (Pöyry 2007), the abandonment of which has led to the decline of numerous meadow specialists, including the endangered *Colias myrmidone* (Esper, 1781) (Konvička et al. 2008a) or charismatic large blue butterflies of the genus *Maculinea* (=*Phengaris*), which are flagships of grassland conservation in Europe (Thomas et al. 2009). The future survival of the aforementioned species is dependent on the application

Table 1 Positive effects of habitat management on European butterflies of conservation concern documented in the literature

Species	Status	Favourable management type					
		Rotational mowing	Extensive grazing	Trampling	Occasional burning	Fallowing	Maintenance of sparse forest stands
Archon apollinus	NT		80				
Aricia anteros	NT					72	
Boloria chariclea	NT	9					
Boloria titania	NT	13, 73	13				
Carcharodus flocciferus	NT	24, 4	24				
Carcharodus lavatherae	NT	86, 4	14, 86				
Chazara briseis	NT		35, 38, 39	26, 35			
Coenonympha hero	VU	11		10			
Coenonympha oedippus	EN	62, 69	17				
Coenonympha orientalis	VU						50
Coenonympha phryne	CR	85	86				
Coenonympha tullia	VU	94	85, 94		20	94	
Colias chrysotheme	VU		48	85, 4			
Colias hecla	NT	85	85				
Colias myrmidone	EN	45	45, 78, 90				
Cupido decoloratus	NT			4, 5			
Erebia christi	VU		52				
Erebia claudina	NT		85				85
Erebia epistygne	NT		87				87
Erebia flavofasciata	NT		15				50
Erebia sudetica	VU						4, 47, 51
Euphydryas desfontainii	NT		63, 63	64			
Euphydryas iduna	NT						49
Euphydryas maturna	VU						1, 19, 29, 93
Gonepteryx cleobule	VU						50
Gonepteryx maderensis	EN						85
Hipparchia bacchus	VU					50	
Hipparchia fagi	NT		66	4	57		
Hipparchia hermione	NT		4		4		4, 66
Hipparchia statilinus	NT		4	65	4		
Hipparchia tilosi	VU						50
Iolana iolas	NT		58	68	58		
Leptidea morsei	NT						4, 18, 34
Lopinga achine	VU		4				6, 7, 46, 77
Lycaena helle	EN	4, 28, 30	3, 30, 31	75			
Maniola halicarnassus	NT	50					
Melitaea aurelia	NT	4	4, 27	27, 40			
Melitaea britomartis	NT		4, 12				
Muschampia cribrellum	NT	41	22				
Pararge xiphia	EN		71				37
Parnassius apollo	NT		8, 70	70			
Parnassius mnemosyne	NT						43, 55, 84
Parnassius phoebus	NT	85					
Phengaris arion	EN	74, 76	76, 82	82			
Phengaris nausithous	NT	32, 36, 59	59	44	60		
Phengaris teleius	VU	36, 83	32, 59, 96, 97	44	60		

Table 1 continued

Species	Status	Favourable management type					
		Rotational mowing	Extensive grazing	Trampling	Occasional burning	Fallowing	Maintenance of sparse forest stands
Plebejus dardanus	NT			50			
Plebejus pylaon	NT	58	4, 58		58		
Plebejus trappi	NT		85				
Plebejus zullichi	EN					2	
Polyommatus eros	NT		95				
Polyommatus galloi	VU		23				
Polyommatus humedasae	EN			87, 89	87		
Polyommatus nephohiptamenos	NT	85					85
Polyommatus damon	NT	4	25	79			
Polyommatus dorylas	NT		86				
Polyommatus nivescens	NT	89					
Polyommatus orphicus	VU				42		
Pseudochazara amymone	VU	16	16				92
Pseudochazara cingovskii	CR			92			
Pseudochazara euxina	EN		50				
Pseudochazara orestes	VU						85
Pseudophilotes panoptes	NT		61, 68	61			
Pseudophilotes vicrama	NT		4, 31, 86	4			
Pyrgus cirsii	VU	33	85				
Thymelicus acteon	NT	4, 81				56	
Tomares nogelii	VU	21					85
Turanana taygetica	EN			89		89	
Zerynthia cerisy	NT	53, 54					

The species conservation status follows the European Red List of Butterflies (van Swaay et al. 2010): *CR* critically endangered, *EN* endangered, *VU* vulnerable, *NT* near threatened. Numbers in the table refer to the papers reporting the effects: (1) AOPK (2011); (2) Barea-Azcón et al. (2014); (3) Bauerfeind et al. (2009); (4) Beneš et al. (2002); (5) Beneš et al. (2003); (6) Bergman (1999); (7) Bergman (2001); (8) Bohlin et al. (2008); (9) Britten and Brussard (1992); (10) Cassel et al. (2001); (11) Cassel et al. (2008); (12) Cerrato et al. (2014); (13) Cozzi et al. (2008); (14) Coutsis and Ghavalás (2001); (15) Cupedo (2000); (16) Cuvelier and Mølgaard (2015); (17) Čelik et al. (2009); (18) Čelik (2013); (19) Čizek and Konvička (2005); (20) Dennis and Eales (1997); (21) Dincă et al. (2009); (22) Dincă et al. (2010); (23) Dinca et al. (2013); (24) Dolek and Geyer (1997); (25) Dolek and Geyer (2002); (26) Dover and Settele (2009); (27) Eichel and Fartmann (2008); (28) Fischer et al. (1999); (29) Freese et al. (2006); (30) Goffart et al. (2010); (31) Grill and Cleary (2003); (32) Grill et al. (2008); (33) Guillaumin (1972); (34) Höttinger (2004); (35) Johannesen et al. (1997); (36) Johst et al. (2006); (37) Jones and Lace (1992); (38) Kadlec et al. (2009); (39) Kadlec et al. (2010); (40) Kleyer et al. (2007); (41) Kolev (2003); (42) Kolev (2005); (43) Konvička and Kuras (1999); (44) Konvička et al. (2005); (45) Konvička et al. (2008a); (46) Konvička et al. (2008b); (47) Konvička et al. (2014); (48) Korb (1994); (49) Kozlov and Kullberg (2008); (50) Kudrna et al. (2015); (51) Kuras et al. (2003); (52) Leigheb et al. (1998); (53) Lelo and Spasojević (2012); (54) Lelo (2000); (55) Luoto et al. (2001); (56) Louy et al. (2007); (57) Möllenbeck et al. (2009); (58) Munguira and Martín (1993); (59) Novák et al. (2007); (60) Nowicki et al. (2015); (61) Obregón et al. (2014); (62) Örvössy et al. (2013); (63) Pennekamp et al. (2013); (64) Pennekamp et al. (2014); (65) Pinzari (2009); (66) Pinzari and Sbordoni (2013); (67) Rabasa et al. (2007); (68) Settele et al. (2008);(69) Settele (2010); (70) Schmeller et al. (2011); (71) Shreeve and Smith (1992); (72) Schurian (1995); (73) Schweiger et al. (2008); (74) Sielezniew and Rutkowski (2012); (75) Skórka et al. (2007); (76) Spitzer et al. (2009); (77) Streitberger et al. (2012); (78) Szentirmai et al. (2014); (79) Šlancarová et al. (2012); (80) Šlancarová et al. (2015); (81) Thomas et al. (1992); (82) Thomas (1995); (83) Thomas et al. (2009); (84) Välimäki and Itämies (2003); (85) van Swaay and Warren (1999); (86) van Swaay (2002); (87) van Swaay et al. (2010); (88) van Swaay et al. (2012); (89) van Swaay et al. (2011); (90) Verovnik et al. (2011); (91) Verovnik et al. (2013); (92) Verovnik et al. (2014); (93) Vrabec (2001); (94) Weking et al. (2013); (95) Wiemers et al. (2010); (96) Witek et al. (2010); (97) Witek et al. (2011)

of meadow management, which should follow the principles of rotational mosaic mowing. These principles involve (i) relatively low mowing intensity, with a single fragment being mown no more than once per year, and (ii) mowing different fragments at different times in order to ensure heterogeneous turf height within meadows (Morris 2000). A higher mowing frequency may be beneficial for xerophilous species, which prefer short vegetation, e.g.,

Table 2 Negative effects of habitat management on European butterflies of conservation concern documented in the literature

Species	Status	Unfavourable management type			
		Afforestation	Drainage	Intensive agriculture	Intensive forestry
Aricia anteros	NT			30, 70	
Boloria chariclea	NT			71	
Boloria improba	EN			80	
Boloria polaris	VU			47	
Boloria titania	NT		12		
Carcharodus flocciferus	NT			4, 20, 45	
Carcharodus lavatherae	NT	4, 81		81	
Chazara briseis	NT	31		34, 66	
Coenonympha hero	VU	10		9	
Coenonympha oedippus	EN	65, 79	56, 65, 79	13, 56	
Coenonympha phryne	CR	80		80, 85	
Coenonympha tullia	VU	16, 61, 90	16, 32, 33		
Colias chrysotheme	VU	4, 80		80	
Colias myrmidone	EN	41, 74		23, 41	
Cupido decoloratus	NT	4			
Erebia christi	VU				84
Erebia claudina	NT			80	
Erebia epistygne	NT			15	
Erebia sudetica	VU	43, 48		43	
Euchloe bazae	VU			80	
Euphydryas desfontainii	NT		57, 58	58	
Euphydryas iduna	NT	46	46		
Euphydryas maturna	VU	24, 40, 88		1, 80, 88	
Gonepteryx cleobule	VU			47	
Gonepteryx maderensis	EN		80		80
Hipparchia bacchus	VU			47	
Hipparchia fagi	NT	53, 60, 68		53	
Hipparchia hermione	NT	4, 60			
Hipparchia leighebi	NT			47, 82	
Hipparchia sbordonii	NT			82	
Hipparchia statilinus	NT	59		4, 59	
Iolana iolas	NT	62		62, 63	
Leptidea morsei	NT	14, 29		14, 80	
Lopinga achine	VU	5, 42		36, 73	
Lycaena helle	EN	3, 27	28	3, 25, 27	
Melitaea aurelia	NT	21, 64		45	
Melitaea britomartis	NT	11, 44, 45	11	4	
Muschampia cribrellum	NT			18, 37	
Oeneis norna	NT		6		
Pararge xiphia	EN	69		69	
Parnassius apollo	NT	17, 50, 55			50
Parnassius mnemosyne	NT	39, 78			39, 51, 52
Parnassius phoebus	NT	80		80	
Phengaris arion	EN		8	72	
Phengaris nausithous	NT		22, 35	91	
Phengaris teleius	VU		22, 35	91	
Pieris cheiranthi	EN		80		

Table 2 Negative effects of habitat management on European butterflies of conservation concern documented in the literature

Species	Status	Unfavourable management type			
		Afforestation	Drainage	Intensive agriculture	Intensive forestry
Aricia anteros	NT			30, 70	
Boloria chariclea	NT			71	
Boloria improba	EN			80	
Boloria polaris	VU			47	
Boloria titania	NT		12		
Carcharodus flocciferus	NT			4, 20, 45	
Carcharodus lavatherae	NT	4, 81		81	
Chazara briseis	NT	31		34, 66	
Coenonympha hero	VU	10		9	
Coenonympha oedippus	EN	65, 79	56, 65, 79	13, 56	
Coenonympha phryne	CR	80		80, 85	
Coenonympha tullia	VU	16, 61, 90	16, 32, 33		
Colias chrysotheme	VU	4, 80		80	
Colias myrmidone	EN	41, 74		23, 41	
Cupido decoloratus	NT	4			
Erebia christi	VU				84
Erebia claudina	NT			80	
Erebia epistygne	NT			15	
Erebia sudetica	VU	43, 48		43	
Euchloe bazae	VU			80	
Euphydryas desfontainii	NT		57, 58	58	
Euphydryas iduna	NT	46	46		
Euphydryas maturna	VU	24, 40, 88		1, 80, 88	
Gonepteryx cleobule	VU			47	
Gonepteryx maderensis	EN		80		80
Hipparchia bacchus	VU			47	
Hipparchia fagi	NT	53, 60, 68		53	
Hipparchia hermione	NT	4, 60			
Hipparchia leighebi	NT			47, 82	
Hipparchia sbordonii	NT			82	
Hipparchia statilinus	NT	59		4, 59	
Iolana iolas	NT	62		62, 63	
Leptidea morsei	NT	14, 29		14, 80	
Lopinga achine	VU	5, 42		36, 73	
Lycaena helle	EN	3, 27	28	3, 25, 27	
Melitaea aurelia	NT	21, 64		45	
Melitaea britomartis	NT	11, 44, 45	11	4	
Muschampia cribrellum	NT			18, 37	
Oeneis norna	NT		6		
Pararge xiphia	EN	69		69	
Parnassius apollo	NT	17, 50, 55			50
Parnassius mnemosyne	NT	39, 78			39, 51, 52
Parnassius phoebus	NT	80		80	
Phengaris arion	EN		8	72	
Phengaris nausithous	NT		22, 35	91	
Phengaris teleius	VU		22, 35	91	
Pieris cheiranthi	EN		80		

Table 2 continued

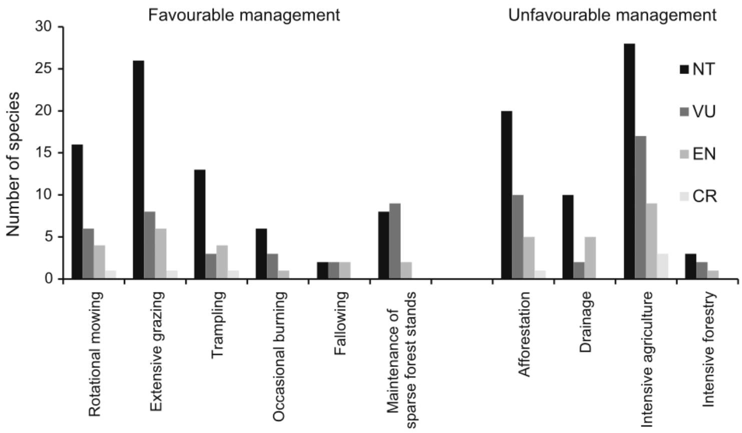
Species	Status	Unfavourable management type			
		Afforestation	Drainage	Intensive agriculture	Intensive forestry
Pieris wollastoni	CR			82	
Plebejus dardanus	NT			47	
Plebejus pylaon	NT			53	
Plebejus trappi	NT		80	80	
Plebejus zullichi	EN			53	
Polyommatus eros	NT	89		89	
Polyommatus galloi	VU			19	
Polyommatus golgus	VU			47, 89	
Polyommatus humedasae	EN	80			
Polyommatus nephohiptamenos	NT		80	80	
Polyommatus damon	NT	4, 75	4	75	
Polyommatus dorylas	NT	80			
Polyommatus nivescens	NT	82			
Polyommatus orphicus	VU	38		38	
Polyommatus violetae	VU	47		47	
Pseudochazara amymone	VU			87	
Pseudochazara cingovskii	CR			87	
Pseudochazara euxina	EN			47	
Pseudophilotes panoptes	NT	55		55, 82	
Pseudophilotes vicrama	NT	4, 26			
Pyrgus cirsii	VU	80		80	
Thymelicus acteon	NT			49, 77, 76	4
Tomares nogelii	VU	17, 80		17	80
Turanana taygetica	EN			82	
Zegris eupheme	NT			47	
Zerynthia cerisy	NT		2		

The species conservation status follows the European Red List of Butterflies (van Swaay et al. 2010): *CR* critically endangered, *EN* endangered, *VU* vulnerable, *NT* near threatened. Numbers in the table refer to the papers reporting the effects: (1) AOPK (2011); (2) Atay (2012); (3) Bauerfeind et al. (2009); (4) Beneš et al. (2002); (5) Bergman (1999); (6) Bolotov (2011); (7) Brommer and Fred (1999); (8) Casacci et al. (2011); (9) Cassel and Tammaru (2003); (10) Cassel et al. (2008); (11) Cerrato et al. (2014); (12) Cozzi et al. (2008); (13) Čelik et al. (2009); (14) Čelik (2013); (15) de Arce-Crespo et al. (2009); (16) Dennis and Eales (1997); (17) Dincă et al. (2009); (18) Dincă et al. (2010); (19) Dinca et al. (2013); (20) Dolek and Geyer (1997); (21) Eichel and Fartmann (2008); (22) Elmes et al. (1998); (23) Freese et al. (2005); (24) Freese et al. (2006); (25) Goffart et al. (2010); (26) Grill and Cleary (2003); (27) Habel et al. (2011a); (28) Habel et al. (2011b); (29) Höttinger (2004); (30) Hüseyinoğlu (2013); (31) Johannesen et al. (1997); (32) Joy and Pullin (1997); (33) Joy and Pullin (1999); (34) Kadlec et al. (2009); (35) Kajzer-Bonk et al. (2013); (36) Kodandaramaiah et al. (2012); (37) Kolev (2003); (38) Kolev (2005); (39) Konvička and Kuras (1999); (40) Konvička et al. (2005); (41) Konvička et al. (2008a); (42) Konvička et al. (2008b); (43) Konvička et al. (2014); (44) Koren et al. (2011); (45) Koren and Jugovic (2012); (46) Kozlov and Kullberg (2008); (47) Kudrna et al. (2015); (48) Kuras et al. (2003); (49); Louy et al. (2007); (50) Łozowski et al. (2014); (51) Luoto et al. (2001); (52) Meier et al. (2005); (53) Möllenbeck et al. (2009); (54) Munguira and Martín (1993); (55) Obregón et al. (2014); (56) Örvössy et al. (2013); (57) Pennekamp et al. (2013); (58) Pennekamp et al. (2014); (59) Pinzari (2009); (60) Pinzari and Sbordoni (2013); (61) Pocewicz et al. (2009); (62) Rabasa et al. (2007); (63) Rabasa et al. (2008); (64) Sang et al. (2010); (65) Settele (2010); (66) Seufert and Grosser (1996); (67) Schmeller et al. (2011); (68) Schmitt and Rákosy (2007); (69) Shreeve and Smith (1992); (70) Schurian (1995); (71) Simonsen (2005); (72) Spitzer et al. (2009); (73) Streitberger et al. (2012); (74) Szentirmai et al. (2014); (75) Šlancarová et al. (2012); (76) Thomas (1995); (77) Thomas et al. (2001); (78) Välimäki and Itämies (2003); (79) van Halder et al. (2008); (80) van Swaay and Warren (1999); (81) van Swaay (2002); (82) van Swaay et al. (2011); (83) van Swaay et al. (2010); (84) van Swaay et al. (2012); (85) Verovnik et al. (2013); (86) Verovnik et al. (2013); (87) Verovnik et al. (2014); (88) Vrabec (2001); (89) Wiemers et al. (2010); (90) Weking et al. (2013); (91) Wynhoff et al. (2011)

Coenonympha phryne (Pallas, 1771) (van Swaay and Warren 1999).

Mowing should optimally take place outside the flight periods of target butterfly species so as to maintain high availability of nectar sources for their imagoes, and a sufficient number of host plants for oviposition (Johst et al. 2006; Mládek et al. 2006; Dover et al. 2010; Wynhoff et al. 2011). This may be a serious limitation if several target

Fig. 1 Numbers of European butterfly species of conservation concern affected by various types of habitat management. *Shades of gray* indicate different conservation status according to the European Red List of Butterflies (van Swaay et al. 2010): *NT* near threatened, *VU* vulnerable, *EN* endangered, *CR* critically endangered



species occur sympatrically at the same site, which is frequently the case with *Maculinea* butterflies (Sliwinska et al. 2006). In fact, mowing timing must be further restricted in the case of *Maculinea* butterflies due to their myrmecophilous lifestyle (Thomas 1995; Witek et al. 2011). Since the adoption of larvae by ants is a key process for *Maculinea* survival (Thomas 1995; Witek et al. 2010; Sielezniew and Rutkowski 2012), mowing should be conducted only after adoption occurs, i.e., in the second half of September at the earliest (Grill et al. 2008); this guideline likely applies for the conservation of other myrmecophilous butterflies. Furthermore, strong association with ants, which depend on microhabitat conditions in the soil (Elmes et al. 1998), precludes the use of mulching mowers for the management of sites inhabited by myrmecophilous species (Marhoul and Turoňová 2007). In general, mulching is the most devastating method of mowing meadows (Humbert et al. 2010), and it should be discouraged. In addition, Humbert et al. (2010), who investigated the effects of different moving techniques on meadows, found that using motor bar mowers is much better than utilizing rotary mowers.

Extensive grazing

Historically, extensive grazing was applied in grasslands and woodlands together with other types of management. It constituted an effective way of suppressing vegetation succession, thus improving the quality of habitats for numerous butterfly species. In order to benefit butterfly communities, grazing has to be appropriately planned according to its load (i.e., number of livestock units per area unit), types of grazing animals, and grazing period (Morris 2000; Háková et al. 2005; Pöyry et al. 2006). A generally accepted rule is that the optimal sampling intensity should be 0.2 livestock units per hectare, and it should not exceed 0.5 livestock units; this was proved by various studies, such as those on *Colias myrmidone* (Konvička et al. 2008a) *Carcharodus flocciferus* (Zeller, 1847) (Dolek and Geyer 1997), *Euphydryas desfontainii* (Godart, 1819) (Pennekamp et al. 2013), *Parnassius apollo* (Linneaus, 1758) (Schmeller et al. 2011).

The principle that grazing intensity needs to be limited is well exemplified in the endangered *Lycaena helle* (Denis & Schiffermüller, 1775) (Habel et al. 2011b). The species is a typical meadow specialist, inhabiting humid, seminatural meadows, which were historically maintained by grazing and haymaking (Konvička et al. 2005; Bauerfeind et al. 2009). The introduction of intensive grazing or mowing resulted in local extinctions of the species. Conversely, leaving such sites without any management leads to meadow overgrowth and the disappearance of the species habitats in the long term perspective (Hula et al. 2004; Habel et al. 2010). An appropriate method of management for *Lycaena helle* involves reducing the intensity of grazing and introducing a mosaic mowing, thereby achieving an imitation of traditional farming methods that used to maintain fine-grained mosaic landscapes with different managements (Skórka et al. 2007). This system is also appropriate for other endangered butterflies, such as *Melitaea aurelia* (Nickerl, 1850) (Kleyer et al. 2007). In contrast, Eichel and Fartmann (2008) argued that intensive grazing can also be beneficial for this species as long as it is done once in a few years and some land fragments are left ungrazed.

The type of farm animals kept is important due to the different ways they graze. Sheep grazing has been shown to have a negative impact on the near threatened species *Polyommatus damon* (Dolek and Geyer 2002). In turn for *Pseudophilotes vicrama* (Christoph, 1887) it is optimal to extensively graze goats and sheep within large fenced enclosures, and to gradually move them over a wide area (Beneš et al. 2002; Grill and Cleary 2003). A related species, *Polyommatus dorylas* (Denis & Schiffermüller, 1775), requires extensive grazing combined with the active removal of bushes and tree seedlings; in contrast, intensive grazing by sheep threatens the persistence of the species (Beneš et al. 2002; van Swaay 2002). The timing and duration of grazing are also important factors (Morris 2000). At localities with endangered butterfly species present it should not be applied during the late spring to mid-summer period as it reduces the availability of larval foodplants and nectar sources for adults. Conversely, grazing is most appropriate during the autumn (September–November) and spring (April) (Konvička et al. 2005).

Trampling

Regular trampling can locally prevent the establishment of vegetation and thus it can supress succession. Historically, butterfly site trampling was caused by grazing animals (Morris 2000; WallisDeVries and Reemakers 2001; Kruess and Tscharntke 2002). Nowadays, artificial trampling by horseback riding, biking or hiking offers a simple and typically costless alternative, which helps to maintain butterfly habitats in early successional stages (Konvička et al. 2005). One species that apparently benefits from trampling is the near threatened *Chazara briseis* (Linnaeus, 1758), which inhabits steppe-like grasslands (Johannesen et al. 1997). After the penetration of its sites by sheep ceased, the species suffered a serious decline in the Czech Republic, most likely due to the expansion of shrubs (Kadlec et al. 2009). The trampling of habitat patches, either through grazing or through various adventurous sports, is also necessary for the near threatened species *Hipparchia statilinus* (Hufnagel, 1766) (Beneš et al. 2002; Pinzari 2009). Contrarily, high intensity trampling can also be detrimental for butterfly populations, as was shown in the case of *Erebia sudetica* (Staudinger, 1861) (Kuras et al. 2003).

Occasional burning

Occasional burning may, in some cases, be beneficial for butterfly populations (McIver and Macke 2014). Burning reduces the expansion of shrubs, and it is particularly useful for vast and abandoned areas. As a disturbance event, burning typically exerts long-term positive consequences by suppressing succession, but it has a negative impact on the affected populations in the short term (Wolf 2002). However, Nowicki et al. (2015) found absolutely no short-term negative impacts of large-scale fires on the metapopulations of *Maculinea teleius* and *M. nausithous*. In any case, two basic rules of the thumb should be followed when applying burning as a conservation management tool in order to minimalize possible negative short-term effects. Firstly, small fragments of land should be left unburnt to serve as refuges from which the neighbouring burnt fragments of land can be recolonized (Konvička et al. 2005; Nowicki et al. 2015). Apart from this, burning should occur in seasons when it is likely to be least harmful, i.e., in winter or early spring. Among other examples, burning in winter months has already been successfully applied for the management of sites occupied by *Pseudophilotes vicrama* (Moore, 1865), *Coenonympha tullia* (Müller, 1764), or *Hipparchia fagi* (Scopoli, 1763) (Dennis and Eales 1997; Marttila et al. 1997; Möllenbeck et al. 2009).

Fallowing

Although (as discussed previously) succession at grassland habitats usually has a negative effect on butterfly communities, there are cases in which vegetation succession can be considered advantageous in its early stages (Skórka et al. 2007; Schirmel and Fartmann 2014). This is particularly true for a relatively large group of butterflies that benefit from the occurrence of high vegetation or shrubs within their grassland habitats. For example, overgrown localities with high grasses and abundant shrubs are optimal sites for *Lycaena helle* (Skórka et al. 2007; Habel et al. 2011b), and *Carcharodus lavatherae* (Esper, 1783) (Coutsis and Ghavalás 2001). *Thymelicus acteon* (Rottemburg, 1775) is another species that profits from succession in its early stages (Beneš et al. 2002; Louy et al. 2007). In all such cases, fallowing may constitute a viable management option; however, it can be utilized only for a limited time period since the continuation of succession, beyond a certain stage, inevitably results in habitat quality deterioration (Skórka et al. 2007).

Maintenance of sparse forest stands

A majority of woodland butterflies are, in fact, restricted to open habitats within woodlands, which in recent decades have become rare. The reason for this is the abandonment of traditional methods of forest utilization, such as regular clearcuts, tree stand thinning, and forest grazing. The absence of these activities has led to closing of tree canopies and changes in forest vegetation (Kodandaramaiah et al. 2012). Consequently, a number of woodland butterfly species are now endangered in Europe. One such species is *Euphydryas maturna* (Linnaeus, 1758), which requires insulated glades, sunny spots with young ash trees, and a high availability of nectar plants for its survival (Vrabec 2001; AOPK 2011). Similarly, vulnerable butterflies such as *Lopinga achine* (Scopoli, 1763), *Leptidea morsei*

(Fenton, 1882), and *Parnassius mnemosyne* (Linnaeus, 1758) need open and sunny habitats within forests, including sparse stands, clearings or road margins (Konvička and Kuras 1999; Luoto et al. 2001; Välimäki and Itämies 2003; Höttinger 2004; Konvička et al. 2008b; Streitberger et al. 2012). Finally, open habitats are also vital for woodland species with caterpillars using grasses as host plants, for instance *Hipparchia hermione* (Linnaeus, 1764) (Beneš et al. 2002; Pinzari and Sbordoni 2013) and *Erebia sudetica* (Staudinger, 1861) (Kuras et al. 2001, 2003).

Prescribed forest management is therefore essential for the conservation of most woodland butterflies. Recommended measures should include opening canopies, supressing the growth of tree seedlings within forest glades, supporting forest grazing and promoting coppice management (Slámová et al. 2013). Optimally, tree density should be low enough to allow open spots, which are spaced at least every 300 meters and interconnected with forest roads and clearings (Marhoul and Turoňová 2007). Coppicing, i.e., forest use focused on the production of relatively small diameter wood, for a range of uses including firewood, together with grazing ensured a diverse mosaic of forest microhabitats and created suitable sites for woodland butterflies in the past (Buček 2010). Since both activities are no longer economically viable, financial incentives may be needed to trigger them. Maintaining a network of forest roads with wide margins and strips of herb-rich grassland at forest edges is also recommended (Marhoul and Turoňová 2007).

Unfavourable management

Afforestation

The afforestation of formerly open habitats began at the turn of the 18th and 19th centuries, along with the development of modern forestry (Konvička et al. 2005). Currently, afforestation of grassland habitats poses one of the biggest problems for butterflies in Europe, threatening numerous species of conservation concern (van Swaay and Warren 2006; Cassel et al. 2008; Augenstein et al. 2012; Cerrato et al. 2014). It is thus highly regretful that this process is often supported by land management authorities through financial incentives in the form of afforestation grants (MZE 2001). Apart from the direct loss of grassland habitats (van Swaay and Warren 2006), the negative consequences of afforestation stem from the increased fragmentation of remaining habitat patches. Because grassland butterflies have difficulties dispersing through forested landscapes (Nowicki et al. 2014), the effective isolation of existing populations increases, causing overall declines of metapopulations (van Swaay and Warren 2006; Augenstein et al. 2012). *Parnassius apollo* (Linnaeus, 1758) and *Pseudophilotes vicrama* (Moore, 1865) are typical examples of butterfly species that suffer from afforestation (Grill and Cleary 2003; Schmeller et al. 2011).

Drainage

Deliberate drainage or any other processes that drain soil, such as construction works in the vicinity of wet habitats, is a common problem for endangered butterfly conservation (WallisDeVries and Ens 2010; Kati et al. 2012). Wet meadow specialists, such as *Melitaea britomartis* (Assmann, 1847) (Cerrato et al. 2014) or *Coenonympha tullia*, are particularly threatened by drainage because their host plants depend on adequate soil water levels (Dennis and Eales 1997). The same is true for myrmecophilous species such as *Maculinea* butterflies (Elmes et al. 1998). While drainage in the past was primarily conducted in order to increase the area of arable land, nowadays it is typically imposed as a flood prevention measure, also within protected areas (Mládek et al. 2006). However, there is little justification for such actions, at least from the conservation point of view. A recent study by Kajzer-Bonk et al. (2013) proved that a large-scale flood had absolutely no negative impact on the metapopulations of *Maculinea nausithous* and *M. teleius*, which provides a strong argument against 'conservation-oriented' drainage works.

Intensive agriculture

In many European countries, the current agriculture policy focuses on the intensification of land use and the application of modern agrotechnical methods in order to maximise economic benefits (Mládek et al. 2006; Pöyry 2007). Obviously, any conversion of former grassland habitats to cultivated farmlands will always imply habitat destruction for grassland butterflies (Konvička et al. 2005). Furthermore, the excessive use of insecticides within farmlands has a negative impact on butterfly communities in neighbouring areas (van Swaay and Warren 2006). Nevertheless, even traditional meadow management in the form of mowing and/or grazing may play a negative role if its intensity is too high. Several studies have demonstrated that intensive grazing and mowing lead to a decrease in butterfly species abundance (Balmer and Erhardt 2000; Hula et al. 2004; Saarinen and Jantunen 2005). This negative effect is caused by a significant reduction in the availability of larval host plants. Therefore, meadow specialists with strict trophic requirements, e.g., *Maculinea arion* (Casacci et al. 2011), *M. nausithous, M. teleius* (Witek et al. 2010), and *Coenonympha hero* (Cassel and Tammaru 2003), tend to suffer most. Intensive agriculture has a negative effect on other species as well, with *Coenonympha oedippus* (Fabricius, 1787) (Örvössy et al. 2013), *Muschampia cribrellum* (Eversmann, 1841) (Dincă et al. 2010), and *Plebejus pylaon* (Fischer, 1832) (Munguira and Martín 1993) serving as examples.

Intensive forestry

Just as farming intensification decimates the populations of many grassland butterflies, forestry intensification can also bring about negative consequences for their forest dwelling counterparts. Intensive forestry has caused the abandonment of traditional practices, such as forest grazing and coppicing that benefited butterfly communities in the past (Slámová et al. 2013). There are numerous species of conservation concern among butterflies negatively affected by forestry intensification, including *Coenonympha tullia, Erebia sudetica*, *Euphydryas maturna*, *Hipparchia hermione*, *Leptidea morsei, Lopinga achine* and *Parnassius mnemosyne* (Dennis and Eales 1997; Luoto et al. 2001; Kodandaramaiah et al. 2012; Streitberger et al. 2012; Čelik 2013; Pinzari and Sbordoni 2013; Konvička et al. 2014). To reverse the current negative trends for all these species, changes in forestry management are highly desirable. Forest management must not be focused exclusively on maximising economic benefits from wood production. Specifically, forest stands should be thinned and occasional sunny enclaves should be created.

Discussion

Prior to any human land use, grasslands as well as open places within forests, i.e., the habitats preferred by a majority of European butterflies, used to be sustained by large herbivore grazing, which prevented forest growth (Konvička et al. 2005; Pöyry et al. 2005; Krauss et al. 2005; Stefanescu et al. 2009). The co-existence of various herbivore species with varying feeding preferences and abundances led to strong spatial heterogeneity in herbal vegetation, while fluctuating grazer densities increased temporal dynamics of habitats (Morris 2000; Saarinen and Jantunen 2005; Öckinger et al. 2006; Rösch et al. 2013). Trampling providing continuous disturbance and the provision of dung which fertilised soils were additional positive impacts. All the aforementioned factors resulted in high plant species richness, which in turn benefited butterfly communities as well as various other insect taxa (Van Klink et al. 2015).

With increasing human population in Europe, wild grazers were decimated or even completely exterminated (e.g., aurochs), but since ancient times their role in maintaining butterfly habitats in favourable state was replaced by human activities (Bakker et al. 2004; Van Klink et al. 2015). Traditional agriculture supported the existence of a diverse mosaic of flower meadows mowed with variable intensity and timing, extensively grazed hillsides, and country roads (Balmer and Erhardt 2000; Morris 2000; Konvička et al. 2005). In turn, forest areas, comprising the second most important butterfly habitat (Warren and Bourn 2011), used to be cut frequently, which provided sufficient amount of sunny places (Kodandaramaiah et al. 2012; Fartmann et al. 2013; Slámová et al. 2013). During the twentieth century, mechanisation in both agriculture and forestry brought the era of intensive land use (Young et al. 2005; Wrbka et al. 2008; Korösi et al. 2014), and the traditional land use practices were no longer economically viable (Konvicka et al. 2005; Henle et al. 2008). Consequently, the land became either intensively used or abandoned, which led to population declines in numerous butterfly species (Dover et al. 2010; Horák et al. 2013; Loos et al. 2014).

In order to reverse the negative impacts of changes in agriculture on biodiversity the European Union has reformed its Common Agricultural Policy, focusing it on achieving an optimal balance between food production and sustaining biodiversity (Henle et al. 2008; Wrbka et al. 2008; EEA 2011). Currently, one of its most important instruments are agri-environmental schemes, which subsidise farmers for applying biodiversity-friendly agricultural practices, often resembling the traditional ones (Wätzold et al. 2008; Wrbka et al. 2008). Nevertheless, the pan-European mechanisms of Common Agricultural Policy have so far failed to improve the situation for butterflies, especially those of conservation concern (Henle et al. 2008; Warren and Bourn 2011).

If no further actions are taken, it is most likely that butterfly populations will keep declining and species extinctions will continue. Therefore, it seems necessary that butterfly persistence in semi-natural habitats of Europe is supported with appropriate conservation-oriented management of their sites, e.g., within nature reserves or Natura 2000 areas (van Swaay and Warren 2006; Pöyry 2007; van Swaay et al. 2012). Our review offers some rule-of-thumb recommendations in this respect.

The baseline should be stopping further destruction or devastation of butterfly habitats through ill-conceived management activities, such as afforestation of open lands or drainage works. However, the elimination of unsuitable management alone is not enough to improve the status of endangered butterfly species (van Swaay and Warren 2006; WallisDeVries and Ens 2010; van Swaay et al. 2012). If left abandoned, their habitats will gradually deteriorate in quality, and eventually they will turn into forest through vegetation succession (Bartel and Sexton

2009). Consequently, it is necessary to implement practices aimed at preventing succession processes.

Extensive grazing and rotational mowing have been demonstrated to be the most suitable types of management in this respect, benefiting various groups of endangered butterflies (Dover et al. 2010). These types of management imitate the traditional way of meadow use (Saarinen and Jantunen 2005; Loos et al. 2014). D'Aniello et al. (2011), who compared the effects of grazing and mowing for meadow butterflies, found that grazing is generally more effective in maximising the number of butterfly species occurring in meadows; however, low intensity mosaic mowing provides almost equally positive results.

Trampling is an integral part of grazing, and it typically supports butterfly communities as well (Morris 2000). In areas lacking grazing, trampling can be achieved through various sport activities, e.g., hiking, biking, or horseback riding (Konvička et al. 2005). It has also been found that occasional small area burning is beneficial for a wide spectrum of butterfly species (Möllenbeck et al. 2009; McIver and Macke 2014). In addition, the active removal of shrubs and young trees may at times be necessary, especially because even their minor expansion threatens the populations of some butterfly species (Stefanescu et al. 2009). Conversely, certain species may actually profit from the presence of bushes within their habitats; therefore, the initial stages of succession should be allowed in such cases (Stuhldreher and Fartmann 2014). Other specific management types are suitable for butterflies associated with forests. For a relatively large group of endangered woodland species, maintaining (or, if necessary, establishing) forest glades and other sunny enclaves, as well as thinning forest stands, is recommended (Slámová et al. 2013; Maes et al. 2014).

It is also worth mentioning that apart from targeted management actions as described above butterflies can also benefit indirectly from various other human activities, specifically those suppressing natural succession. A classic example here are military training grounds, characterised by relatively frequent disturbances caused by blasts or heavy vehicles on one hand and the exclusion of intensive agriculture and forestry on the other (Ferster and Vulinec 2010; Rivers et al. 2010). Such conditions result in the formation of heterogeneous landscapes, which often support high diversity of butterflies with various habitat requirements (Warren et al. 2007; Čížek et al. 2013). Abandoned quarries are also known to provide a favourable, if atypical, environment for many animal and plant species (Tropek et al. 2010; Verovnik et al. 2013). Although quarry operations represent a dramatic land degradation; shortly after their abandonment spontaneous succession turns them into diverse habitat mosaics supporting a rich butterfly fauna (Novák and Konvička 2006; Tropek et al. 2010, Čermáková et al. 2010). A similar situation can be observed in other artificial environments especially those created by infrastructure development, such as road margins, railway embankments, gravel pits, or ruderal habitats in suburbia (Van Geert et al. 2010; Lenda et al. 2012; Moron et al. 2014; Nowicki et al. 2013). Nevertheless, it must be underlined that such man-made environments offer favourable conditions only in their early successional stages, hence only in the short-term, and later on they require management just like natural habitats in order to prevent overgrowing.

In our paper, we primarily dealt with management types that should be promoted or prevented at the local scale of butterfly sites. Therefore, it is important to note that the actions favouring butterfly populations at the small-scale, will not necessarily be similarly favourable if applied at the large-scale. The most obvious example is the case of prescribed burning, but the same principle is true also for most other management types discussed. Besides, it should be kept in mind that many suitable management types are interconnected and that there can be no general recommendations on how to manage a particular habitat type. Finding a clear solution concerning the most appropriate management practice for any butterfly species depends on its species-specific habitat requirements, and for this reason it demands profound knowledge of the focal species ecology. Hence deciding an optimal management may be difficult, especially for species with highly specialised requirements (Schirmel and Fartmann 2014). Furthermore, the management of a locality must take into account the requirements of all of the species of conservation concern inhabiting it. These species may in fact have conflicting needs, and prioritising selected species would be essential in such cases (cf. Schmeller et al. 2008).

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Bubová, T., Kulma, M., Vrabec, V., Nowicki, P. 2016. Adult longevity and its relationship with conservation status in European butterflies. Journal of Insect Conservation, 20(6), 1021-1032. [Délka života dospělců a její vztah k ochranářskému statusu u evropských druhů motýlů]

Článek popisuje vliv délky života dospělců evropských motýlů a délky letového období na jejich ochranářský status. Tato studie obsahuje souhrn výsledků z monitoringu „„mark-release-recapture““ metodou publikovaných v časopisech indexovaných v databázích Web of Knowledge, Scopus nebo Google Scholar, na jejichž základě bylo prokázáno, že více ohrožení motýli mají kratší délku života než méně vzácné druhy. Tento fenomén lze vysvětlit nízkou schopností druhů vzácných migrovat, dále krátkým časem na hledání partnera, reprodukci a ovipozici samic. Přestože délka letové sezóny se též výrazně lišila mezi jednotlivými druhy motýlů, její vliv na ochranářský status potvrzen nebyl. Výsledky této práce ukazují, které druhy jsou náchylnější k vyhynutí a mají význam „včasného varování“ pro ochranářskou praxi.

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ORIGINAL PAPER

Adult longevity and its relationship with conservation status in European butterflies

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Abstract Many European butterfly species are currently experiencing serious declines, and may be threatened with extinction. Nevertheless, due to limited knowledge on the species biology and ecology, detailed assessments of endangerment level are not possible, and instead identifying species of conservation concern has to rely on proxies. Earlier studies suggested several characteristics, including host plant specificity, overwintering stage, patch size requirements or mobility, as potentially useful indicators of butterfly species vulnerability, but the usefulness of adult longevity in this respect has not been considered so far. Based on the information gathered through an extensive literature search we investigated the relationship between adult life span, flight period length or the temporal fragmentation index calculated as the ratio of the two parameters, and conservation status of European butterflies. We found that the species classified in one of the IUCN conservation concern categories (i.e. Endangered, Near Threatened, or Vulnerable) lived shorter as adults and were characterised by higher values of the temporal fragmentation index, while there was no particular pattern concerning flight period length. We believe that the apparent effects detected reflect the fact that shorter adult life span, and thus increased temporal fragmentation, in combination with protandry, i.e. earlier emergence of males, decrease individual chances of finding mating partners. Such a situation leads to lower effective population size and reduced viability, especially in the case of small populations. All concerned, the investigated parameters reflecting adult longevity may serve as 'early warning' indicators, helping to flag-up butterfly species possibly at risk.

Keywords Extinction risk · Flight period · Life span · Species vulnerability · Temporal fragmentation · Threat level

Introduction

Butterfly populations in Europe have declined drastically in recent decades (Thomas et al. 2004; EEA 2011). These negative trends have prompted the launching of numerous programmes for butterfly conservation (Warren and Bourn 2011). However, for effective conservation, it is necessary to properly identify species threatened with extinction, and Red Lists are compiled for this purpose. Assessments of species positions on such lists should ideally be based on thorough knowledge of their biology and ecology and how these affect the species vulnerability to threats (Margules and Pressey 2000; Mattila et al. 2006). Despite the fact that butterflies comprise one of the most studied invertebrate groups, such knowledge is nevertheless available for only a very limited number of butterfly species (van Swaay 2002; Wenzel et al. 2006; Müller et al. 2010). Therefore, for their successful conservation, it is important to identify traits that predispose butterfly species to extinction risk (Mattila et al. 2006). Life histories traits and/or ecological characteristics could be used as indicators of potential vulnerability to threats as many of these characteristics are common among species of conservation concern (Statzner et al. 2001; Mattila et al. 2006; Nylin and Bergström 2009).

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Studies conducted to date have identified a wide range of butterfly characteristics that can potentially act as proxies for extinction risk. One of the most commonly addressed aspects in this respect is the division into generalists and specialists (Nylin and Bergström 2009; Ali and Agrawal 2012; Bartoňová et al. 2014). The latter group includes a disproportionally high number of threatened species due to their stricter habitat and host plant requirements (Hodgson 1993; Purvis et al. 2000; Fontaine et al. 2007). Another well-established pattern relates to life history and voltinism, where univoltine species and/or those overwintering in the egg or larval stage are more susceptible to climate change, which has recently become one of the most serious drivers of butterfly declines (Hodgson 1993; Conrad et al. 2004; Mattila et al. 2006; Nylin and Bergström 2009). In addition, low mobility was typically reported for threatened butterflies (Kotiaho et al. 2005; Mattila et al. 2006; Nylin and Bergström 2009; Habel et al. 2015). This is not surprising, because less mobile species with low colonization success rates are more vulnerable to the effects of habitat fragmentation, which is nowadays a crucial threat for butterflies (Thomas 1995; Novacek and Cleland 2001; Baguette and Schtickzelle 2006; Franzen and; Johannesson 2007). Also as a consequence of habitat fragmentation, butterflies with greater patch size requirements are highly represented among species of conservation concern (Cowley et al. 1999; Kotiaho et al. 2005; Baguette and Stevens 2013).

It has recently been pointed out that the extinction risk of butterfly populations is likely to depend not only upon classic spatial fragmentation of their habitats, but also upon their fragmentation over time (Nowicki et al. 2005b). The latter derives from the fact that the individual life span of the adult butterflies is usually much shorter than is the length of adult occurrence season dubbed as the flight period. Consequently, groups of individuals from different parts of a season do not have the chance to mate with one another. This problem is further exacerbated by protandry, i.e. earlier emergence of males in the season as compared with females, which is typical for butterflies (Wiklund and Fagerström 1977). The extent of and a temporal fragmentation (*sensu* Nowicki et al. 2005b) depends upon adult life span and flight period length and so it is highly variable among butterflies. Surprisingly, the effects of temporal fragmentation on butterfly species extinction risk have not been investigated so far.

In the present study we evaluated the relationships between adult life span, flight period length, and a temporal fragmentation index (defined as the ratio of flight period length to adult life span) on one hand, and the species conservation status, as reported in the European Red List (van Swaay et al. 2010), on the other hand. Butterflies that have short life spans and long flight season length will have fewer opportunities for males and females representing different daily cohorts to meet together (Nowicki et al. 2005b). Consequently, we hypothesised that a higher level of threat should be associated with: (a) shorter adult life span; (b) longer flight period length; and (c) higher values of the temporal fragmentation index. We tested the above hypotheses using the data gathered through an extensive literature review.

Methods

Literature search

In order to gather information on adult life span and flight period length in butterflies, we searched for mark-recapture studies (which typically assess both these parameters) within the following databases: ISI Web of Science (http://apps.webofknowledge.com/), Scopus (http://www.scopus.com/home.url), and Google Scholar (http://scholar.google.com/). We used "(re)capture" and "butterfly/butterflies" or "Lepidoptera" as the searched keyword combinations. We restricted our search results to only European species, because relatively little information was available for all others, with the slight exception of a small number of North American species. We also utilised relevant grey literature on the subject, in particular academic theses or reports known to us.

We considered only adult life span data originating from field studies, and thus we excluded literature on adult life span measured in controlled conditions, i.e. physiological longevity. This is because when butterflies are raised in enclosures or laboratories, their recorded longevity is known to overestimate the life span actually achieved in nature (Karlsson and Wiklund 2005; Nowicki et al. 2005b). We likewise excluded mark-recapture estimates of butterfly residence time assessed in clearly open populations, which are subject to substantial emigration and thus considerably underestimate butterfly longevity (Nowicki et al. 2005a). An exception was made for the cases in which the emigration rate could be estimated and accounted for, e.g. using the Virtual Migration model (Hanski et al. 2000). Consequently, we believe that the life span estimates we used in our study represent true adult longevities and not just adult residence times within the surveyed populations.

Quite often the literature sources did not explicitly report adult life span but instead they gave adult survival rate estimates. In such cases we converted survival rate (ϕ) into adult life span (e) using the following formula: $e = (1 - \phi)^{-1} - 0.5$ (Nowicki et al. 2005b). Whenever the studies reported adult life span or survival estimates for males and females separately, we used the mean of the two values because they typically differed rather little, i.e. by less than 10%. It should be noted that adult life span values provided by mark-recapture studies are restricted to a single season, and thus they do not account for the fact that certain individuals flying during the season might also be on the wing in the previous or in the following year in species overwintering as adults. Nevertheless, due to the high mortality of overwintering adults such individuals are relatively few. Furthermore, their existence does not undermine the usefulness of single-season data for our study, in which adult life span is understood as the time during which an adult flies and breeds within a single generation, and not as its total life expectancy, including the period of inactive overwintering.

Regarding flight period length, we calculated it as the total number of days of adult occurrence inclusive of the first and last day. Thus, for example, a flight period from 1 to 30 July corresponds to a length of 30 days (and not 29 days). We excluded the studies that did not cover the entire flight period, which was either explicitly mentioned by the authors or was clear from the reported results, with relatively high daily numbers of individuals recorded at the beginning or the end of the study period. In a few cases, we also used information on flight period length from studies other than mark-recapture surveys, e.g. behavioural observations conducted from the very start to the very end of the season. On the other hand, we decided not to use the information on flight period provided by several general books on butterflies (e.g. Settele et al. 1999; Beneš et al. 2002). Even though such information was easily available for most European species, it turned out to be too superficial to be applicable in our analyses. Specifically, the information was given in the form of general statements, mentioning that e.g. species X flies from early July to mid-August, thus allowing only very coarse assessments of flight period length with margins of error of as much as 10–15 days.

Data handling and analysis

The extent of temporal fragmentation was calculated as the ratio between flight period length and average adult life span, both measured in days. In the case of studies spanning several years (e.g. Schtickzelle et al. 2002; Nowicki et al. 2009), we treated the data from each year separately. In making the calculations, we endeavoured to use data on life span and flight period length from the same population and year. This usually was possible because most mark-recapture studies reported both. Otherwise, we paired together data on adult life span and flight period from the closest-lying locations, but in rare cases these locations were quite distant from one another and represented different biogeographic regions. For multivoltine species, we relied on information provided by studies conducted on the first generation whenever possible. This is because the population size of the first generation is generally much smaller than those of later generations and it is thus critical for species persistence, and furthermore the occurrence of later generations is sometimes facultative (Franzen and Johannesson 2007; Fric et al. 2010; Nabielec and Nowicki 2015).

For species with more than one record available, we calculated median values of adult life span, flight period length, and temporal fragmentation index and used them in the subsequent analyses, since the distributions of records were often non-normal (right-skewed). In turn, it is worth noting that the distribution of median values among species was normal for all of the three parameters considered.

For the purpose of the analyses, we adopted the Red List status of European butterflies in accordance with van Swaay et al. (2010). Despite our original expectations, the sample sizes of species for which we were able to gather data were small in the three conservation concern categories, i.e. Endangered (EN), Vulnerable (VU), and Near Threatened (NT). We therefore decided to pool these into a single 'conservation concern' category (CC) and analyse it against the species categorized as Least Concern (LC).

We analysed the relationship between adult life span, flight period length or temporal fragmentation index, and conservation status (CC vs. LC, dichotomous dependent variable) using logistic regression analysis. For each predictor, we conducted two separate analyses, using the full data set gathered as well as what we term a 'core data set'. The core data set excluded species for which data quality was problematic for various reasons. These included cases of (a) species for which data on adult life span and flight period length came from distant populations, representing different biogeographic regions; (b) species for which only the data for the second generation was available; and (c) ***Maculinea alcon***, the conservation status of which is questionable, apparently due to its uncertain systematic status, with two distinct forms existing, namely ***M. alcon*** '***alcon***' and ***M. alcon*** '***rebeli***' (Als et al. 2004; Steiner et al. 2005; Pecsenye et al. 2007; Sielezniew et al. 2012). It is classified as LC by van Swaay et al. (2010), however many authors regard both forms to be under threat in Europe (Wallis-DeVries 2004; Tartally et al. 2008; Czekes et al. 2014).

Obviously, a common problem with cross-species analyses is that records for related species may not be fully independent from one another. The standard solution in such cases is controlling for phylogenetic autocorrelations (Martins and Hansen 1996). However, this was not possible in our study, because a full phylogenetic tree with scalable inter-specific distances is not yet available for European butterflies (cf. Cowley et al. 2001; Bartoňova et al. 2014). Therefore, in order to verify the risk of phylogenetic autocorrelation biases, we instead applied the intraclass correlation coefficients (Stanish and Taylor 1983; Lessells and

Boag 1987) to test for potential repeatability of our records within species as well as higher taxa, namely tribes, subfamilies, and families (but not at the genus level, because we rarely had data for more than one species per genus). The testing yielded significant results for species (life span: $r_I = 0.7379$, $P < 0.0001$; flight period length: $r_I = 0.5513$, $P < 0.0001$), but not for any higher taxa (life span: $r_I = 0.0539$, $P = 0.6691$ for tribes; $r_I = 0.1166$, $P = 0.4962$ for subfamilies; $r_I = 0.0408$, $P = 0.6386$ for families; flight period length: $r_I = 0.1922$, $P = 0.2835$ for tribes; $r_I = 0.1401$, $P = 0.4468$ for subfamilies; $r_I = 0.1312$, $P = 0.4213$ for families). Such an outcome indicates that our data records were highly repeatable within species, but fairly independent among them. This, in combination with the fact that despite a relatively small sample size we managed to gather data for a wide range of European butterfly species, makes us believe that the results of our analyses are not biased by potential phylogenetic autocorrelations.

Results

We successfully gathered relevant information for 50 species of European butterflies, including 4 classified as EN, 5 as VU, and 6 as NT, as well as 35 species classified as LC (Table 1). The average adult life span of these species ranged from ca. 2.5 to 15 days. The flight period length was between 20 and 50 days in most cases, although some clear outliers could also be noticed. The shortest adult occurrence season was reported for ***Pseudophilotes bavius*** (median value of 16.5 days), whereas in satyrid butterflies it sometimes approached or exceeded 70 days (in ***Maniola jurtina*** and ***Coenonympha pamphilus*** respectively). There was no apparent correlation between flight period length and adult life span (Pearson's correlation: $r = 0.1794$, $P = 0.2125$), and consequently the ratio of the two parameters, which we defined as the temporal fragmentation index, varied greatly from ca. 2 to more than 12 (Table 1).

As indicated by Red List categories, extinction risk generally increased with increasing adult life span (Fig. 1a). Similarly, butterflies in the three categories collectively characterized as CC had higher temporal fragmentation index values (Fig. 1c). On the other hand, there was no clear pattern concerning flight period length, which turned out to be slightly elevated among NT species. The latter result was partly due to strong variation within this particular group (Fig. 1b).

The logistic regression analyses confirmed the above patterns, revealing significant relationships with species conservation status (CC vs. LC) in the case of adult life span and temporal fragmentation index, but no effect whatsoever for flight period length (Table 2). It is noteworthy that the effect of adult longevity and temporal fragmentation increased (despite considerably smaller sample sizes) when the analyses were restricted to the core data set, thus excluding species for which the quality of the data gathered was problematic. The threshold value for adult life span at which a species had 50% probability of being listed in one of the CC categories was 3.53 days for the full data set and 4.31 days for the core data set. In the case of temporal fragmentation index, the respective thresholds were 9.19 and 8.01.

Discussion

The selection of species on which we based our investigation may not be fully representative for the whole spectrum of European butterflies. In particular, an underrepresented group are the species from Mediterranean region as well as from northern Europe, the conservation status of which is potentially less related to adult longevity and more to the life history parameters beyond the scope of the present study, such as wintering stage and voltinism (Mattila et al. 2006; Nylin and Bergström 2009). Another limitation of our database is the fact that it lacked very common species, mostly of Nymphalidae and Pieridae families, which are neglected in mark-recapture studies, apparently because of the lack of scientific and conservation interest in them. Nevertheless, based on anecdotal information such species are reported to have long-living adults (Settele et al. 1999; Beneš et al. 2002), and thus we believe that their inclusion would have actually strengthened the outcome of our analyses.

Our results indicated the existence of clear relationships between adult life span and temporal fragmentation index on one hand and conservation status of European butterflies on the other. Moreover, the relationships proved to be significant, regardless of whether the full data set or the core data set was used, which increases our confidence in these findings. In contrast, butterfly conservation status was not linked in any way to flight period length as defined in our study.

Butterfly adult life span is a part of an adaptive life history which involves mating and egg laying strategy (Carey 2001; Beck and Fiedler 2009). The average life span reported in our study typically reached only a few days. Such a short life span implies that the mating must take place shortly after eclosion to minimise delay and allow most of the females to oviposit their eggs before they die (Scott 1973; Beck and Fiedler 2009). Hence, in species with short life spans adult butterflies have a very narrow time window to copulate. Adult life span also affects realised fecundity, i.e. the number of eggs laid, which in turn can have a critical impact on population viability (Fischer et al. 2006; Pijpe 2007; Haeler et al. 2014). Low quantities

Table 1 Summary information on adult life span, flight period length, and the temporal fragmentation index (i.e. ratio of flight period length to adult life span) gathered for European butterflies

Species	Status	Life span (days)	Flight period (days)	Temporal fragmentation	Sources
Colias myrmidone[a]	EN	3.54 (3.32–3.75)	26 (21–31)	7.47 (5.60–9.34)	Szentirmai et al. (2014)
Lycaena helle	EN	7.19 (5.45–7.83)	39 (29–64)	5.87 (3.87–8.90)	Fischer et al. (1999), Bauerfeind et al. (2009), Reymond (2014), Turlure et al. (2014), Nabielec and Nowicki (2015)
Phengaris (=*Maculinea*) *arion*	EN	3.53 (3.07–4.26)	37 (32–39)	10.28 (9.15–10.92)	Bonelli et al. (2013)
Coenonympha oedippus	EN	3.00 (2.50–4.20)	27.5 (18–28)	7.98 (6.67–10.00)	Örvössy et al. (2013)
Phengaris (=*Maculinea*) *teleius*	VU	3.01 (1.61–4.16)	38 (28–56)	12.01 (9.12–40.00)	Nowicki et al. (2005a, b, 2009, 2014), Vodă et al. (2010)
Euphydryas maturna	VU	6.53 (4.75–8.30)	42.5 (36–49)	7.33 (4.34–10.32)	Wahlberg et al. (2002), Konvička et al. (2005)
Lopinga achine	VU	6.30 (5.95–6.64)	28 (25–31)	4.43 (4.20–4.67)	Bergman and Landin (2002), Streitberger et al. (2012)
Coenonympha tullia	VU	3.05 (2.80–3.30)	24.5 (21–28)	7.99 (7.50–8.48)	Turner (1963), Warren (1992), Komonen et al. (2004)
Erebia sudetica	VU	4.00	29	7.25	Nowicki et al. (2005a)
Thymelicus acteon[b]	NT	7.00	41	5.86	Thomas (1983), Buszko and Masłowski (2008)
Parnassius mnemosyne	NT	9.06 (5.05–11.15)	51 (31–51)	5.66 (4.57–10.10)	Schmidt (1989), Seufert (1990), Konvička and Kuras (1999)
Parnassius apollo	NT	3.73 (3.20–4.26)	29 (25–33)	8.08 (5.87–10.30)	Brommer and Fred (1999), Komonen et al. (2004), Fred et al. (2006)
Iolana iolas	NT	5.88 (3.63–8.12)	49.5 (49–50)	9.83 (6.16–13.50)	Rabasa et al. (2005, 2007), Heer et al. (2013)
Phengaris (=*Maculinea*) *nausithous*	NT	2.84 (2.02–5.74)	40 (23–56)	12.64 (4.36–23.76)	Pfeifer et al. (2000, 2007), Nowicki et al. (2005a, b, 2014), Vodă et al. (2010)
Euphydryas desfontainii	NT	5.55	36	6.49	Pennekamp et al. (2014)
Pyrgus sidae	LC	9.20	25	2.72	Hernándes-Roldán et al. (2009)
Hesperia comma	LC	4.40 (3.00–10.30)	35 (23–50)	7.95 (2.23–15.63)	Thomas (1983), Komonen et al. (2004), Soulsby and Thomas (2012)
Zerynthia polyxena	LC	5.28 (4.40–6.17)	29 (20–38)	5.94 (3.24–8.64)	Örvössy et al. (2005), Batáry et al. (2008), Celik (2012)
Leptidea sinapis[a]	LC	8.35 (6.50–10.20)	38.5 (33–44)	5.00 (3.24–6.77)	Warren et al. (1986), Komonen et al. (2004), Friberg et al. (2008)
Leptidea reali[a]	LC	7.60	44	5.79	Friberg et al. (2008)
Anthocharis cardamines	LC	6.95 (5.60–8.30)	28.5 (21–34)	4.30 (2.53–6.07)	Courtney and Duggan (1983), Dempster (1997)
Lycaena virgaureae	LC	6.43 (6.20–6.65)	31 (29–33)	4.84 (4.36–5.32)	Fjellstad (1998), Komonen et al. (2004), Haaland (2015)
Lycaena hippothoe	LC	9.60 (7.00–10.00)	28 (28–32)	2.92 (2.80–4.57)	Fischer (1998), Fischer and Fiedler (2001), Komonen et al. (2004)
Satyrium w-album[b]	LC	6.90	28	4.06	Warren (1992), Komonen et al. (2004)
Cupido minimus[b]	LC	15.00	31	2.07	Morton (1985), Komonen et al. (2004)
Pseudophilotes bavius	LC	2.80 (2.40–5.40)	16.5 (12–28)	5.47 (2.50–11.67)	Crişan et al. (2014)
Phengaris (=*Maculinea*) *alcon*[c]	LC	2.44 (1.62–5.98)	29 (18–60)	11.88 (5.59–29.27)	Seufert (1993), Nowicki et al. (2005a, 2009), Timuş et al. (2013)
Plebejus argus	LC	3.35 (3.20–3.50)	30 (20–40)	9.11 (5.71–12.50)	Warren (1992), Lewis et al. (1997), Cormont et al. (2011)

Table 1 (continued)

Species	Status	Life span (days)	Flight period (days)	Temporal fragmentation	Sources
Aricia eumedon[b]	LC	3.59	19	5.30	Seufert (1993), Komonen et al. (2004)
Polyommatus icarus[a]	LC	4.40 (3.40–5.40)	26 (18–34)	5.80 (5.29–6.30)	Dowdeswell et al. (1940), Scott (1973), Komonen et al. (2004)
Polyommatus bellargus	LC	9.10 (8.10–10.10)	26.5 (24–29)	2.98 (2.38–3.58)	Davis et al. (1958)
Polyommatus coridon	LC	5.52 (3.70–8.70)	56.5 (24–63)	10.79 (4.21–14.86)	Davis et al. (1958), Nowicki et al. (2005a), Schmitt et al. (2006)
Argynnis paphia[b]	LC	11.50	38	3.30	Magnus (1954), Komonen et al. (2004)
Argynnis aglaja	LC	8.20	58	7.07	Zimmermann et al. (2009)
Brenthis ino	LC	9.74 (5.70–13.79)	40.5 (35–46)	5.30 (2.54–8.07)	Zimmermann et al. (2005), Fric et al. (2010)
Boloria eunomia	LC	8.23 (3.00–11.55)	35.5 (26–45)	4.32 (2.68–11.00)	Schtickzelle et al. (2002), Turlure et al. (2010)
Boloria euphrosyne	LC	9.00 (5.97–11.10)	29 (24–32)	3.39 (2.61–4.02)	Baguette and Neve (1994), Komonen et al. (2004), Al Dhaheri (2009)
Boloria aquilonaris	LC	4.26	21	4.93	Turlure et al. (2010)
Euphydryas aurinia	LC	6.40 (2.24–15.37)	31 (15–42)	3.16 (2.60–12.95)	Munguira et al. (1997), Wahlberg et al. (2002), Anthes et al. (2003), Komonen et al. (2004), Schtickzelle et al. (2005), Fric et al. (2010), Zimmermann et al. (2011), Casacci et al. (2015)
Melitaea cinxia	LC	5.80	33	5.69	Wahlberg et al. (2002)
Melitaea Didyma	LC	7.00 (5.50–8.00)	46 (30–51)	5.75 (5.45–7.29)	Vogel and Johannesen (1996)
Melitaea diamina	LC	8.59 (6.35–10.61)	29 (29–51)	4.57 (3.38–4.81)	Hanski et al. (2000), Wahlberg et al. (2002), Fric et al. (2010)
Melitaea athalia	LC	10.00 (5.45–11.26)	35 (30–55)	4.88 (3.50–5.50)	Warren (1987), Wahlberg et al. (2002), Fric et al. (2010), Cormont et al. (2011)
Pararge aegeria[a]	LC	9.50	23	2.42	Warren (1992), Komonen et al. (2004)
Lasiommata megera[a]	LC	4.20	40	9.52	Parr et al. (1968), Harker and Shreeve (2008)
Coenonympha pamphilus[a]	LC	7.30	76	10.41	Wickman (1985)
Aphantopus hyperantus	LC	3.95 (3.60–4.29)	36.5 (35–38)	9.29 (8.86–9.72)	Sutcliffe et al. (1997), Soulsby and Thomas (2012)
Maniola jurtina	LC	6.55 (6.51–10.83)	67 (51–78)	7.84 (6.19–11.91)	Tudor and Parkin (1979), Brakefield (1982), Lörtscher et al. (1997), Cormont et al. (2011)
Erebia aethiops	LC	6.50	33	5.08	Slámova et al. (2013)
Minois dryas	LC	4.47 (3.50–6.74)	25.5 (19–32)	5.17 (4.75–6.75)	Pellet and Gander (2009), Bilnicki (2015)

Whenever more than one record was available for a species, we present the median with the range (min–max) in parentheses. Conservation status follows the European Red List of Butterflies (van Swaay et al. 2010): *EN* endangered, *VU* vulnerable, *NT* near threatened, *LC* least concern. Superscripts indicate species which were excluded from the core data set used in the analyses for various reasons

[a] Data available only for the second or third generation

[b] Data on adult life span and flight period length came from different regions

[c] Questionable conservation status—see Methods for the rationale

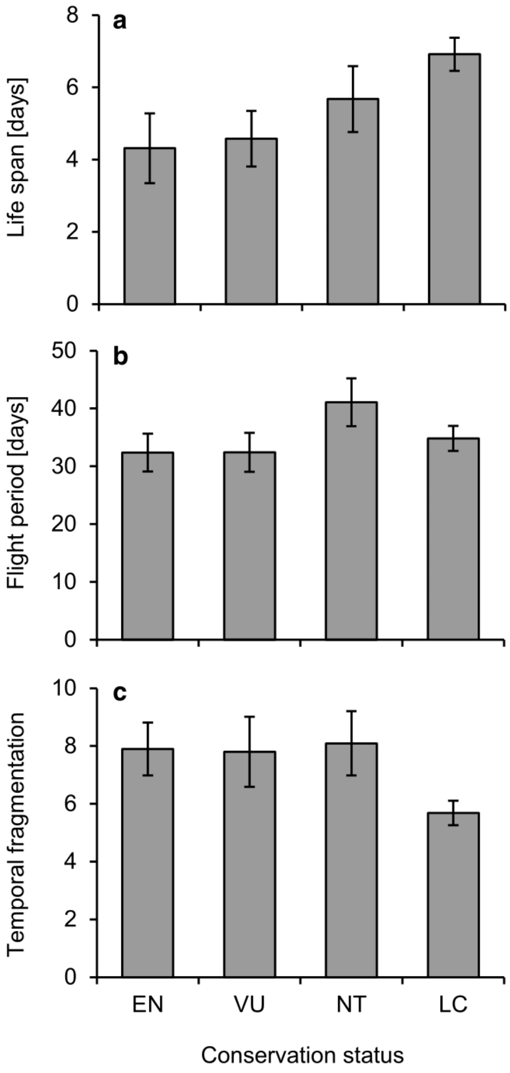

Fig. 1 Adult life span (**a**), flight period length (**b**), and temporal fragmentation index (**c**) in relation to the conservation status of European butterflies (*EN* endangered, *VU* vulnerable, *NT* near threatened, *LC* least concern). The values shown represent means (with their SEs) across all the investigated species in each category. Different conservation concern categories (EN, VU, NT) are treated separately only for the graphic presentation, but they were pooled together in the analyses (see Table 2)

of oviposited eggs over the long term are bound to result in population decline. It is thus not surprising that the results of our study confirmed that short life span corresponds to higher threat level in European butterfly species.

Alternative hypotheses explaining the relationship between adult life span or temporal fragmentation index and conservation status involve mobility and predation. Since both emigration probability and movement distance are typically time-dependent (Hanski et al. 2000), short-lived butterflies may be expected to emigrate in lower numbers and move shorter distance throughout their adult lifetime. Species with such characteristics are more likely to experience the negative effects of habitat fragmentation, and hence be more prone to being threatened (Kotiaho et al. 2005; Franzen and; Johannesson 2007; Habel et al. 2015), especially in the highly fragmented landscapes of Europe. However, the plausibility of the above explanation is undermined by empirical studies indicating that dispersal capabilities and butterfly life span may be negatively correlated, since investing in mobility and longevity is subject to a developmental trade-off (Hanski et al. 2006; Niitepõld and Hanski 2013).

Additionally, the better conservation status of species with long-living adults might possibly be attributed to their lower mortality due to predation. It has been reported that butterflies with anti-predator defence features (such as aposematism, eye-spots, etc.) have longer life spans, but it must be stressed that the effect of these anti-predator defences on longevity was rather weak and mostly restricted to tropical butterflies (Beck and Fiedler 2009). Furthermore, although predation on adult butterflies may sometimes be considerable, it is nonetheless of relatively little importance for population dynamics as compared with predation experienced at the egg, larval or pupal stages (Dempster 1984; Warren 1992).

An increased level of temporal fragmentation was also found to correspond to higher species vulnerability in our study. A possible explanation is that if butterflies live for only a limited part of the flight period then they may have lower chances of finding mates. These low mating opportunities are further decreased by the fact that males emerge earlier in the season than females, usually by several days, due to protandry (Pfeifer et al. 2000; Petit et al. 2001; Nowicki et al. 2005b). This phenomenon is common in insects, particularly in species for which the flight season is very long and the majority of the population occurs at the beginning of the flight season, such as is the case of the mayfly (Gibbs and Siebenmann 1996; Takemon 2000). Protandry prevents inbreeding and ensures that only strong males survive long enough to mate. Moreover, it also supports immediate female fertilisation, which minimises the risk of females dying before mating (Fagerström and Wiklund 1982; Zonneveld and Metz 1991; Zonneveld 1992; Morbey and Ydenberg 2001).

Although all the aforementioned effects are considered positive, some negative consequences of protandry are also known. Since a fraction of males may not survive

Table 2 Results of multiple logistic regression analyses of factors affecting conservation status (conservation concern vs. least concern) of European butterflies

Predictor	Data set (with sample size)	Parameter value (± SE)		Model fit		
		Intercept	Estimate	χ^2	P	R^2
Life span	Full ($n=50$)	1.29 ± 0.91	−0.37 ± 0.16	6.83	0.0089	0.27
	Core ($n=37$)	1.82 ± 1.10	−0.42 ± 0.19	6.05	0.0138	0.33
Flight period length	Full ($n=50$)	−1.12 ± 0.98	0.01 ± 0.03	0.09	0.7685	0.01
	Core ($n=37$)	−1.18 ± 1.20	0.02 ± 0.03	0.25	0.6192	0.01
Temporal fragmentation	Full ($n=50$)	−3.18 ± 0.99	0.35 ± 0.13	7.85	0.0051	0.29
	Core ($n=37$)	−3.85 ± 1.31	0.48 ± 0.18	9.44	0.0021	0.41

long enough to mate with the later-emerging females, some females will consequently go unmated, and will thus be unable to lay eggs. This lost reproductive potential can significantly decrease population size and could also lead to population extinctions. The protandry effect might therefore be evolutionarily advantageous at higher population densities but, if the population density decreases, it can be harmful (Calabrese and Fagan 2004). Additionally, weather conditions could affect the proper timing of male and female emergence by up to several days (Schtickzelle et al. 2002; Robinet and Roques 2010). For instance, rainy days at the beginning of the flight period in 1983 were found to influence individual development in ***Euphydryas editha bayensis***, resulting in the first females emerging 14 days after males, which had a clear negative effect on the population dynamics of the species (Dobkin et al. 1987; Baughman 1991). Furthermore, a high level of temporal fragmentation, namely short individual life span in relation to long flight period, in combination with protandry seriously reduced the effective population size (***sensu*** Lande and Barrowclough 1987; Hill 1972), thus accelerating the loss of genetic variability in small populations.

In contrast to several earlier studies, which suggested that extinction risk in butterflies decreases with lengthening flight period (Komonen et al. 2004; Kotiaho et al. 2005; Franzen and; Johannesson 2007), our analyses did not reveal any link between flight period length alone and species conservation status. The most straightforward explanation for such a result could be that flight period length per se is unimportant for species extinction risk, and it only matters in combination with adult longevity. Nevertheless, we hypothesise that the situation is more complex, namely a longer time of adult occurrence has both positive and negative consequences for species viability. On the one hand, as discussed above, it increases the temporal fragmentation of butterfly populations and may reduce mating opportunities for both sexes. On the other hand, a longer flight period allows for the compensation of the negative effects of stochastic changes during the flight period such as unfavourable weather conditions, and inappropriate management interventions, e.g. inappropriate timing of meadow mowing (Cormont et al. 2011). An extended adult occurrence season improves the resilience of butterfly populations to catastrophic events such as floods or fires (cf. Konvička et al. 2002; Kajzer-Bonk et al. 2013; Nowicki et al. 2015), because only a small fraction of individuals is affected if a short-term disturbance happens during the flight period.

Our findings demonstrate that both adult life span and temporal fragmentation index may serve as useful ‘early warning’ indicators, helping to flag-up butterfly species possibly at risk from among those for which detailed information essential for evaluating threat level is lacking. Regretfully, as our literature search implies, the estimates of adult life span and flight period length (needed for calculating temporal fragmentation index) are not readily available for most butterflies either. However, they are relatively easy to get through mark-recapture studies. These are simple to plan and conduct, and they may be carried out with the help of amateur naturalists. This gives the longevity-related parameters analysed in the present study a substantial advantage over the proxies for species vulnerability previously suggested by other authors (Kotiaho et al. 2005; Mattila et al. 2006; Nylin and Bergström 2009), such as host plant specificity, overwintering stage, patch size requirements or mobility (see Introduction for their rationale), because assessing the latter characteristics typically requires specialist expertise.

Obviously, the main drawback of mark-recapture studies is their labour-intensity. Therefore, it would be highly desirable if the information on adult longevity and flight period length could be extracted from the well-established butterfly monitoring schemes based on transect counts. Evaluating flight period length with transect counts requires increased frequency of the transect surveys, because biweekly counts, as currently adopted in most monitoring schemes (van Swaay et al. 2008), are not enough for this purpose. In turn, assessing adult longevity with transects counts appears more difficult to accomplish. Zonneveld (1991) developed a theoretical model for the estimation of life span from transect count data, but its applicability has so far been hampered by rigorous assumptions, which are difficult to meet in real world situations (Nowicki et al. 2008). Nevertheless, more recent developments based on this model, such as the Insect Count Analyzer (INCA) software (Longcore et al. 2003), are promising and give some hope that transect counts can be reliably used to derive butterfly life span estimates in the near future.

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[Vyhodnocení účinnosti aktivního managementu na loukách s výskytem modrásků rodu *Phengaris*]

V článku bylo provedeno vyhodnocení účinnosti managementových zákroků, které měly vést k podpoře populací modrásků *Phengaris teleius* a *P. nausithous* na lokalitě Děčín-Dolní Labe. Monitoring probíhal metodou „mark-release-recapture" na celkem 17 lokalitách. Na třech z nich byl aplikován management (seč jednou ročně ve vhodný termín, tzn. mimo letovou sezónu studovaných druhů) po čtyři roky 2012-2015 a na dalších třech stanovištích po dobu dvou let 2014-2015. Na základě odhadu velikostí populací na jednotlivých lokalitách se nepodařilo prokázat zvýšení početnosti populace na žádné ze studovaných lokalit s aplikovaným managementem. Na druhou stranu je nutné říci, že kolonie motýlů z ostatních lokalit (bez údržby) během těchto let stagnovaly. Ukázalo se, že management lokality formou jedné seče za rok je pro modrásky rodu *Phengaris* vyhovující, nicméně efekt na populace není okamžitý.

EVALUATION OF ACTIVE MANAGEMENT APPLIED TO MEADOWS WITH *PHENGARIS* BUTTERFLIES OCCURRENCE[*]

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In recent decades, changes in meadows maintenance have reduced the populations of endangered butterfly species *Phengaris nausithous* (Bergsträsser, 1779) and *P. teleius* (Bergsträsser, 1779). Currently, meadows are either abandoned or intensively used. Unfortunately, both these managements are considered unfavourable for grassland butterfly species. In this study, the effect of suitable meadow management on population sizes of both the above mentioned *Phengaris* species was investigated. The experiment was performed at the locality Dolní Labe (Děčín, Czech Republic). The most suitable models, based on the lowest values of Akaike's information criterion corrected for small sample sizes, were selected using MARK statistical software. The results were subsequently compared with data obtained from this locality prior to the management application. Unexpectedly, no significant positive effects were found. To reach the desirable status, suitable management practices should be applied for long-term. To verify the management effect on the population size, the meadows were divided into three groups: (*i*) application of favourable management, (*ii*) mowing in inappropriate term, (*iii*) without management. Based on the statistical evaluation, the management application proved to be the most favourable option for both studied butterflies species.

Phengaris teleius, *Phengaris nausithous*, Dolní Labe, active protection, mowing, succession

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INTRODUCTION

The majority of European butterfly species are vitally dependent on meadows and open grasslands with large plant diversity (van Swaay, 2002; Jansen et al., 2012). The management methods of these localities belong among the most important factors affecting the density of butterfly populations (Wallis De Vries et al., 2007; D'Aniello et al., 2011). During the recent decade, changes of the management have occurred – the meadows are used intensively. Agricultural intensification causes the biodiversity reduction, abandonment of traditional land-use types (Kruess, Tscharntke, 2002; Benton et al., 2003; Saarinen, Jantunen, 2005; Young et al., 2005), and increased land fragmentation, which is considered to be the main threat to reduce the number of butterfly species (Krauss et al., 2005; Pöyry, 2007). On the other hand, the absence of agricultural interventions leads to the onset of succession (Hula et al., 2004; Skórka et al., 2007).

Active protection of the butterfly species consists in the understanding of their requirements and demands, such as the quantity and distribution of their necessary resources (Dennis et al., 2006; Jansen et al., 2012). The blue butterflies of the genus *Phengaris* are considered as European flagship species for butterfly protection in open grasslands (Spitzer et al., 2009; Thomas et al., 2009; van Swaay et al., 2010). The Scarce large blue, *Phengaris teleius* (Bergsträsser, 1779) and the Dusky large blue, *Phengaris nausithous* (Bergsträsser, 1779) are sympatric living species, occurring in wet meadows (Kajzer-Bonk et al., 2013). Their host plant is Great Burnet (*Sanguisorba officinalis*) (e.g. Thomas, 1984) and both of these species are also myrmecophilous (Elmes et al., 1998;

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Wynhoff et al., 2011). According to the European Red List of Butterflies (van Swaay et al., 2010) and Red List of the Czech Republic (Farkač et al., 2005), *P. teleius* belongs among vulnerable species and *P. nausithous* among near threatened species.

To select favourable meadows management, it is necessary to monitor many factors that affect the survivability and the size of butterfly populations (Van Langevelde, Wynhoff, 2009). The establishment of varied networks including grasslands, patch and road edges, is also very important (Hanski et al., 1994; Nowicki et al., 2013). These measures reduce the fragmentation and support the metapopulation density system (Nowicki et al., 2014). The timing and quantity of blue butterfly meadows interventions belong among essential factors as well. The meadows should be ideally cut once a year, but it is also possible to do the cut every two or three years (Johst et al., 2006; Novák et al., 2007; Vrabec et al., 2008). The negative impact on blue butterflies was reported when meadows were cut twice a year (Dierks, Fischer, 2009). According to Beneš et al. (2002) and Konvička et al. (2005) the optimal time for cutting meadows is May or September. September is also reported by Kőrösi et al. (2014) as an ideal cutting time for *P. teleius* prosperity. Mowing meadows amid the flying season is inconvenient, because it causes not only the lack of nectar sources and oviposition opportunities for adults, but it mainly leads to egg destruction and larval mortality as well (Johst et al., 2006; Dover et al., 2010). Therefore, it is important to make the first mowing in a suitable term providing the host plant sufficient time to restore before the beginning of the flight season (Dierks, Fischer, 2009). The proper mowing timing also influences the host ants (Wynhoff et al., 2011; Kőrösi et al., 2014). When cutting in the second half of September, abundance of *Myrmica* ants in meadows increases (Grill et al., 2008). Beneš et al. (2002) and Konvička et al. (2005) considered mosaic mowing as most suitable for blue butterflies. This cutting method basically replaces traditional agriculture (Pöyry, 2007) with the principle in combination of both unmaintained and maintained vegetation – mowing at different times per year (Morris, 2000; Saarinen, Jantunen, 2005; Farruggia et al., 2012). However, using no management in meadows is also unsuitable, because it causes succession onset, consequently it leads to overgrowing of meadows by woody plants (Provoost et al., 2011; Schirmel, Fartmann, 2014).

The populations of blue butterflies at the Dolní Labe locality have been subjected to long-term monitoring. A favourable meadow management was designed with mowing mode supporting the growth of population and applied in selected meadows in 2014 and 2015. To evaluate the designed management, these hypotheses were tested: (a) yearly mowing in a suitable term will in long-term increase the blue butterfly populations; (b) blue butterfly populations will not be able to survive in meadows which are mown in inappropriate time or in meadows without management.

MATERIAL AND METHODS

Study species

According to the European red list of butterflies (van Swaay et al., 2010), *Phengaris teleius* and *P. nausithous* belong to vulnerable and/or near-threatened species categories. Both these butterflies belong among particularly protected species in the Czech Republic and *P. nausithous* being more distributed (Beneš et al., 2002).

The investigated species are both social parasites with similar life cycles, however, some different aspects in their behaviour are known as well (Beneš et al., 2002). Both species are monophagous, their only host plant is Great Burnet (*Sanguisorba officinalis*) (e.g. Thomas, 1984). Females lay their eggs on the host plant head, larvae hatch and live there till L3 stadium. Immediately after ecdysis to L4, they fall down to the ground, where they are subsequently adopted by *Myrmica* ants (Thomas et al., 1989; Pech et al., 2007). *P. nausithous* larvae could be adopted only by *M. rubra*, while *P. teleius* larvae were found mainly in anthills of *M. scabrinodis*, but also in those of *M. rubra*, *M. ruginodis* or *M. rugulosa* (Tartally, Varga, 2005; Witek et al., 2008, 2011; Wynhoff et al., 2011). Parasitic larvae live for 10–22 months, until they pupate (Thomas, 1984; Sliwinska et al., 2006). Flying season of adult butterflies is the same for both species – since the beginning of July to the end of August. However, certain variability within various regions has been described (Batáry et al., 2009). It is known that the choice of location for oviposition is affected by host plants developmental stages and the host ants presence. While *P. teleius* females oviposit rather to young flower heads, those of *P. nausithous* prefer older host plant heads (Figurny, Woyciechowski, 1998). Both species oviposit only to host plants near *Myrmica* anthills (Van Dyck et al., 2000; Wynhoff et al., 2008; Van Dyck, Regniers, 2010). Ensuring the presence of all resources of vital importance to the blue butterfly genus *Phengaris* is thus largely influenced by meadows management.

Study area

The suitable meadows management research was realized at the locality Dolní Labe (502.34´51°´´N, 1450.99´12°´´E), which is a part of the Protected Landscape Area (PLA) Labské pískovce, near the

Table 1. Management methods applied to *P. teleius* and *P. nausithous* at the Dolní Labe locality in 2009–2015

Patch No.	2009	2010	2011	2012	2013	2014	2015
1	*iii*	*iii*	*iii*	i^{ad}	i^{ac}	i^{ac}	i^{ac}
2	*iii*	*iii*	*iii*	*iii*	*iii*	*iii*	*iii*
3	*iii*	*iii*	*iii*	i^{ad}	i^{ac}	i^{bc}	i^{ac}
4	*iii*	*iii*	*iii*	i^{ad}	i^{ac}	i^{ad}	i^{bc}
5	*ii*	*ii*	*ii*	*ii*	*ii*	*ii*	*ii*
6	*ii*	*ii*	*ii*	*ii*	*ii*	*ii*	*ii*
7	*ii*	*ii*	*ii*	*ii*	*ii*	*ii*	*ii*
8	*ii*	*ii*	*ii*	*ii*	*ii*	*ii*	*ii*
9	*iii*	*iii*	*iii*	*iii*	*iii*	i^{ad}	i^{ac}
10	*iii*	*iii*	*iii*	*iii*	*iii*	*iii*	*iii*
11	*iii*	*iii*	*iii*	*iii*	*iii*	i^{ac}	i^{ac}
12	*ii*	*ii*	*ii*	*ii*	*ii*	*ii*	*ii*
13	*iii*	*iii*	*iii*	*iii*	*iii*	i^{ac}	i^{ac}
14	*iii*	*iii*	*iii*	*iii*	*iii*	*iii*	*iii*
15	*iii*	*iii*	*iii*	*iii*	*iii*	*iii*	*iii*
16	*iii*	*iii*	*iii*	*iii*	*iii*	*iii*	*iii*

(*i*) mowing 1× per year (mowing entire patch^{a}, rotation^{b}), applied in spring^{c} or autumn^{d}; (*ii*) mowing in inappropriate term (during flight season); (*iii*) localities without management

town of Děčín (Czech Republic). Population parameters research of the genus *Phengaris* in this area started in 2008 when only 6 patches were monitored. This flight season therefore was not included into the calculations and statistical analyses. The area is divided into 16 patches with confirmed occurrences of blue butterflies (Fig. 1). The area of the investigated patches ranges from 140 to 19 084 m^2 and maximum distance between the patches is 5.21 km. To compare the results of the management practices, all the patches were divided into three groups/types: (*i*) mowed 1× per year (mowing entire patch, rotation – applied in spring or autumn), rotation mowing implies successive mowing of different meadow fragments (Bubová et al., 2015); (*ii*) mowed in inappropriate term (during the flight season); (*iii*) patches without any management (Table 1).

Patches of type (*i*) (formerly belonging to types (*ii*) and (*iii*)) were firstly mowed in 2012. These patches are mowed periodically once a year in the spring (May) or autumn (September). The first patches mown were the meadows near the Labe River. Depending on the meadows condition, they were mowed once or twice per year since 2012. Currently, six meadows are managed in this way. Another five patches belonging to type (*ii*) are mainly privately owned and uncoordinatedly mown several times per year. The six remaining patches of type (*iii*) are unkept and weed overgrown, vegetation at this places consists mainly of *Urtica dioica*, *Impatiens glandulifera royle, Rubus idaeus* or gone wild fruit trees. Since the beginning of monitoring, no management interventions were observed there.

During monitoring of the Dolní Labe locality in 2008–2011 (2008 excluded from calculations), no targeted management interventions were applied there. In autumn 2012, parts of selected meadows were mowed in cooperation with PLA Labské pískovce. Since 2013, other meadows have been involved in the suitable management program. Therefore, they are mowed once per year in spring or autumn, according to the actual situation. In 2015, the Directorate of Waterways of the Czech Republic started to support this project. Currently, suitable management has been applied at six patches, and enrolment of further patches in the immediate vicinity of the Labe River is scheduled in the following years. The owners of the meadows with *Phengaris* butterfly occurrence outside the Labe River valley, with lacking or inappropriate management, are intended to be addressed, too.

Field methods

We used Mark-Release-Recapture method to find out *Phengaris* butterflies population size at the investigated patches. The monitoring was carried out in the following terms: 2009 (9/7–19/8), 2010 (9/7–12/8), 2011 (8/7–5/8), 2012 (14/7–10/8), 2013 (5/7–15/8), 2014 (3/7–18/8), and 2015 (6/7–14/8). During these days, all investigated patches were observed daily, except continuous rain days. The blue butterflies were captured with an entomological hand-held net. A waterproof pen was used to identify the caught unmarked specimens with a unique code on the ventral side of hind-wings. Capture time, sex, wing wear, weather conditions, butterfly behaviour, and patch were recorded. In case of recapturing marked individuals, we enrolled aforementioned parameters to the recording sheet as well.

Data analysis

Population estimates. To estimate the size of population, all obtained data for both studied butterfly species *P. teleius* and *P. nausithous* were evaluated in the statistical program MARK, which is able to provide information about population size using Capture-Mark-Recapture method (White, Burnham, 1999; Cooch, White, 2007). Concretely, we used Cormack-Jolly-Seber model (Live Recaptures), whose principle is a live animal capturing (Schwarz, Seber, 1999). Basic parameters of the models are survival (φi) and capture probability (pi). For each parameter there exist different standard patterns assumed, for example: (.) parameter constant over time and equal for all groups; (g) parameter constant over time, but varying among groups; (t) parameter changing over time, but equal for all groups; (g*t) parameter changing over time and varying among groups; (g+t) parameter changing over time and varying among groups too, but over time it is constant. There exist 25 different combination models. The selection of the most appropriate model for each data set was based on Akaike's information criterion corrected for small sample sizes (AICc) (Hurvich, Tsai, 1989). The model with the lowest AICc is the one which best fits the empirical data (Nowicki et al., 2005). The best model for population estimation was selected after parameters customization at two patches with the highest density of investigated species. In the case that selected models for these patches were different, we preferred the model used for the patch with the highest captured butterflies number and AICc, ΔAICc, Estimate, Standard Error, 95% Confidence Interval (CI)–, 95% CI+ values as well (see Table 2). After we had selected the most suitable model, the final value of real ($\hat{N}_{total}$) indicating the seasonal population size was calculated (Nowicki et al., 2005).

Statistical analysis

To determine the efficiency of the management, $\hat{N}_{total}$ values calculated in program MARK were used. Subsequently, the $\hat{N}_{total}$ values were statistically analyzed. The significance level $\alpha = 0.05$ was selected for all tests.

Population size comparison after the management change. Verification of the first hypothesis (a – yearly mowing in a suitable term will in long-term increase the population of butterfly species *P. teleius* and *P. nausithous*) was performed in two steps. The analysis

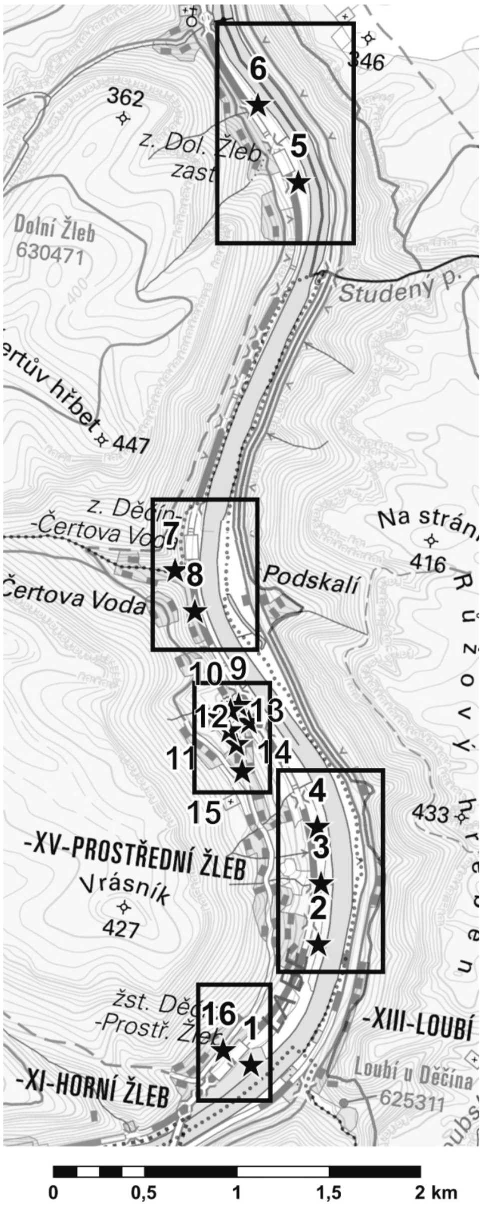

Fig. 1. Locality Dolní Labe (50 51´ 2.34´´N, 14 12´ 50.99´´E) near the town of Děčín, dislocation patches 1–16

Table 2. The best models calculated by MARK software and the individual parameters for *P. teleius* and *P. nausithous* during flight seasons at Dolní Labe locality in 2009–2015

	Selected model		$\hat{N}_{total}$	Best patch*	AICc	ΔAICc	Estimate		Standard error		95% CI			
											95% CI–		95% CI+	
	survival *(φi)*	capture probability *(pi)*					*(φi)*	*(pi)*	*(φi)*	*(pi)*	*(φi)*	*(pi)*	*(φi)*	*(pi)*
2009														
P. teleius	(.)	(.)	368	4	91.42	0.00	0.85	0.28	0.06	0.08	0.69	0.15	0.93	0.47
P. nausithous	(.)	(.)	1370	13	381.14	24.24	0.60	0.44	0.04	0.06	0.52	0.33	0.67	0.55
2010														
P. teleius	(.)	(.)	889	4	138.48	0.00	0.75	0.21	0.06	0.06	0.60	0.12	0.86	0.35
P. nausithous	(t)	(t)	1952	4	376.85	19.95	0.87	0.73	0.00	0.22	0.86	0.24	0.86	0.96
2011														
P. teleius	(.)	(.)	196	4	69.86	0.00	0.84	0.62	0.08	0.11	0.61	0.39	0.95	0.80
P. nausithous	(.)	(.)	273	6	191.50	0.00	0.83	0.53	0.04	0.07	0.74	0.41	0.89	0.66
2012														
P. teleius	(.)	(.)	233	4	67.21	0.00	0.60	0.46	0.13	0.16	0.35	0.19	0.81	0.76
P. nausithous	(g)**	(.)	298	4	119.14	1.57	0.83/ 0.51	0.36	0.54/ 0.16	0.09	0.70/ 0.24	0.21	0.91/ 0.78	0.55
2013														
P. teleius	(.)	(.)	406	15	59.86	2.93	0.81	0.12	0.13	0.08	0.45	0.03	0.96	0.36
P. nausithous	(.)	(.)	952	4	514.08	1.89	0.77	0.28	0.03	0.04	0.70	0.21	0.82	0.36
2014														
P. teleius	(.)	(.)	391	13	159.13	1.74	0.70	0.55	0.06	0.08	0.58	0.39	0.80	0.71
P. nausithous	(.)	(t)	1486	6	1883.98	0.66	0.81	0.89	0.01	0.01	0.78	0.54	0.83	0.98
2015														
P. teleius	(.)	(.)	371	3	467.51	0.00	0.87	0.30	0.02	0.03	0.81	0.24	0.91	0.37
P. nausithous	(.)	(.)	602	3	946.55	1.48	0.81	0.46	0.18	0.03	0.77	0.41	0.84	0.52

$\hat{N}_{total}$ = real population size, AICc = Akaike's information criterion corrected for small sample sizes, CI = confidence interval

*patch with the highest number of captured butterflies, which was essential in the final model selection; the following parameters are provided for this patch; the population estimation was evaluated using data from this locality

**estimate, standard error, and CI values are presented both for males and females

principle was a comparison between flight seasons with management and those without any management. Firstly, we statistically evaluated (independent two-sample *t*-test) data from the patches under management since 2012 (No. 1, 3, and 4 in Table 1). In the next step, patches managed since 2014 (No. 9, 11, and 13) were evaluated in the same way.

Comparison of targeted management efficiency. To verify the second hypothesis (b – the blue butterflies populations will not be able to survive in meadows mowed in inappropriate time or in those without management), we compared the population sizes at patches which were divided into three groups from the management viewpoint: (*i*) suitable management, (*ii*) inappropriate management, and (*iii*) no management (see Table 1). The data were subjected to the analysis of variance (ANOVA) test. We used one-way ANOVA and Scheffé's test (for patches comparison) and main effect ANOVA, Fisher's LSD test, and confirmation Scheffé's test for dependence verification of population size in the management types (*i*); (*ii*); (*iii*) and flight season.

RESULTS

Population estimates

Using the MARK program, the most suitable models were selected (Table 2) and $\hat{N}_{total}$ values for studied butterfly species and flight seasons calculated. For the survival parameter (φ) and the capture probability (p), the model (.)(.) was the most suitable. This model is

Table 3. Statistical analyses for verification of two hypotheses regarding flight seasons 2009–2015 of *P. teleius* and *P. nausithous* at Dolní Labe locality. The calculations were performed using two-sample *t*-tests and the analysis of variance (one-way and the main effects; significance level α = 0.05)

	F	*P*	*P* (sided)*	*P*
(a) Comparison of population size after management changes[a]				
After 2012				
Phengaris teleius	2.887	0.119		> 0.05
Phengaris nausithous	2.295	0.202		> 0.05
After 2014				
Phengaris teleius	5.118	0.082		> 0.05
Phengaris nausithous	18.310	0.005	0.229*	> 0.05
(b) Comparison of targeted management efficiency				
Comparison of type management on patches[b]				
Phengaris teleius	10.727	0.000		< 0.05
Phengaris nausithous	5.750	0.004		< 0.05
Comparison of population size, depending on management type and flight season[c]				
Phengaris teleius				
Year	0.497	0.799		> 0.05
Type of management	2.469	0.126		> 0.05
Phengaris nausithous				
Year	0.766	0.610		> 0.05
Type of management	0.733	0.500		> 0.05

[a] two-sample *t*-test, independent

[b] ANOVA one-way and Scheffé's test

[c] ANOVA main effects and Fisher's LSD test

*newly calculated level of significance for the case of inhomogeneous variances

constant over time and equal for all groups, with equal daily survival and capture probability. It was selected for populations of both investigated butterfly species in 2009, 2011, 2013, and 2015 and for *P. teleius* in 2010, 2012, and 2014. Three other models were applied to *P. nausithous*. For the flight season 2010, model (t)(t) with both daily survival and capture probability varying among sampling days was applied. This model varies over time, but for all groups. Model (.)(g), i.e. the model constant over time and varying for the group, was selected for the season 2012. Finally, (.)(t) model was selected as the most suitable for 2014, with equal daily survival and capture probability varying among sampling days. This model varies over time but equally for all groups. Using the selected models, the $\hat{N}_{total}$ value (real population size) was calculated for all patches and individual flight seasons. These values have been subsequently used to verify the suggested hypotheses.

Statistical analysis

The results of the statistical analyses are shown in Table 3.

Population size comparison after the management change. In the first step of the hypotheses verification, data obtained at patches No. 1, 3, and 4 were analyzed. Statistical analyses were performed separately for both investigated species and no significant effects of management on the investigated patches were found (*P. teleius* and *P. nausithous*: $P > 0.05$). Significant differences between flight seasons before and after management changes were not found in the next step (patches No. 9, 11, and 13) as well (*P. teleius* and *P. nausithous*: $P > 0.05$). However, the population size comparison between the patches with management onset in 2012 and in 2014 reveals certain differences (see Table 3). Meadows managed since 2012 are more successful (*P*-value) than the patches managed since 2014. The trend of differences between population sizes is displayed in Fig. 2.

Comparison of targeted management efficiency. Statistical analyses used for the comparison of management types efficiency (Scheffé's test) demonstrated statistically significant differences ($P > 0.05$) between the management method (*i*) – yearly mowing, and methods (*ii*) and (*iii*) (unsuitable mowing period and no mowing, respectively). The suitable management (*i*) was found most effective for *P. teleius,* on the other hand, method (*ii*) appeared to be the worst one. For *P. nausithous*, significant differences ($P > 0.05$) were found between methods (*i*) and (*iii*). The worst for this species is when the meadows are unmanaged (*iii*). The analyses performed in order to demonstrate the investigated butterfly population dependence on management type and flight season did not show significant differences. Even though Fisher's LSD test showed some deviations during flight season in methods (*ii*) and (*iii*), Scheffé's test and homogeneity of variance test found out no significant differences.

DISCUSSION

In terms of increasing long-term viability of studied butterflies at the locality Dolní Labe, the results of our study showed that timing monitoring and suitable mowing management selection are necessary for the long term. We also verified years mowing efficiency but, unfortunately, no significant effects were found during our four-year-long observation. This fact is in contradiction with results reported by Kőrösi et al. (2014), who found out positive effects of management on *Phengaris teleius* population during just a three-year period. The same authors also recommended mowing in September as the most suitable management. On the contrary, Novák et al. (2007), after three years of investigation, did not find clear effects of management on the blue butterfly population sizes. In our study, we determined noticeable differences between meadows managed since 2012 and those managed since 2014. The positive influence of long-term appropriate meadows mowing is shown in Fig. 2. The patches, which were involved in management only since 2014, had been unkempt and weed overgrown, so the transformation to an optimum condition is still in progress (Pöyry et al., 2005; Stefanescu et al., 2009). However, low population sizes in 2015 could play a significant role in the unclear effect of management which had been firstly applied the previous year 2014. For *P. nausithous*, the $\hat{N}_{total}$ value was more than half lower than in 2014 (see Table 1 – 2014: $\hat{N}_{total} = 1486$; 2015: $\hat{N}_{total} = 602$). For *P. teleius* the values were similar. The population size may be reduced even due to climatic change, which has currently been highly discussed (Cormont et al., 2013; Kajzer-Bonk et al., 2013; Nieto-Sánchez et al., 2015), however this factor has not been investigated at the Dolní Labe locality. To detect a climate change would require a several-year local monitoring. Although the effect on the population size of the investigated butterflies was not statistically confirmed, the host plants number increased noticeably using management. The *Sanguisorba officinalis* presence is essential for the studied species development (Figurny, Woyciechowski, 1998; Batáry et al., 2009; Dierks, Fischer, 2009), however, according to Nowicki et al. (2005) the correlation between the host plant density and the blue butterfly populations size does not exist, which is also demonstrated by our results. *Myrmica* ants are the other essential factor, which could be influenced by meadows management practices (Elmes et al., 1998; Johst et al., 2006; Wynhoff et al., 2011). Positive effects were found out by Dauber et al. (2006). On the other hand, no effect of management is reported by Dahms et al. (2005).

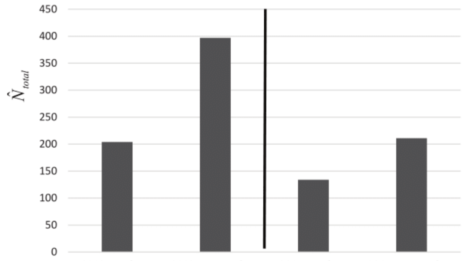

Fig. 2. Graphical comparison of management application success rates at localities mown since 2012 and those managed since 2014. The data shown are for *P. teleius* and *P. nausithous* at the locality Dolní Labe

The present research revealed that patches with active suitable management are highly sought for by the studied butterflies. The lowest occurrence of *P. teleius* individuals was found at meadows mowed in the middle of flight season while meadows without management were evaluated as worst patches for *P. nausithous* survival. For *P. teleius*, our results correspond with those reported by Skórka et al. (2007), who did not consider succession as a serious threat. No significant influence of primary succession on blue butterfly populations was also reported by Dover et al. (2010). On the other hand, period without management longer than three years could have fatal consequences for blue butterflies (Bergman et al., 2004; Kőrösi et al., 2014). The hypothesis (b) – butterfly species *P. nausithous* and *P. teleius* are unable to survive in a long-term at patches with inappropriate or no management – was confirmed.

CONCLUSION

To protect the butterflies of meadow habitats, the natural environment must be maintained. There is a need to replace the intensive mowing regime and ensure a return of traditional land-use practices. Butterfly populations of *P. teleius* and *P. nausithous* are decreasing due to inadequate mowing regime changes. Their meadows are either mowed in inappropriate terms (in the middle of flight season) or even not mowed at all. Unfortunately, meadows abandonment is usually connected with successional changes and landscape fragmentation increase. To determine a management favourable for blue butterflies, it is necessary to take into account all landscape requirements and the mowing regime selection should be realized on total compromises basis, which reflects the character of a particular locality.

P. teleius and *P. nausithous* population parameters have been monitored on 16 patches at Dolní Labe locality since 2008. To increase the studied butterfly populations, targeted management was applied to selected localities. Since 2012, three patches near the Labe River have been annually mowed (mowing entire patch, rotation) in spring or in autumn. In 2014, other three patches, which are located off the Labe River shores, were involved into the management. The population sizes were analyzed using program MARK, which allowed us to compare the population success at the patches before and after the mowing application. The effects of both applied managements (since 2012 and since 2014) on the population size were not significant. However, butterfly population increases were evident in patches mowed since 2012 compared to those mowed since 2014. These results showed that the long-term management at investigated localities is essential and its effect will be apparent in a time horizon longer than four years. To comprehensively compare all the investigated meadows at the Dolní Labe locality, the patches were divided from the management viewpoint into three groups: (*i*) application of favourable management, (*ii*) mowing in inappropriate term, (*iii*) without management. The statistical analyses results confirmed the (*i*) variant as the most favourable option for both butterfly species studied.

For protection and enhancing the number of blue butterflies on the area of Dolní Labe, it is also necessary to properly set the mowing term. Therefore, it will be important to verify the mowing effect in spring and in autumn to determine more appropriate management methods. Several new localities, mainly near the Labe River, are planned to be involved into the mowing regime in the next flight season. Also negotiations with the owners of meadows mowed during the flight season or left without management will be continued. Similarly, we will extend the cooperation with the PLA Labské pískovce and the Directorate of Waterways of the Czech Republic, which should follow our common objective – to increase the size of investigated butterfly populations by suitable management application and mowing optimization.

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Monitoring modrásků *Phengaris teleius* a *P. nausithous* byl u Přelouče zahájen na začátku století v souvislosti se stavbou vodního kanálu na řece Labe. Do roku 2010 probíhal monitoring pouze na 10 stanovištích na pravém břehu, které dle plánu stavby měly být z velké části poničeny. V roce 2011 však byly objeveny další populace na opačném břehu řeky. V této práci byly zveřejněny a vyhodnoceny výsledky monitoringu těchto motýlů metodou „mark-release-recapture“ z let 2011-2015. Průměrná metapopulační velikost za toto období 2800 jedinců *P. teleius* a 1400 pro *P. nausithous* byla rovnoměrně rozložená po obou březích řeky včetně lokalit, kterých by se stavba průplavu neměla dotknout. Místo původně odhadované ztráty 40,1 - 64,3 % populace *P. teleius* a 20,2 - 47,4 % *P. nausithous* se tak nový odhad potenciálních ztrát důsledků zániku patchů na pravém břehu řeky snížil na 13,9 – 25,7 % pro *P. teleius* a 8,5 – 20,0 % pro *P. nausithous*. Při aplikaci vhodného managementu na plochách na levém břehu tak prognóza pro celkovou místní metapopulaci vypadá v porovnání s předchozím odhadem příznivě.

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ORIGINAL PAPER

Long-term monitoring of *Phengaris* (Lepidoptera: Lycaenidae) butterflies in the Přelouč surroundings (Czech Republic): is the waterway construction a serious threat?

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Abstract The monitoring of sympatrically occurring *Phengaris teleius* and *P. nausithous* metapopulations in the surroundings of Přelouč, Czech Republic, was launched over a decade ago in connection with the planned waterway construction on the Elbe river. The mark-recapture surveys were initially restricted to 10 habitat patches on the right river bank, but in 2010 three new patches were discovered on the opposite bank. We use the monitoring results for 2011–2015 to assess how the discovery of additional populations alters the impact evaluation of the prospective construction. The overall abundance of *P. teleius* in the recently discovered populations was about twice as high as on the right bank (ca. 2,800 vs. 1,400 adults on average), while the numbers of *P. nausithous* on both banks proved to be balanced (at ca. 600 adults on average). Furthermore, we confirmed a substantial exchange of butterflies between population representing both banks, which indicates a well-integrated metapopulation in both species. Since the waterway would partly destroy the two largest populations on the right bank, the potential loss due to its construction is estimated at 40.1–64.3% *P. teleius* individuals and 20.2–47.4% *P. nausithous* individuals occurring there. Nevertheless, concerning the entire metapopulation, the predicted decline is considerably smaller, reaching 13.9–25.7% in *P. teleius* and 8.5–20.0% in *P. nausithous*. Consequently, a long-term survival of the species is likely even in the case of the waterway construction, as long as appropriate management is applied on the unaffected habitat patches.

Keywords Extinction risk · *Phengaris teleius* · *Phengaris nausithous* · Population size · Natura 2000 · Threat assessment · Waterway construction

Introduction

Large-scale land use changes have resulted in serious declines of many European butterflies in recent decades (Thomas et al. 2004). Two species of the genus *Phengaris* Doherty 1891 (syn. *Maculinea* Van Eecke 1915): *P. nausithous* (Bergstässer 1779) and *P. teleius* (Bergstässer 1779) are among endangered butterfly species in Europe (Settele et al. 2005). In the European Red List of Butterflies both species are considered 'Vulnerable/Near Threatened' (van Swaay et al. 2010), they are also listed in the Annex of the Habitats Directive. Due to their unique life cycle, including social parasitism in *Myrmica* ants, sedentary larval stages that feed exclusively on the single host plant *Sanguisorba officinalis*, and their short adult life span, these species are considered to be grassland biodiversity indicators as well as the flagships for nature conservation (Settele et al. 2005; Thomas et al. 2009). The *Phengaris* butterflies typically live as metapopulation systems formed by spatially separated local populations within mosaic landscapes (Nowicki et al. 2005, 2007, 2014; Bonelli et al. 2013). The landscape capacity to support a viable metapopulation depends on the number and sizes of local habitat patches as well as on their spatial locations, which affect habitat connectivity (Hanski and Ovaskainen 2000). Therefore, each local habitat loss could act as a threat for metapopulation

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stability, and thus also for the persistence of these butterfly species in a wider region.

A significant impact of human activities on butterfly populations and their biotopes is well known. The constructions of roads, quarries or human settlements are some of the most common reasons for the destruction of habitats of endangered species (van Swaay et al. 2006). All of the aforementioned constructions also lead to habitat fragmentation at the landscape scale, which is considered the most significant reason for butterfly population declines (Kotiaho et al. 2005). Accordingly, the diversity of butterfly species in many European regions is negatively influenced by urban sprawl (Lizeé et al. 2011; Concepción et al. 2016) and modern agricultural practices (Stefanescu et al. 2004). ***Phengaris*** butterflies constitute one of the most spectacular examples of species retreat due to large-scale land-use changes, although in some regions they have been reintroduced recently (Wynhoff 2001; Wynhoff et al. 2011).

In the surroundings of the town of Přelouč, the Czech Republic, the monitoring of ***Phengaris*** populations was initiated over a decade ago in relation to the construction of the navigable waterway that leads to the city of Pardubice. The continuous research there is focused mainly on identification of new localities of ***Phengaris*** butterflies (Vrabec and Rychlíková 2006; Horák et al. 2008; Vrabec and Hanykýřová 2011; Vrabec and Šebková 2011). Moreover, several valuable ecological studies into the functioning of ***Phengaris*** metapopulations were based on the data obtained in the region (Witek et al. 2008; Nowicki and Vrabec 2011; Bonelli et al. 2013; Nowicki et al. 2014).

The preliminary mark-recapture survey in 2002 was performed on only one main habitat patch at the Slavíkovy ostrovy locality on the right (north) bank of the Elbe river, even though several other ***Phengaris*** populations were known in the Přelouč surroundings at that time (cf. Vrabec et al. 2005). In 2004, the monitoring was extended to eight closely lying patches within the same locality, and two other patches there were included in the monitoring in 2005. Thus, between 2005 and 2010, the research area comprised 10 patches (Nowicki and Vrabec 2011). This extension allowed the initial assessment of the metapopulation size, as well as of the possible impact of the waterway construction. Moreover, appropriate management, although irregular and with varying intensity, was set up on a few patches (see Table 1). In 2010 we confirmed the occurrence of ***Phengaris*** butterflies on the opposite (left) bank of the Elbe (Vrabec et al. 2005; see Fig. 1), and from the following year, our monitoring programme was extended to these newly discovered localities. Taking into account the left bank populations, the imminent threat to the entire metapopulation represented by the waterway construction should not be as serious as initially expected, although this is dependent on the size of these populations and their integration, through butterfly dispersal, with the rest of the metapopulation.

Therefore, in the present study we aim at assessing the current status of both ***Phengaris*** species in the region, with a special focus on how it is affected by the recently discovered additional populations. We also evaluate how the existence of these populations alters the level of threat to the metapopulation caused by the planned waterway

Table 1 Management of the habitat patches of *Phengaris* butterflies in the Slavíkovy ostrovy locality on both banks of the Elbe river

Year	Right bank patches										Left bank patches		
	1	2	3	4	5	6	7	8	9	10	11	12	13
2004	AP	AP	AP	No	No	IN	IN	No	No	IN[a]	IN	IN	No
2005	AP	AP[a]	AP/IN	AP[a]	AP[a]	IN	IN	No	AP[a]	IN[a]	IN	IN	No
2006	AP	AP[a]	AP[a]	AP[a]	AP[a]	IN	IN	No	AP[a]	IN[a]	IN	IN	No
2007	AP[a]	AP[a]	AP	AP[a]	No	IN	IN	No	AP[a]	IN[a]	IN	IN	No
2008	AP[a]	AP	AP	AP[a]	No	AP[a]	AP[a]	No	AP[a]	IN[a]	IN	IN	No
2009	AP[a]	AP[a]	AP	AP[a]	No	IN	IN	No	IN	IN[a]	IN	IN	No
2010	IN[a]	AP[a]	AP[a]	AP[a]	No	IN	IN	No	AP[a]	IN[a]	IN	IN	No
2011	No	No	No	No	No	IN	IN	No	No	IN[a]	IN[a]	IN	No
2012	AP[a]	AP[a]	No	AP	No	IN	IN	No	AP[a]	IN[a]	AP/IN	IN	AP[a]
2013	AP[a]	IN[a]	No	AP[a]	IN[a]	IN	IN	No	AP[a]	IN[a]	AP/IN	IN	No
2014	AP[a]	No	IN[a]	IN	No	IN	IN	No	AP	IN[a]	AP/IN	IN	AP[a]
2015	AP[a]	AP	No	AP	No	IN	IN	No	AP/IN	IN[a]	AP	IN	AP[a]

AP appropriate management, concerning the requirements of the investigated ***Phengaris*** butterflies, i.e. mowing either in spring or in autumn, with occasional removal of shrubs and tree seedlings, ***IN*** inappropriate management, i.e. mowing during the butterfly flight period in summer season, ***AP/IN*** appropriate and inappropriate management applied in different fragments of a patch, ***No*** no management, i.e. meadow abandonment

[a] Cases in which management was applied only in a part of a patch

Fig. 1 Map of the study area with the locations of the habitat patches of *Phengaris* butterflies marked in *yellow*. The *blue line* delimits the fragment of the meadow complex to be destroyed by the planned waterway and associated constructions. The background satellite image is freely available from http://www.mapy.cz. (Color figure online)

construction in the near future. In a broader context, we thus answer the question of how the assessments of habitat destruction impact on metapopulation persistence depend on defining the spatial extent of a focal metapopulation. Finally, we provide recommendations for the conservation actions needed in the light of this prospective construction.

Methods

The study area is located in the Czech Republic, Eastern Bohemia, in the surroundings of the town of Přelouč in the Pardubice district (GPS 50°03′N 15°34′E, 207 m a.s.l.). The locations of all the investigated patches are shown in Fig. 1. The long-term monitoring of the sympatrically occurring *P. teleius* and *P. nausithous* is based on intensive mark-recapture performed at the selected localities. The results concerning the metapopulation dynamics and dispersal among the local populations based on the data collected for the ten initially known habitat on patches (numbered 1–10) on the right bank of the Elbe were published by Nowicki and Vrabec (2011). In the present study, we analyse the data provided by the surveys conducted since 2011, which included all the previously known populations as well as the three additional ones (numbered 11–13), discovered on the opposite bank of the river.

The mark-recapture sampling evaluated in this paper was conducted from 6 July to 13 August 2011, 10 July to 4 August 2013, 3 July to 11 August 2014, and 8 July to 13 August 2015. The sampling was typically conducted on a daily basis, with few gaps due to unfavourable weather. Each capture day was performed approximately between 09:00 and 17:00. Every specimen captured was marked with a unique code on the right back wing. Data obtained for the 2012 season, when the survey intensity was very low due to the lack of manpower available, were not used, because their quality was insufficient for the envisaged analyses.

For the purpose of this study, population sizes of both species for patches 1–10 (on the right bank of the Elbe river) and patches 11–13 (on the left bank) were estimated individually for years 2011 and 2013–2015. In addition, we estimated the size of the fraction of the metapopulation present on each bank, based on the mark-recapture data pooled for both groups of populations. Whenever possible, the seasonal population size estimates (N_{total}) were derived together with their standard errors (SE), using Jolly–Seber models (Arnason and Schwarz 1999) in MARK 8.1 software and the approach described by Nowicki and Vrabec (2011). For small populations, in which the sample size of capture records was too small for applying Jolly–Seber models (typically below ten individuals), we approximated the seasonal population size through dividing the total number of captured butterflies by the average seasonal proportion of individuals captured in large populations estimated with Jolly–Seber models.

In addition, we investigated the distribution of adult butterfly movements among the investigated populations. Due to relatively low numbers of inter-patch movements recorded, and the fact that their directions were consistent between years, we analysed the movements jointly for the whole period of our study. However, we distinguished between movements among patches 1–10 or among patches 11–13, i.e. the flights performed between populations on the same river bank, and movements between the two aforementioned groups of patches, which must have involved flights across the Elbe.

Based on the waterway project documentation, its construction would destroy 60% of the area of patch 1, 20% of patch 8, and 25% of patch 9. These data were subsequently used to assess potential declines of the metapopulations of both investigated *Phengaris* species caused by the construction. The potential decline was calculated as the proportion of the metapopulation present within the area to be destroyed; the calculations were done separately for each year to account for possible inter-annual variability of population sizes, which is typical for the focal butterflies (Nowicki et al. 2009). We considered two scenarios. In the first one (A), only the fraction of the metapopulation on the right bank was taken into consideration, and the potential decline was calculated, based on the fractions of populations 1 and 9 to be lost, as:

$$\%\,\mathrm{Decline}(A) = \left(0.6 \times N_{\mathrm{total}\ 1} + 0.25 \times N_{\mathrm{total}\ 9}\right) / N_{\mathrm{total}\ 1\text{–}10} \times 100\%$$

In the second scenario (B), the metapopulation was assumed to include also the left bank populations, and the potential decline was defined as:

$$\%\ \mathrm{Decline(B)} = \left(0.6 \times \mathrm{N}_{\mathrm{total}\ 1} + 0.25 \times \mathrm{N}_{\mathrm{total}\ 9}\right) / \left(\mathrm{N}_{\mathrm{total}\ 1--10} + \mathrm{N}_{\mathrm{total}\ 11--13}\right) \times 100\%$$

It should be explained that patch 8 was not included in the numerator of the above formulas even though it is going to be partly lost, because it is mostly overgrown with shrubs and the number of butterflies recorded there over the last 5 years was always close to zero. Furthermore, to simplify the calculations, the densities of the focal butterflies at the investigated patches were considered to be uniform within for the entire area of any given patch. We thus disregarded the possible preferences of ***Phengaris*** butterflies for patch edges (Kőrösi et al. 2012; Nowicki et al. 2013), however, the resulting bias in our calculations is negligible, because the patch fragments to be destroyed by the waterway construction contain their margins and core areas alike. Finally, by using the sum of the metapopulation fractions on both banks in the denominator (for the scenario B), we probably slightly overestimate the size of the entire metapopulation, because the individuals dispersing between both banks are included twice in the calculations. Nevertheless, the overestimation should be minor, because the numbers of flights across the Elbe were relatively low as compared with those among populations located on the same river bank (see "Results" section), and the estimation of the entire metapopulation size based on pooled data would be problematic due to the occasional differences in surveys on both bank (e.g. different sampling days were used sometimes).

Results

The population sizes of ***Phengaris*** butterflies at individual habitat patches on both banks of the Elbe river are presented in Tables 2 and 3. Based on these results, the highest population sizes of ***Phengaris teleius*** on the right bank were found on patch 1 (Table 2), which would be mostly destroyed, as well as on patch 9, which would be also significantly affected by the waterway construction, i.e. partially destroyed and transformed into an island. For ***P. nausithous***, the butterflies on the right bank were more or less equally distributed among all patches (Table 3), but the highest abundances were again found on patches 1 and 9 as well.

The comparison of the sizes of metapopulation fractions present on the right and left bank is shown in Figs. 2 and 3. In the case of ***P. teleius***, it is evident that its overall abundance on the left bank (i.e. within the recently discovered patches) predominates over the right bank (i.e. within the previously known patches), with ca. 2,800 vs. 1,400 adults per year on average. In turn, the abundance of ***P. nausithous*** is roughly similar on both banks (ca. 600 adults per year on average in each case). Moreover, the exchange of individuals between the populations on both banks was confirmed (Figs. 4, 5). For both investigated species, we recorded a substantial number of flights over the Elbe (more than 150 cases in ***P. teleius*** and nearly 40 cases in ***P. nausithous***). Although their frequencies were relatively low as compared with those of the flights among patches located on the same river bank, this fact apparently reflects the decrease in dispersal probability with increasing movement distance.

The hypothetical butterfly loss due to the waterway construction, caused by the partial destruction of patches 1 and 9, is shown in Table 4. Assuming a uniform population density within both aforementioned patches, 40.1–64.3% of ***P. teleius*** and 20.2–47.4% of ***P. nausithous*** occurring on the right bank would be sacrificed. On the other hand, the inclusion of the left bank populations into the calculations revealed that the potential butterfly

Table 2 The numbers of the individuals captured (n) and the estimated sizes of ***Phengaris teleius*** populations ($\mathrm{N}_{\mathrm{total}}$, whenever possible presented with their standard errors) on both banks of the Elbe river (right bank populations: 1–10; left bank populations: 11–13)

Patch	Area (ha)	2011		2013		2014		2015	
		n	$\mathrm{N}_{\mathrm{total}}$ (±SE)	n	$\mathrm{N}_{\mathrm{total}}$ (±SE)	n	$\mathrm{N}_{\mathrm{total}}$ (±SE)	n	$\mathrm{N}_{\mathrm{total}}$ (±SE)
1	1.57	430	884 (±185)	307	1188 (±225)	426	957 (±90)	255	929 (±145)
2	0.29	2	5	3	10	4	9	5	14
3	0.28	5	13	0	0	3	7	5	14
4	0.54	1	3	0	0	14	31	19	54
5	0.45	9	23	1	3	8	18	0	0
6	1.24	4	10	1	3	2	4	4	11
7	0.52	4	10	2	7	12	27	10	29
8	0.21	13	33	2	7	0	0	1	3
9	1.13	83	296 (±33)	152	386 (±54)	296	746 (±73)	294	754 (±92)
10	1.51	2	5	0	0	0	0	0	0
11	4.01	195	695 (±73)	321	2259 (±383)	939	3214 (±285)	1209	2768 (±365)
12	0.90	127	366 (±56)	162	662 (±220)	341	1308 (±232)	381	736 (±107)
13	0.31	5	10	11	55	50	265 (±17)	2	4

Table 3 The numbers of the individuals captured (n) and the estimated sizes of ***Phengaris nausithous*** populations (N_{total}, whenever possible presented with their standard errors) on both banks of the Elbe river (right bank populations: 1–10; left bank populations: 11–13)

Patch	Area (ha)	2011		2013		2014		2015	
		n	N_{total} (±SE)	n	N_{total} (±SE)	n	N_{total} (±SE)	n	N_{total} (±SE)
1	1.57	48	262 (±44)	28	107 (±54)	74	186 (±81)	75	143 (±33)
2	0.29	4	20	5	11	7	13	4	7
3	0.28	16	80	39	130 (±51)	48	57 (±16)	34	51 (±8)
4	0.54	0	0	0	0	44	107 (±47)	22	36 (±6)
5	0.45	2	10	3	7	7	13	0	0
6	1.24	5	25	7	16	18	33 (±14)	12	20
7	0.52	25	125	48	117 (±44)	23	42 (±19)	11	18
8	0.21	3	15	0	0	0	0	0	0
9	1.13	20	100	76	221 (±50)	202	440 (±137)	115	216 (±30)
10	1.51	4	20	0	0	0	0	0	0
11	4.01	64	362 (±106)	28	140	54	148 (±74)	126	310 (±79)
12	0.90	23	100 (±72)	111	689 (±239)	93	444 (±145)	49	104 (±22)
13	0.31	7	35	13	65	24	60	6	13

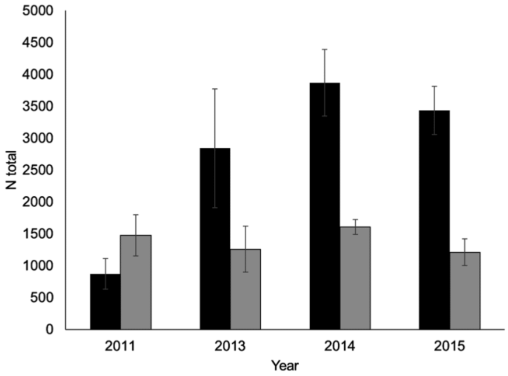

Fig. 2 The estimated sizes of *Phengaris teleius* metapopulation fractions present on both banks on the Elbe river (***grey bars*** left bank patches; ***black bars*** right bank patches; ***whiskers*** represent standard errors of the estimates). It should be noted that the estimates were derived independently of the sums of the population size estimates given in Table 2 and are usually considerably lower, because of a number of dispersing individuals, which are included in the population sizes for more than 1 patch. The estimates for 2012 are not available due to inadequate quality of the mark-recapture data gathered. The estimate for the left bank metapopulation fraction in 2013 is less reliable, as indicated by its relatively large standard error, due to relatively poor fit of the best performing Jolly–Seber model

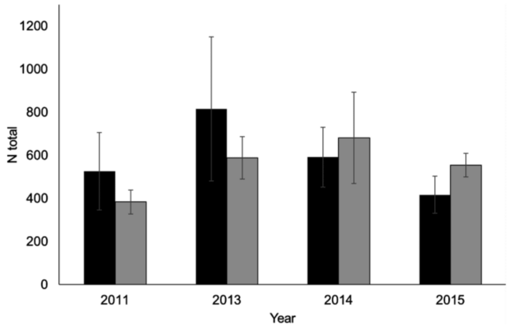

Fig. 3 The estimated sizes of *Phengaris nausithous* metapopulation fractions present on both banks on the Elbe river (***grey bars*** left bank patches; ***black bars*** right bank patches; ***whiskers*** represent standard errors of the estimates). It should be noted that the estimates were derived independently of the sums of the population size estimates given in Table 2 and are usually considerably lower, because of a number of dispersing individuals, which are included in the population sizes for more than one patch. The estimates for 2012 are not available due to inadequate quality of the mark-recapture data gathered

losses may represent only 13.9–25.7% of the entire metapopulation for *P. teleius* and 8.5–20.0% for *P. nausithous*.

Discussion

The knowledge of the actual metapopulation size and its functional structure (i.e. local populations linked with dispersal or isolated) is substantial for assessing the chances of species persistence in fragmented landscapes and its vulnerability to threats. In our specific case, the estimates of population sizes in individual patches combined with the information on butterfly movements enable us to predict the potential decline in metapopulation size that could happen if some of the patches are impacted by the construction of the waterway.

The waterway construction project is routed directly through patch 1 and it will partially disrupt patch 9 as well. If the metapopulation was restricted only to the right bank of the Elbe, then the planned construction would imply a

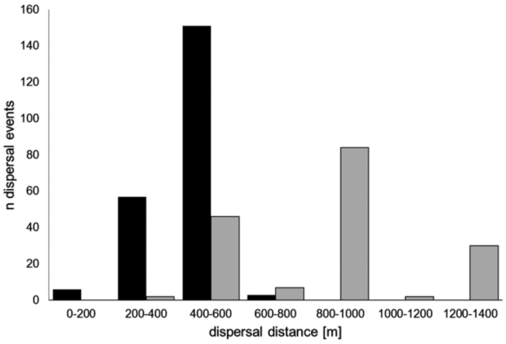

Fig. 4 Frequency of *Phengaris teleius* dispersal events among habitat patches located on the same bank of the Elbe river (***black bars***) and between patches on the opposite banks, which must have involved flights across the Elbe (***grey bars***)

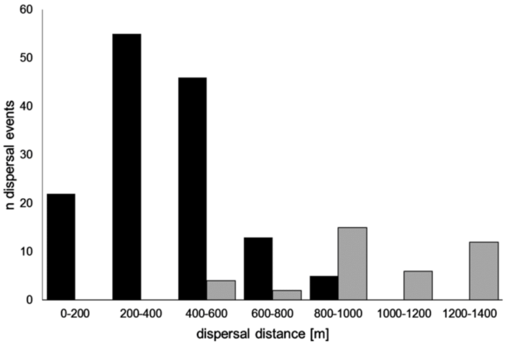

Fig. 5 Frequency of ***Phengaris nausithous*** dispersal events among habitat patches located on the same bank of the Elbe river (***black bars***) and between patches on the opposite banks, which must have involved flights across the Elbe (***grey bars***)

significant risk of the local extinctions of both butterflies, because patches 1 and 9 host the majority of the individuals in both investigated species present on the right bank. The destruction of large fragments of these patches would mean the loss of about half the overall of the population on the right bank in the case of ***P. teleius***, and around one-third in the case of ***P. nausithous***. Such a substantial decline might lead to a subsequent collapse of the entire system as a result of stochastic processes at the remaining smaller populations (Cormont et al. 2011; Bouhours and Lewis 2016). To our knowledge, no study describing minimal abundance necessary for maintaining genetic variability is available for the ***Phengaris*** butterflies; however, it is generally known that population decline causes the gene loss due to genetic drift and inbreeding, increasing the risk of negative genetic impacts on the population survival (Reed et al. 2003; McLaughlin et al. 2002a, b; Hellmann et al. 2003). The loss of genetic variability should therefore be considered highly likely in the substantially reduced metapopulations of ***Phengaris*** butterflies.

The recent discovery of additional populations on the left bank of the Elbe completely change the impact evaluation for the waterway construction project. Our observations show that the river does not represent an obstacle for dispersal (see Figs. 4, 5). In the present study we have not considered dispersal among patches located on the same river bank, but it was the subject of detailed analyses in our earlier papers (Nowicki and Vrabec 2011; Nowicki et al. 2014), which documented a substantial exchange of individuals. The gene flow is therefore ensured, and with the inclusion of the left bank patches the metapopulations of ***Phengaris*** butterflies appear to be at least twice larger than initially expected. It is worth noting that these recently discovered patches should not be affected by the waterway construction. Moreover, their appropriate management could lead to an increase in local abundances of both species, which would compensate for the losses in the populations on the right bank. In the light of these facts, the prospective patch 1 destruction as well as the partial devastation of patch 9 do not appear as much of a tragedy for the metapopulations of both investigated species. The predicted decline for the entire metapopulation should not be higher than ca. one-fourth for ***P. teleius*** and ca. one-fifth for ***P. nausithous***.

Table 4 Comparison of the overall abundances of *Phengaris* butterflies within the area to be spared by the planned waterway construction (unaffected N_{total}) and the hypothetical decline in metapopulation size caused the construction (% decline) under the two scenarios considered: A = metapopulation restricted to the right bank populations; B = metapopulation comprising the populations on both banks

Scenario	Species	2011		2013		2014		2015	
		Unaffected N_{total}	% decline	Unaffected N_{total}	% decline	Unaffected N_{total}	% decline	Unaffected N_{total}	% decline
A	*P. teleius*	874	40.1	450	64.3	848	47.3	644	53.6
	P. nausithous	202	47.4	469	20.2	459	32.6	412	25.4
B	*P. teleius*	1746	25.7	3291	19.7	4716	13.9	3398	18.0
	P. nausithous	728	20.0	1284	8.5	1051	17.4	999	12.3

On the other hand, if the waterway construction is launched, proper management of all the remaining patches, unaffected by construction, will play a crucial role in the the conservation of *Phengaris* butterflies in the region. This is especially true for patches 11–13 on the left bank of the Elbe. In the ideal case, appropriate management should be implemented also in the neighbouring localities with *Phengaris* butterflies situated several kilometres apart, such as Labišťata, Lohenice, and Semín-Polábek (authors' unpubl. data), which were not included in the present study, but are within the maximum dispersal distance of both species (Nowicki et al. 2014).

From a wider landscape perspective, such a comprehensive approach proves to be a necessity, because the waterway construction is not the only threat to the blue butterflies. The availability and quality of patches with enough resources will always be the limit for *Phengaris* butterflies. Small and isolated populations of the *Sanguisorba officinalis* host plant are vulnerable to environmental stochasticity and sudden management changes, whereas larger ones are more stable (Wärner et al. 2014). Therefore, the same effects could be expected for the studied butterflies, which are completely dependent on their host plants.

Furthermore, granting the construction permission should be accompanied by the declaration of the preserved sites with *Phengaris* butterflies in the Přelouč surroundings as a Site of Community Importance (SCI). The SCI status will allow us to influence the land use at a wider spatial scale and will also provide us with legal means of management control on the relevant habitat patches, regardless of when the waterway construction is finalized.

Conclusions

Assessment of the potential impact of the planned waterway construction on *Phengaris* butterflies, restricted to the ten previously known habitat patches on the right bank of the Elbe, indicated that such a limited system would be prone to extinction in the case of a partial destruction of its main populations, occupying patches 1 and 9. The discovery of additional patches inhabited by *Phengaris* butterflies on the left bank of the Elbe, and proving that they are well integrated in the metapopulation through butterfly dispersal across the river, changed the evaluation of the waterway construction impact on the locality. Concerning the entire metapopulation in a broader area (i.e. all 13 patches), a long-term survival of the butterflies is likely even in the case of the waterway construction, as long as appropriate management is applied on the unaffected habitat patches. Furthermore, the declaration of a Site of Community Importance, including a sufficiently large area outside of the construction zone, seems an optimal solution.

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[První etablované populace invazní rybenky *Ctenolepisma longicaudata* (Escherich) v České republice]

V textu je popsán prvonález a nálezové okolnosti invazní rybenky *Ctenolepisma longicaudata* na území České republiky. Tato rybenka původem ze Střední Ameriky se v počátku 21. století rychle šíří Evropou. Na rozdíl od původních druhů nemá speciální nároky na teplotu a vlhkost. Navíc dokáže dlouho hladovět, a má proto do budoucna potenciál stát se dominantní synantropní rybenkou. Na základě hlášení typických škod byli zástupci tohoto druhu objeveni ve skladu a přilehlých kancelářích soukromé firmy v Praze. Vzhledem k masivní infestaci a přítomnosti ve všech stádiích byla populace považována za etablovanou a byla doporučena eradikace. Rybenky byly na toto místo zavlečeny v kartonových obalech se zbožím. Tato publikace také naznačuje, že takovéto sklady by se do budoucna mohly stát primárním habitatem tohoto druhu.

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Rapid Communication

The first established population of the invasive silverfish *Ctenolepisma longicaudata* (Escherich) in the Czech Republic

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Abstract

The silverfish *Ctenolepisma longicaudata* (Escherich) (Zygentoma, Lepismatidae) is an invasive, synanthropic, warehouse, and economic pest, probably of Central American origin. During recent decades, its occasional occurrence has been recorded from some European countries. Here, we report the first established population of *C. longicaudata* within the territory of the Czech Republic. In the autumn 2017, the population was discovered in a warehouse and surrounding office buildings in Prague. Since this species causes damage to starch components and fabrics as well as food contamination, we strongly recommend further monitoring and possibly eradication.

Key words: Lepismatidae, insect, invasive species, biological invasion, Prague

Introduction

Ctenolepisma longicaudata (Escherich), also known under the common names giant silverfish, long-tailed silverfish, and paperfish, is strictly synanthropic and a successful invasive insect species belonging to the order Zygentoma, family Lepismatidae. Since *C. longicaudata* is of no serious medical importance apart from the potential for food contamination, it is considered to be an economic pest (Smith 2017), especially due to its ability to feed on cellulose and starchy materials (Van der Weijden et al. 2007), including wallpapers, books, photo albums, and archives. Moreover, they can also cause damage to cotton or silk (Mallis et al. 1958).

Although first described in South Africa (Escherich 1905), its native range and origin is unclear. This species may originate from central parts of America (Zettel 2010) while Wygodzinsky (1972) marked it as non-native in this region. From there it has been introduced indirectly by human activities, especially as a contaminant of transported commodities, to all continents excluding Antarctica. In Europe, the first individual was captured in France in 1914 (Paclt 1967). However, the nocturnal and hidden way of life probably caused *C. longicaudata* to remain under-recorded and the establishment of this silverfish in several European countries was only confirmed in recent decades. For instance, in the Netherlands, where *C. longicaudata* is currently the dominant species in the modern household environment (Schoelitsz and Brooks 2014), this invasive species was first identified in 1989 (Nierop and Hakbijl 2002). Subsequently, it was reported in Austria (Zimmermann 2016), Belgium (Lock 2007), Germany (Meineke and Menge 2014), Italy (Molero-Baltanás et al. 2000), Sweden (Pape and Wahlstedt 2002), and the United Kingdom (Goddard et al. 2016). Considering relatively intensive trade among the aforementioned countries and the Czech Republic, the introduction of these cardboard package inhabitants was rather a question of time. In the summer 2017, several local Czech online mass media (idnes.cz or euro.cz) even informed about potential risks associated with an invasion of this species.

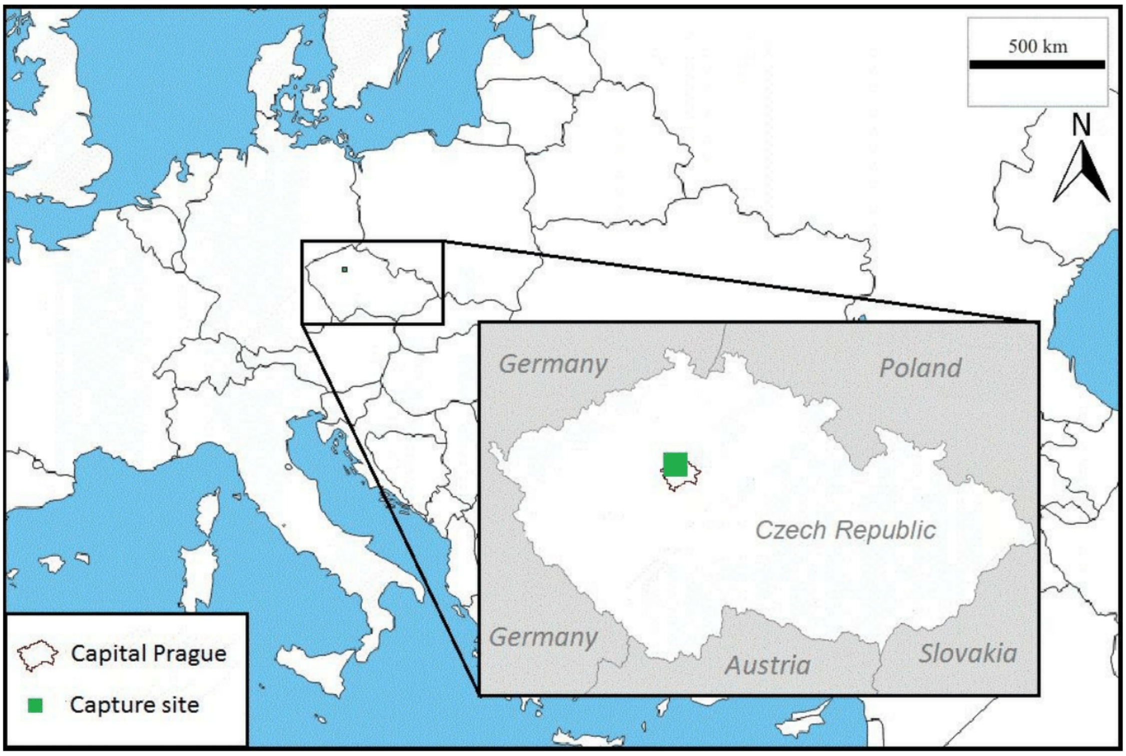

Figure 1. Location of the established population of invasive *Ctenolepisma longicaudata* in Prague, Czech Republic.

Methods

Site

On 18 October 2017, a private company contacted the Department for Vector Control of the National Institute of Public Health (Czech Republic) to report the occurrence of an unusual silverfish species in their offices, archive, and the adjacent warehouse located in the suburb of Prague (Staré Bohnice district, GPS: 50.1346N, 14.4154E, Figures 1 and 2A), where it was causing damage by feeding on documents. Afterwards, the area was visited by the authors, who detected dozens of silverfish, collected some individuals using an aspirator, and preserved them in plastic vials with 70% ethanol for further identification.

Species identification

The collected individuals were examined with an Olympus SZ61 stereomicroscope, and measurements were taken with an ocular grid. Photos were taken using Canon MP-E 65mm/2.8 1–5x macrolens on bellows attached to a Canon EOS 550D camera. Partially focused images of each specimen were stacked using Helicon Focus 3.20.2 Pro software. All individuals were morphologically identified using recent keys (Theron 1963; Wygodzinsky 1972). The species identification was then confirmed by Patrizia Visentin (Entostudio s.r.l., Italy) and Fabrizio Montarsi (Istituto Zooprofilattico Sperimentale delle Venezie, Italy). The voucher specimens are deposited at the National Institute of Public Health in Prague, Czech Republic and in the collection of the Czech University of Life Sciences, Prague, Czech Republic.

Results

During the onsite inspection in the warehouse, we captured 14 individuals (six adults and eight juveniles). Based on the morphological features, all captured silverfish were identified with certainty as *Ctenolepisma longicaudata*. The highest density of *C. longicaudata* was found in the warehouse, on shelves with empty paper boxes (Figure 2A). Additionally, species-specific damage (Figure 2B, C) was observed throughout the entire area of the company, including administrative offices. During the collection of the samples, the humidity in the warehouse was 35%

Figure 2. (A) A warehouse as the habitat of *Ctenolepisma longicaudata*; (B, C), damage caused by silverfish feeding in a neighbouring office building. Photo: J. Patoka (A), J. Dvořáková (B, C).

and air temperature was 23 °C. The captured adult individuals had a body length of more than 12 mm (Figure 3A), with a dark grey dorsal abdomen, uniform in color, antennae, and tails (cerci) longer than the body. The head bore numerous setae on the front margin (Figure 3B), and the last abdominal segment was truncate with slightly emarginate posterior margin. Unlike the other local silverfish species, every collected individual had 3+3 bristle combs of macrosetae on the II–VI urotergites. Finally, nine sensory papillae on the terminal segment of the labial palpus (Figure 3C) supported the identification as *C. longicaudata*.

Discussion

In the Czech Republic, four silverfish species have been reported to date: *Atelura formicaria* Heyden, *Ctenolepisma lineata* Fabricius, *Lepisma saccharina* L., and *Thermobia domestica* Packard (Rusek 1977). While *L. saccharina* is very common in households throughout the country, the other species mentioned above are quite rare. *Thermobia domestica* Packard occurs only in warmer artificial habitats, such as bakeries, boiler rooms, hot-water heaters, or heating plants, and is also commercially reared for use as live prey for small insectivorous pets. *Ctenolepisma lineata*, which was confirmed in the South Moravian Region (Štys and Rozkošný 1996; Král and Davidová-Vilímová 2001), is very rare and is listed as a critically endangered species (Rusek 2005). The last silverfish species, *A. formicaria* (family Nicoletiidae), lives in ant colonies, especially of the genus *Lasius* (Parmentier et al. 2013). Here, we record for the first time the occurrence of *C. longicaudata* in the Czech Republic. Given the observed abundance and presence of all developmental stages of *C. longicaudata* including small nymphs, the species is considered established.

In the past, households, libraries, and museums used to be the main habitats for *C. longicaudata*. Unlike other European anthropophilic Zygentoma species, *C. longicaudata* does not necessarily need high air humidity and temperature for its development. The temperature optimum lies at around 24 °C; however, *Ctenolepisma longicaudata* might also survive in temperatures as low as 1 °C for several months (Lock 2007). This species feeds mainly on cellulose (paper) or starch including glue (Van der Weijden et al. 2007) and has also been reported to be attracted by food residues (Goddard et al. 2016). Additionally, it can survive long periods of starvation (Lindsay 1940). Therefore, it is able to spread easily with the aid of parcel and post services and, consequently, readily colonizes new areas. Its introduction

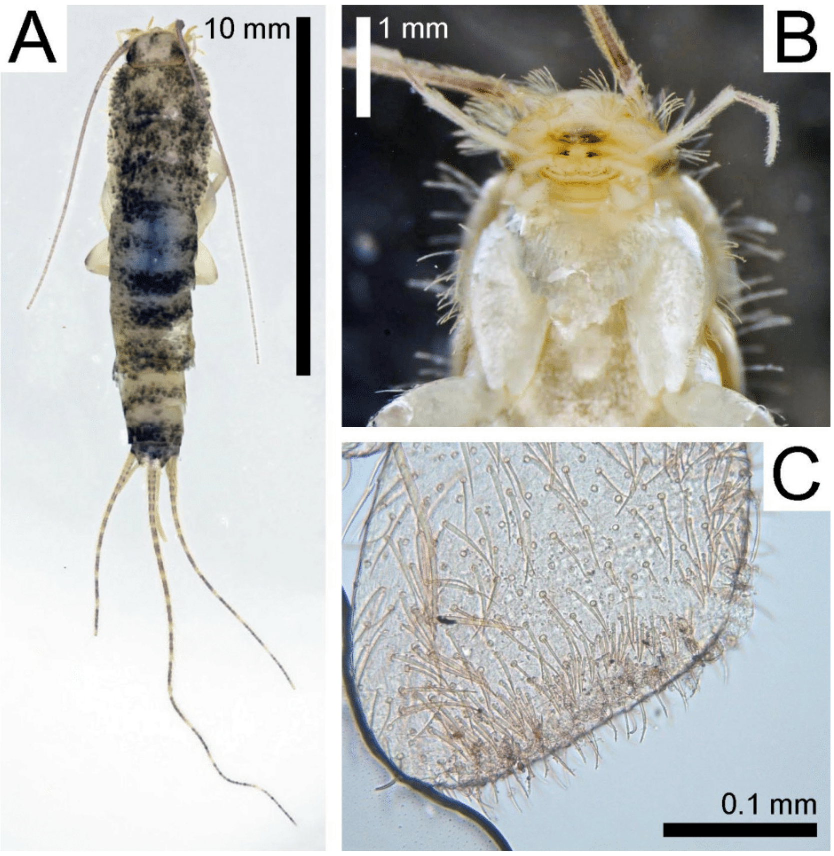

Figure 3. A captured specimen of *Ctenolepisma longicaudata:* (A) dorsal view; (B) head, ventral view; (C) terminal segment of the labial palpus. Photo: R. Sehnal (A), M. Petrtýl (B), P. Visentin (C).

into the rest of Europe, where offices and warehouses may become its primary habitat, is thus expected in the years to come. Now, the risk is re-emerging for archives, museum collections, and libraries, naturally involved in exchanges, in the remaining European countries as well as in the Czech Republic. We strongly recommend that further monitoring and possibly eradication should be conducted and the awareness of conservationists, wild life managers, and other stakeholders should be raised about these risks.

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[Nutriční hodnota tří druhů švábů používaných jako krmivo pro zvířata]

Studie je zaměřena na rozbor obsahu živin u běžně komerčně dostupných švábů *Blaptica dubia*, *Blaberus discoidalis* a *Blatta lateralis.* Od každého druhu byli analyzováni dospělci a subadultní jedinci. Analýzy nutričních hodnot prokázaly, že se jedná o zdroj poměrně kvalitních bílkovin. Zajímavé bylo zjištění, že dospělci všech tří testovaných druhů měli v porovnání se subadulty vyšší obsah bílkovin (602 – 678 g/kg sušiny > 489 – 547 g/ kg sušiny) a popelovin (42 – 51 g/kg sušiny > 36 – 40 g/kg sušiny), ale naopak nižší množství tuku (145 – 214 g/ kg < 236 – 363 g/kg). Majoritními mastnými kyselinami byly u všech skupin kyselina olejová (38,0 – 44,2 %), palmitová (21,6 – 26,8 %) a linoleová (8,5 – 15,3 %). Index esenciálních aminokyselin byl 0,4 - 0,9. Výsledky pokusu indikují, že kvantita živin se v závislosti na vývojovém stádiu liší, zatímco jejich kvalita se příliš nemění.

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Short communication

Nutritional value of three *Blattodea* species used as feed for animals

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ABSTRACT. Nutrient contents of subadult and adult *Blaptica dubia*, *Blaberus discoidalis* and *Blatta lateralis* roaches, commonly used as feed source for insectivorous animals in captivity, were determined. Contents of crude protein, fat, ash, chitin, carbohydrates, calcium and phosphorus were analysed. Adults of all species contained more crude protein (602–678 g · kg^{-1} DM > 489–547 g · kg^{-1} DM) and ash (42–51 g · kg^{-1} DM > 36–40 g · kg^{-1} DM) but less fat (145–214 g · kg^{-1} DM < 236–363 g · kg^{-1} DM) than subadults. Chitin content ranged 53–86 g · kg^{-1} DM and the calcium to phosphorus ratio was 1:1.9–23.5. Amino and fatty acid profiles were also determined. Very high levels of glycine (66.4–166.2 g · kg^{-1} DM) and alanine (81.5–118.4 g · kg^{-1} DM) were found in all examined samples. On the other hand, the lowest amino acid levels were determined in such sulphuric amino acids as cysteine (0.5–2.1 g · kg^{-1} DM) and methionine (4.0–17.2 g · kg^{-1} DM). Regarding essential amino acids, very high lysine (48.6–94.0 g · kg^{-1} DM) and valine (53.3–84.0 g · kg^{-1} DM) levels were determined. Essential amino acid index was found at the level of 0.4–0.9. In analysed cockroaches high oleic (38.0–44.2%), linoleic (8.5–15.3%) and palmitic (21.6–26.8%) fatty acids levels were determined. The obtained results indicate that quantity of protein and lipids varied between subadult and adult cockroaches within the species, while quality of these nutrients remained stable.

Introduction

Unlike the past when insects were primarily used as feed for companion animals, today the usage of insects as an alternative source of nutrients in farm animal diets is also considered. The insects are generally known to be a sufficient source of protein and lipids. However, the nutritional requirements of animals are specified not only by nutrients quantity but also quality. Although the amino acid profile is the decisive characteristic of protein quality, it´s composition is known only for a few insect species. Yi et al. (2013) reported insect protein quality to be higher than soyabean and lower than casein proteins. Barroso et al. (2014) stated that amino acid profile of some *Diptera* is superior to soyabean meal.

From this point of view, the insect protein quality could also be compared with some conventional feed protein sources. On the other hand, Bosch et al. (2014) found that *in vitro* digestibility of insect protein is lower than that of commercially available protein feedstuffs. The quality of lipids depends on their fatty acid profiles and therefore the determination of lipids composition in insects is also important. Fatty acid composition of insects was analysed by several authors, e.g., Finke (2002), Barroso et al. (2014) and Oonincx et al. (2015). It is also known, that fatty acid profile could vary significantly, because it reflects the fatty acid composition of animal diet (Sánchez-Muros et al., 2014).

Carbohydrates in insects are present basically in the form of chitin, usually indigestible for monogastric animals, in very low quantities (Finke, 2002). On the other hand, this long chain polymer of N-acetyl glucosamine may exert positive effect on the immune system (van Huis et al., 2013) and its antioxidative, hypocholesterolaemic and prebiotic effects are also known (Halder et al., 2013).

Insects are also a relatively good source of minerals, such as phosphorus, magnesium, sodium or chloride, and trace elements (Finke, 2002). Nevertheless, certain vital element levels are insufficient for the nutritional requirements of vertebrates. One of such elements is calcium (Barker et al., 1998; Finke, 2013).

There are many insects that are suitable for animal feeding. Sánchez-Muros et al. (2014) summarized that there are currently more than 150 commercially available species, whose nutrient content is species-dependent. Furthermore, not only various species, but also different developmental stages could be used as feed according to individual requirements. Despite the fact that nutritional value of more than 60 insect species is currently known (Sánchez-Muros et al., 2014), the information regarding the differences in insect chemical composition between several stages is only available for a limited number of species. *Acheta domestica* is one of such species – as an adult it contains more fat and protein but less ash than the nymphs (Barker et al., 1998; Finke, 2002). Regarding *Blattodea*, Oonincx and Dierenfeld (2012) found in *Eublaberus distanti* adults higher contents of ash and protein and lower levels of fat than their nymph counterparts, and in adults of *Gromphadorhina portentosa* approximately the same levels of fat and crude protein as the nymphs.

The investigated cockroaches varied in size, weight, sexual dimorphism and reproduction. In comparison to adults, subadult nymphs are smaller and their wings are not yet fully developed. Adult males of *Blatta lateralis* are 19–22 mm long and winged, whereas adult females are 22–25 mm long, oviparous and brachypterous. *Blaptica dubia* adults can reach 40 mm in length. Adult males of this species have long wings that cover the abdomen entirely, whereas ovoviviparous females have strongly reduced wings. Finally, *Blaberus discoidalis* are ovoviviparous and their winged adults can reach the length up to 55 mm. They all are considered as suitable feed for animals and are commercially available. Additionally, they are unable to fly or climb on smooth surfaces, which makes them significantly easier to manipulate and prevents from their accidental escape or contamination. Till this time, the information regarding nutritional values of above-mentioned species was either unavailable or concerned only adults, which made the comparison among different developmental stages impossible.

Due to the limited information, this study presents the nutritional value of subadults and adults of three cockroach species (*B. dubia*, *B. lateralis, B. discoidalis*) and compares the differences between their last two developmental stages from the perspective of nutritional composition. This knowledge will also allow to better understand the dietary habits of insectivorous animals or possibly to meet the requirements of animals, whose nutritional demands have already been standardized.

Material and methods

Sampling

All cockroaches were kept in plastic boxes (dimensions: 59 × 39 × 28 cm = 45 litres for *B. dubia* and 39 × 28 × 28 cm = 22 litres for *B. discoidalis* and *B. lateralis*) filled with flat egg trays. The boxes were covered by a lid equipped with aluminium insect mesh and no substrate was used. Heating foils were used to maintain a constant temperature of 27 °C, which was thermostatically controlled. All the cockroach colonies were fed dog granules (Brit Care Medium Adult Breed, Vafo, Chrášťany, Czech Republic) and old bread *ad libitum*. Slices of fresh vegetables and fruits (i.e. carrot, cucumber, turnip and apple) were provided daily. The vegetable and fruits scraps were constantly removed to prevent the occurrence of mildew. A water source was present in each of the breeding containers. Approximate populations of the colonies were as follows: *B. discoidalis* – 700 specimens, *B. dubia* – 1500 specimens and *B. lateralis* – 1700 specimens (all stages were kept together). Randomly selected subadults and adults (20–40 insects) of all investigated species were individually weighed to determine average weight per insect. Due to the difference in the weight of males and females, these values are reported separately according to sex. Subsequently, samples (about 300 g) were removed from each colony and frozen alive (at −18 °C). The samples were then lyophilized and homogenized.

Analysis of proximate composition

All investigated parameters were determined using the methods of European Commission Regulation (EC) No. 152/2009. The dry matter (DM) content was determined after 5 h of drying at 103 °C. The total amount of nitrogen was evaluated by the Kjeldahl method (ISO 5983-1:2005) using the Kjeltec 2400 analyzer unit (Foss, Hilleroed, Denmark) and crude protein (CP) level was then calculated (6.25 × total nitrogen). Crude fat (petroleum ether extract; EE) content was determined gravimetrically after extraction with petroleum ether using Soxhlet apparatus SER 146 (Velp, Usmate, Italy). The samples were then mineralized in a muffle furnace at 550 °C and the total amount of ash was measured. Additionally, chitin content was analysed according to Liu et al. (2012) using hydrolysis with 1M hydrochloric acid and 1M sodium hydroxide. Because chitin is a nitrogenous polysaccharide, the nitrogen content was also analysed in these hydrolysed samples to refine total crude protein content. Nitrogen-free extract (NFE) was calculated as:

$$\mathrm{NFE} = 100 - (\mathrm{moisture} + \mathrm{CP} + \mathrm{EE} + \mathrm{ash} + \mathrm{chitin}).$$

Finally, the energy content was evaluated using the standard calculation used by other authors such as Finke (2002):

$$\text{Energy content, kJ} \cdot \text{kg}^{-1} = \\ = (\mathrm{CP} \times 17) + (\mathrm{EE} \times 37) + (\mathrm{NFE} \times 17).$$

Analysis of amino acid composition

To describe the protein quality, the amino acid profile was analysed performing acid oxidation in Amino Acid Analyser 400 (INGOS, Prague, Czech Republic). Subsequently, protein quality was evaluated by the essential amino acid index (EAAI), which is based on the content of all essential amino acids except tryptophan in comparison to the reference (eggwhite) protein:

$$\mathrm{EAAI} = \sqrt[10]{\frac{\text{mg of lysine in 1 g of test protein}}{\text{mg of lysine in 1 g of eggwhite protein}}} \times \\ \times \text{(etc. for other determined amino acids).}$$

The determined amino acids were: valine, leucine, isoleucine, tyrosine, phenylalanine, histidine, lysine, arginine, cysteine and methionine.

Analysis of fatty acids

To determine the lipid quality, the fatty acid profile was analysed by gas chromatography-mass spectrometry technique (GC-MS). Prior to that, the Soxhlet extraction of lipids from samples was performed for 4 h, using petroleum ether. The base esterification method by 0.5M methanolic KOH and BF_3-methanol was used for fatty acids derivatization (ISO 12966-2:2011). Methyl esters of fatty acids were analysed by GC-MS using Agilent 7890A GC (Agilent, Wilmington, DE, USA) equipped with a Restek Rt-2560 column (length – 100 m; ID – 0.25 mm; film – 0.25 µm; Restek Corporation, Bellefonte, PA, USA) and coupled to Agilent 5975C single-quadrupole mass detector (Agilent, Wilmington, DE, USA). Hexane was used as a solvent and the sample volume of 1 µl was injected in split mode (ratio 50:1) into the injector heated to 225 °C. The initial oven temperature was 70 °C (hold 2 min), ramp1 was up to 225 °C at 5 °C · min^{-1} (hold 9 min) and ramp2 was up to 240 °C at 5 °C · min^{-1} (hold 25 min). Helium was used as a carrier gas with the flow rate of 1.2 ml · min^{-1}. The mass spectrometry analysis was carried out in full scan mode, the electron ionization energy was set at 70 eV. Methylated fatty acids were identified using a Restek Food Industry FAME mix (cat no. 35077; Bellefonte, PA, USA) and by the comparison of their mass spectra with the National Institute of Standards and Technology Library (NIST, Gaithersburg, MD, USA). The proportions of fatty acids were calculated by area normalization method.

Results

As expected, the weight of roaches was proved to be species-specific. In all examined species the adult females weighed more than the subadult females, while weights of adult males were always lower than those of the subadults. The DM content amounted 307–422 g · kg^{-1} of the specimen fresh weight. In all adults it was found more crude protein but less crude fat and ash than in the subadults of respective species (Table 1). On the other hand, chitin levels showed some species-specific differences: whereas in adult roaches of *B. discoidalis* less chitin content was found than in their subadult counterparts, concentrations were higher in adults of *B. lateralis*. The difference between chitin levels in *B. dubia* adults and their subadult counterparts was less than 1 g · kg^{-1} DM. Concentration of calcium and phosphorus (Table 2) was lower than 50 g · kg^{-1} DM. In the investigated

Table 1. Nutrient content of three cockroaches (*Blattodea*) in adult and subadult stages

Species	Stage	Average weight		Nutrients content									Energy, MJ $\cdot \text{kg}^{-1}$ DM
		female	male	moisture	DM	EE	N × 6.25	chitin protein	CP	chitin	ash	NFE	
		mg per insect		g $\cdot \text{kg}^{-1}$		g $\cdot \text{kg}^{-1}$ DM							
Blaptica dubia	subadult	2034	1659	578	422	328	547	22	525	65	40	42	22
	adult	2213	1279	626	374	214	650	20	630	64	49	43	19
Blaberus discoidalis	subadult	3290	2612	659	341	236	536	33	503	86	36	139	20
	adult	3612	2212	657	343	203	678	26	652	66	42	37	19
Blatta lateralis	subadult	398	290	640	360	363	489	18	470	53	37	77	23
	adult	552	267	693	307	145	602	18	584	59	51	161	18

DM – dry matter; EE – petroleum ether extract of crude fat; N × 6.25 – total nitrogen amount; CP – crude protein; NFE – nitrogen-free extract

Table 2. Calcium and phosphorus content and their ratio of three cockroaches (*Blattodea*) in adult and subadult stages

Species	Stage	Content, g $\cdot \text{kg}^{-1}$ DM		Ca:P ratio
		Ca	P	
Blaptica dubia	subadult	2	43	1:21.5
	adult	2	47	1:23.5
Blaberus discoidalis	subadult	7	20	1:2.9
	adult	8	30	1:3.8
Blatta lateralis	subadult	7	12	1:1.8
	adult	9	25	1:1.9

Table 3. Non-essential amino acid content of three cockroaches (*Blattodea*) in adult and subadult stages

Amino acid	Non-essential amino acid content, g $\cdot \text{kg}^{-1}$ DM					
	Blaptica dubia		*Blaberus discoidalis*		*Blatta lateralis*	
	subadult	adult	subadult	adult	subadult	adult
Aspartic acid	26.3	28.0	33.0	25.3	22.4	39.3
Threonine	16.5	16.5	21.4	13.9	13.6	23.8
Serine	21.2	17.7	26.1	13.9	17.0	27.9
Glutamic acid	47.4	48.0	57.7	48.3	43.4	72.2
Proline	38.3	39.3	49.3	28.4	34.3	45.7
Glycine	98.4	166.2	116.1	69.2	66.4	128.6
Alanine	100.0	108.9	140.1	81.5	83.8	118.4

Table 4. Essential amino acid content and essential amino acid index of three cockroaches (*Blattodea*) in adult and subadult stages

Amino acid	Essential amino acid content, g $\cdot \text{kg}^{-1}$ DM					
	Blaptica dubia		*Blaberus discoidalis*		*Blatta lateralis*	
	subadult	adult	subadult	adult	subadult	adult
Valine	58.8	66.3	84.0	54.4	53.3	81.3
Isoleucine	68.3	37.2	89.1	34.6	57.2	101.3
Leucine	36.0	64.6	26.6	61.5	18.9	9.7
Tyrosine	58.5	46.9	62.2	42.2	47.3	59.9
Phenylalanine	31.2	31.9	38.9	30.6	26.6	46.7
Histidine	34.8	36.6	50.7	30.8	29.6	47.7
Lysine	57.7	62.8	73.5	60.4	48.6	94.0
Arginine	53.8	56.1	71.3	57.2	44.9	89.8
Cysteine	2.1	1.7	1.4	2.0	0.5	0.9
Methionine	17.2	13.6	13.4	15.5	4.0	7.8
EAAI	0.4	0.7	0.9	0.9	0.7	0.6

EAAI – essential amino acid index

roaches more phosphorus than calcium was detected and the lowest Ca:P ratio was found in *B. dubia.*

In the examined cockroaches high levels of such non-essential amino acids as glycine and alanine were found (Table 3). In comparison to eggwhite, calculated EAAI values ranged from 0.4 to 0.9, and the highest EAAI was found in adult and subadult *B. discoidalis* roaches (Table 4). Conversely, contents of methionine and cysteine were the lowest.

In all cockroaches the oleic acid (C18:1*cis-9*) content was the highest among fatty acids, and ranged from 38.0 to 44.2% of total fatty acids (Table 5). Only in *B. lateralis* species more saturated (SFA) than monounsaturated (MUFA) fatty acids were determined. In the remaining two species there were more MUFA. The lowest polyunsaturated fatty acids (PUFA) levels (9.5% and 9.6% for adult and subadult stage, respectively) were found in *B. lateralis.* On the other hand, the highest PUFA amount (20.9%) was found in adults of *B. discoidalis*.

Table 5. Fatty acid profile of three cockroaches (*Blattodea*) in adult and subadult stages

Fatty acid	Fatty acid profile, % of total fatty acids					
	Blaptica dubia		*Blaberus discoidalis*		*Blatta ateralis*	
	subadult	adult	subadult	adult	subadult	adult
C4:0	ND	ND	ND	ND	ND	ND
C6:0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
C8:0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
C10:0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
C12:0	0.3	0.3	0.1	0.1	0.2	0.1
C14:0	2.6	2.2	1.2	1.3	1.9	1.3
C14:1 *cis-9*	0.1	0.1	< 0.1	<0.1	<0.1	<0.1
C15:0	0.2	0.2	0.2	0.3	0.1	0.2
C16:0	22.8	21.6	23.4	22.9	26.8	24.7
C16:1 *trans-9*	0.4	0.5	0.7	0.7	0.2	0.3
C16:1 *cis-9*	9.8	7.7	4.4	2.6	3.3	4.5
C17:0	0.5	0.6	0.3	0.6	0.4	0.5
C17:1 *cis-10*	0.3	0.5	0.2	0.3	0.1	0.2
C18:0	7.8	8.9	6.8	8.9	18.1	17.9
C18:1 *trans-9*	0.1	0.1	0.2	0.5	0.2	0.2
C18:1 *cis-9*	40.3	42.5	44.2	40.2	38.0	39.1
C19:0	0.1	0.1	ND	0.1	0.1	0.1
C18:2 *cis-9,12*	10.6	11.9	15.3	17.9	8.7	8.5
C20:0	0.2	0.3	0.3	0.3	0.6	0.9
C20:1 *cis-11*	0.3	0.3	0.3	0.3	0.2	0.3
C18:3 *cis-9,12,15*	3.2	1.8	1.7	1.9	0.7	0.7
C22:0	0.1	0.1	ND	ND	<0.1	0.1
C20:4 *cis-5,8,11,14*	0.2	0.3	0.5	1.0	0.2	0.4
C24:0	0.1	<0.1	<0.1	<0.1	<0.1	<0.1
SFA	34.7	34.3	32.4	34.6	48.3	45.8
MUFA	51.3	51.6	50.1	44.6	42.2	44.7
PUFA	14.0	14.1	17.5	20.9	9.6	9.5

ND – not detected; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

Discussion

Proximal composition. Information concerning the nutrient content of the investigated species is only available for the adults of *B. dubia* (Yi et al., 2013; Bosch et al., 2014) and young nymphs of *B. lateralis* (Oonincx and Dierenfeld, 2012). Therefore, the results should be compared mainly with other cockroaches or insect species. In all of the examined cockroaches high concentrations of crude protein (470–652 g · kg^{-1} DM) as well as high levels of crude fat (145–363 g · kg^{-1} DM) were determined. This means that both subadult and adult cockroaches used in our study are a similar source of protein and lipids like other species of cockroaches (Oonincx and Dierenfeld, 2012; Bosch et al., 2014), house cricket (*Acheta domesticus*) (Barker et al., 1998; Finke, 2002) and other insects.

The ash content was 36–51 g · kg^{-1} DM, which also corresponds with the findings of other authors (Barker et al., 1998; Finke, 2002; Oonincx and Dierenfeld, 2012). Additionally, we found rather low Ca:P ratios that are insufficient for the vertebrate diets (NRC, 1995, 2003; Finke, 2002); but these ratios are similar to those reported for most insect species (Barker et al., 1998; Finke, 2002; Oonincx and Dierenfeld, 2012). The above-mentioned results confirm the need for calcium supplements in the insectivore diets. In order to better serve the nutritional requirements of vertebrates, further research should be focused on possibilities of increasing Ca content in insects (such as gut loading, etc.) to be able to influence the unfavourable Ca:P ratio as well as the calcium accumulation in the body of insects.

The chitin content (53–86 g · kg^{-1} DM) was similar to that of crickets (*Gryllus testaceus*) (Wang et al., 2005) and grasshoppers (*Acrida cinerea*) (Wang et al., 2007). Significantly lower chitinin contents were reported by Finke (2013). However, these differences may result from different methodologies used. Additionally, crude protein content in chitin was 18–33 g · kg^{-1} DM, which is clearly more than 4 g · kg^{-1} DM reported by Bernard and Allen (1997).

Amino acid profile. In all tested samples contents of glycine (66.4–166.2 g · kg^{-1} DM) and alanine (81.5–118.4 g · kg^{-1} DM) were very high. It is more than in other studies focused on the protein quality in cockroaches, e.g., Yi et al. (2013) and Bosch et al. (2014). These differences were probably caused by feed residues in the cockroach digestive tract.

The examined cockroaches, especially *B. discoidalis*, also seem to be a good source of essential amino acids (EAA). The EEAI without tryptophan was 0.4–0.9, mainly because of high lysine level (48.6–94.0 g · kg^{-1} DM). On the other hand, the lowest levels of sulphuric amino acids: cysteine (0.5–2.1 g · kg^{-1} DM) and methionine (4–17.2 g · kg^{-1} DM) were found. These findings are more or less in line with the results published by other authors. For adult females of the same species, Bosch et al. (2014) reported high contents of valine, leucine, histidine, lysine and arginine, which is consistent with the levels found in *B. dubia* in this study. Moreover, Józefiak et al. (2016) studied the EAA composition of *B. lateralis* subadult nymphs and found that tyrosine, arginine, valine and lysine were among the first five EAAs, which corresponds to the results obtained in this study. On the other hand, the main EAA reported by Józefiak et al. (2016) was leucine, while in this paper the most abundant EAA is isoleucine (101.3 g · kg^{-1} DM for adult stage of *B. lateralis*).

Fatty acid profile. Finke (2002) and Oonincx et al. (2015) analysed the fatty acid profile of cockroaches and determined the same major fatty acids (mainly the oleic acid) in *B. lateralis* and *B. dubia*. Moreover, the presented fatty acids profiles were very similar to those obtained in our study. With a few exceptions, the dominant values of oleic, palmitic and linoleic fatty acids presented in this paper also correspond with the results of other authors (Finke, 2002; Barroso et al., 2014), who investigated fat composition of other terrestrical insect species. In line with these authors, no contents of omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were detected. In this work, some differences in lipids composition were observed between the investigated species, but values within the one species varied only marginally. The results indicate that the fat of tested insects contained high proportion of SFA and MUFA. From this point of view, its nutritional quality is not so high and close to other animal fats. On the other hand, the proportion of PUFA is comparable to commonly used oils such as the olive, sunflower or rapeseed oil (Velíšek, 2014). Moreover, it could even be optimalized or improved during the rearing process by adding suitable supplements to the feeding mixtures for insects.

Comparison of developmental stages. Oonincx and Dierenfeld (2012) comparing two consecutive developmental stages of Turkestan roaches (*B. lateralis*) found that younger nymphs (2nd instar) contained more crude protein (760.5 > 628.5 g · kg^{-1} DM), more ash (78.8 > 68.9 g · kg^{-1} DM) and less crude fat (144.5 < 265.0 g · kg^{-1} DM) than older nymphs (3rd instar). In our study less crude protein (470 < 584 g · kg^{-1} DM), less ash (37 < 51 g · kg^{-1} DM) and more crude fat (363 > 145 g · kg^{-1} DM) were found in younger subadult nymphs than in adults. However, Oonincx and Dierenfeld (2012) did not determine these concentrations for adult roaches. Therefore, their presumption that earlier developmental stages contain more protein and less fat than older specimens of the same species seems dubious in the light of our findings. Nonetheless, further research is needed. We assume that the nutrient content might be significantly influenced by the various diets of the insects (e.g., total fat concentrations correlate with the energy value of the feed), ecdysis and the timing of the analysis.

In the dry matter of adults *B. dubia* roaches 630 g of crude protein and 214 g of crude fat per kg of DM were found, and these concentrations are similar to those reported by Yi et al. (2013) for this species (592 g of crude protein and 214 g of total fat per kg of DM) and Tzompa-Sosa et al. (2014) who reported 7.6 g of total fat from 100 g of fresh samples. The nutritional composition of roaches at the subadult developmental stage has been unknown until now.

This study was the first to determine the nutrient content of *B. discoidalis*. This content can be compared with that of *E. distanti,* which was selected mainly because it belongs to the same family (*Blaberidae*) and similar dimorphism (all adults are winged). The chemical composition of this species was reported by Oonincx et Dierenfeld (2012), whose findings partly correspond with our results. *E. distanti* adults contained more crude protein and ash but less fat than 4–5 cm long nymphs. However, most of the nutrient concentrations determined in this study (especially crude fat and crude protein) differed from those of *E. distanti.*

Conclusions

The obtained results showed differences in nutritional value among three studied species (*Blaptica dubia*, *Blaberus discoidalis* and *Blatta lateralis*). However, they were not as clear as the differences between adult and subadult cockroaches of the same respective species. All adults were better source of crude protein, ash and calcium, but contained less fat, than the subadults. On the other hand, it was determined that the quality (unlike the quantity) of lipids and proteins remained essentially the same.

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[Vliv pohlaví na nutriční hodnotu cvrčka domácího, *Acheta domestica* L.]

Článek popisuje na příkladu cvrčka domácího rozdíl v kvantitě a kvalitě základních živin u hmyzu. Na základě zjištěné nutriční hodnoty bylo stanoveno, že bez ohledu na pohlaví je tento druh hmyzu bohatým zdrojen bílkovin a tuků. Kvalita těchto živin je pak srovnatelná s konvenčními zdroji. Nutriční hodnota se ukázala být ovlivněna pohlavím, kdy samice obsahovaly v porovnání se samci méně bílkovin (61,2 – 64,9 vs. 66,3 – 69,6 g/100 g sušiny, $p = 0.0001$) a chitinu, ale více tuku (18,3 – 21,7 vs. 12,9 – 16,1 g/100 g sušiny, $p = 0{,}0001$). Co se týče kvality bílkovin a tuku, vyjádřené indexy esenciálních aminokyselin (72,3 – 77,1), atherogenicity (1,22 – 1,45) a thrombogenecity (0,53 – 0,58) se v závislosti na pohlaví nelišily. Limitující aminokyselinou byl pro obě pohlaví methionin.

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Effect of sex on the nutritional value of house cricket, *Acheta domestica* L.

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ABSTRACT

Since January 2018, insects have been recognised as novel foods in the EU, but their nutritional value varies, and factors affecting their nutritional composition have been debated. We investigated the effect of sex on the nutritional value and chemical composition of the house cricket (*Acheta domestica* L.). Both sexes were rich in protein and lipids. The proximate composition was partly influenced by sex; females contained a significantly higher amount of lipids (18.3–21.7 vs 12.9–16.1 g/100 g dry matter, $p = 0.0001$) and fewer proteins than males (61.2–64.9 vs 66.3–69.6 g/100 g dry matter, $p = 0.0001$). Males contained more chitin ($p = 0.0015$) and nitrogen chains ($p = 0.0003$) than females. Only the ash ($p = 0.4314$) and nitrogen-free extract ($p = 0.4871$) were uninfluenced by sex. Furthermore, nutrient quality expressed as essential amino acid (72.3–77.1), thrombogenicity (1.22–1.45), and atherogenicity indices (0.53–0.58) did not differ between sexes ($p > 0.05$).

1. Introduction

The growing global population, hand in hand with increasing per capita consumption and changes in dietary habits, poses a higher than ever demand on available resources to meet the nutrient needs of both animals as well as for humans (Van Huis et al., 2013). Livestock production is currently one of the most important factors influencing the global climate. This industrial sector is one of the largest sources of greenhouse gases, a major driver of the use and pollution of freshwater, and a contributor to the loss of biodiversity (Sakadevan & Nguyen, 2017). In the light of the limited and exploited resources, livestock farming thus will have to become more sustainable. To provide sufficient high-quality protein and minimise the environmental footprint of livestock production, its optimisation is undoubtedly a critical strategy and has become a major challenge to humans.

Insects, consumed either as human food or as insect meal, were believed to be able do their share in easing the problem of world protein shortage since 1975 (Meyer-Rochow, 1975). Recently, they have frequently been reported to be a potential alternative protein source (Sánchez-Murós, Barroso, & Manzano-Agugliaro, 2014). This species-rich class of arthropods contains more than a million described species and represents more than 50% of all living organisms on the Earth. Insects also play an important role in the nutrition of many animals as well as people. Totally, 1745 insect species have been documented to be part of the human diets in 113 countries of the world, particularly in Asia and the Pacific, Africa and Latin America (Durst, Johnson, Leslie, & Shono, 2010). Several hundred of such species are known to be reared (Sánchez-Murós et al., 2014), but only a few are suitable for large-scale breeding for use as food or animal feed (Payne, Scarborough, Rayner, & Nonaka, 2016).

The insects, especially those with short life cycle and high reproduction rate, are generally considered to be ecologically and environmentally friendly food or feed alternatives, especially because of their low greenhouse gas emissions (Oonincx et al., 2010). In addition, they can be reared on low-cost by-products of the food industry, expired food (Finke, Rojo, Roos, van Huis, & Yen, 2015; Oonincx, Van Broekhoven, Van Huis, & van Loon, 2015), or even organic waste (Čičková, Newton, Lacy, & Kozánek, 2015). As insects are poikilothermic animals, they do not require energy for thermoregulation and their feed conversion rate might thus be very high. Moreover, their requirements on water and space are lower than in the livestock (Akhtar & Isman, 2018). Finally, their nutritional value is reported to be comparable to that of conventional protein sources (Payne et al., 2016; Yi et al., 2013). On the other hand, their chemical composition is known not to be constant and, so, it varies significantly among different species and within the same insect species.

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The house cricket (*Acheta domestica* L.) has been used as feed for insectivores worldwide for a very long time and; therefore, its large-scale breeding system is well described. The house cricket belongs to the order Orthoptera, which includes species that are reported to be among the best insect-based protein sources (Sánchez-Murós et al., 2014). Specifically, they are rich in protein content, which can reach up to 70 g/100 g dry matter (DM) and their lipid level is usually not much higher than 20 g/100 g DM (Barroso et al., 2014; Bosch, Zhang, Oonincx, & Hendriks, 2014; Chakravorty, Ghosh, Jung, & Meyer-Rochow, 2014; Finke, 2002; Ghosh, Lee, Jung, & Meyer-Rochow, 2017). Therefore, crickets have been studied as supplementary material in the experimental feed for animals (Nakagaki, Sunde, & DeFoliart, 1987). Recently, house crickets have been also associated with entomophagy (Caparros Megido et al., 2017; Grabowski, Nowak, & Klein, 2008). In countries with long traditions of eating insects such as Thailand, crickets have also been commonly and commercially mass-produced for food purposes (Halloran, Roos, & Hanboonsong, 2017). Although insect-based foods have generally not been well accepted in developed countries, the willingness to adopt dietary insect products under certain circumstances has been reported in North American and European countries (Caparros Megido et al., 2017; Hartmann, Shi, Giusto, & Siegrist, 2015; House, 2016; Ruby, Rozin, & Chan, 2015). Moreover, at the beginning of 2018, the new Regulation (EU) No 2015/2283 was introduced. The document updated the categories of food which constitute novel foods and included the whole insects and their parts there. Interestingly, the bread including cricket meal is commercially available in some of European supermarket chains nowadays. Therefore, increased interest in this commodity can be expected in the near future, which prompted the present investigation. The nutritional value of insects depends on their feed (Oonincx & Van der Poel, 2011), developmental stage (Kulma et al., 2016), and how they are reared, prepared and processed (Akhtar & Isman, 2018). However, many other factors could play a role in determining the nutritional value of insects. Recent research (Naseema Begum, Rakesh, Sudhakara Rao & Mamatha, 2014; Ademolu et al., 2017) indicates, that quantity of basic nutrients, some vitamins and micronutrients in insects could be affected by the gender. However, more data are needed to confirm that phenomenon. For this reason, the objective of this study was to reveal effect of sex on the macronutrients of the insects as potential novel protein-rich food sources. Therefore, we investigated this phenomenon using the house cricket (*A. domestica* L.), which was recommended by European Union as one of the novel food edible insect species, to be the experimental model.

2. Materials and methods

2.1. Experimental model and grouping

For the purpose of this study, the house cricket (*A. domestica*) was selected as an appropriate species. In the Czech Republic, house crickets belong to the most accessible insect species with well-known technology of rearing at household level as well as large scale conditions. Moreover, females and males can be easily recognized according to the ovipositor presence in females. The adults of *A. domestica*, used for the experiment were purchased in three batches (1–3) from local commercial sources (Scorpion Export-Import s.r.o., Novosedly nad Nežárkou, Czech Republic) at approximately 3-month intervals in January, April, and June 2016. The insects were euthanised by freezing (−38 °C) and then divided into two groups (males and females). As females were kept together with males before the experiment, they were considered to be gravid. Each group was further subdivided into four parallel repetitions and, finally, 24 samples were analysed. Twenty randomly selected specimens of each sex were individually weighed to determine the difference in fresh weight. Then, the samples were lyophilised, homogenised, and stored in the refrigerator until the analysis.

2.2. Methods

All the prespecified parameters were determined using the methods of the European Commission Regulation (EC) No 152/2009. The DM content was determined after lyophilisation by drying for 5 h at 103 °C. The nitrogen content (N) was evaluated using the Kjeldahl method (International Organization for Standardization [ISO] 5983-1:2005) with the Kjeltec 2400 analyser unit (FOSS, Hilleroed, Denmark), and the crude protein (CP) level was subsequently calculated using a nitrogen-to-protein conversion factor of 6.25. The total fat content (TFC, petroleum ether extract) was determined using the Soxhlet method with the Gerhardt Soxtherm SOX414 (C. Gerhardt GmbH and Co. KG, Königswinter, Germany). Briefly, Dried and homogenised samples (5 g) were cold-water extracted with petroleum ether (program: 70 °C for 120 min). The extracted samples were then dried at 103 °C and weighted until a constant sample weight was achieved. To determine the ash content, the samples were mineralised in a muffle furnace LAC (Verkon, Praha, Czech Republic) at 550 °C. The chitin content was analysed according to the method of Liu et al. (2012) involving hydrolysis with 1 M hydrochloric acid (HCl) and 1 M sodium hydroxide (NaOH). To determine the digestible protein content (DPC), the chitin-bound nitrogen content of the hydrolysed sample was also analysed using the Kjeldahl method. To evaluate the nitrogen-free extract (NFE) and energy value (E), standard calculations were performed using the following formulas (Finke, 2002).

$$NFE = 100 - (crude\ protein + lipids + ash + chitin)$$

$$E\ (MJ \cdot kg-1) = [(DPC \times 17) + (TFC \times 37) + (NFE \times 17)]/100$$

The amino acid profile was determined by acidic and oxidative hydrolysis of the samples, followed by evaluation using an Amino Acid Analyser 400 (INGOS, Prague, Czech Republic) with Na-citrate buffers and ninhydrin detection. Tryptophan was not determined because it was completely destroyed during the classical acid hydrolysis with 6 M HCl. To evaluate the protein quality, the essential amino acid index (EAAI) was calculated (Velisek, 2013) to determine the following essential amino acids: valine, leucine, isoleucine, threonine, phenylalanine, methionine, and lysine (Voet & Voet, 2010). This index is based on the determined content of all the above-mentioned amino acids compared with those of a reference protein, which was whole egg protein in this study.

$$EAAI = \sqrt[2]{\frac{g\ of\ lysine\ in\ 100\,g\ of\ analysed\ protein \times 100}{g\ of\ lysine\ in\ 100\,g\ of\ reference\ protein}} \times (etc.\ for\ other\ 6\,EAA)$$

The base esterification method using 0.5 M methanolic potassium hydroxide (KOH) and boron trifluoride (BF_3)-methanol was used for fatty acid derivatisation. Methyl esters of the fatty acids were then analysed using gas chromatography-mass spectrometry (GC–MS) using an Agilent 7890A GC coupled to Agilent 5975C single-quadrupole mass detector equipped with an Rt-2560 column (100 m × 0.25 mm ID, 0.25 µm film, Restek Corporation, Bellefonte, USA). Hexane was used as the solvent and 1 µL of the sample was injected in the split mode (ratio 50:1) into the injector, which was heated to 225 °C. Starting at 70 °C for 2 min, the oven temperature was increased at a rate of 5 °C/min to 225 °C where it was kept constant for 9 min, and then subsequently increased at a rate of 5 °C/min to a maximum of 240 °C where it was maintained for 25 min. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The MS analysis was carried out in full scan mode with a mass range of 40–400 m/z, and the electron ionization energy was set at 70 eV. The methylated fatty acids were identified using a Restek Food Industry FAME mix (cat#35077) and by comparing their mass spectra with those reported in the National Institute of Standards and Technology Library (NIST, USA). The proportions of the fatty acids were calculated using the area normalisation method and expressed as relative percentage of all fatty acids.

The data from the fatty acid identification was used to calculate the atherogenicity (IA) and thrombogenicity (IT) indices according to the following formulas.

$$IA = \frac{C12{:}\,0 + 4 \times C14{:}\,0 + C16{:}\,0}{\sum MUFA + \sum PUFA\ n{-}6 + \sum PUFA\ n{-}3}$$

$$IT = \frac{C14{:}\,0 + C16{:}\,0 + C18{:}\,0}{0.5 \sum MUFA + 0.5 \sum PUFA\ n{-}6 + 3 \sum PUFA\ n{-}3 + \sum \left(\frac{n-3}{n-6}\right)}$$

IA indicates the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated fatty acids. TI is defined as the relationship between the pro-thrombogenic (saturated) and anti-thrombogenic fatty acids (Ulbricht & Southgate, 1991). These indexes were then used to compare the fatty acid profiles of analysed insects and conventional lipid sources.

The results are expressed as the arithmetical means ($\bar{x}$) and standard deviations (SD). The fatty acid profiles showed SD < 0.01 and, therefore, they are not displayed in Table 2. The data were statistically evaluated using the Statistica 13.2 software (StatSoft, Inc., Tulsa, OK, USA) using a factorial analysis of variance (ANOVA) and Tukey's post-hoc analyses with a significance level of α = 0.05.

3. Results

As could be expected, the average fresh weight of adult females was significantly ($p = 0.0001$) higher than that of males (354.4–408.7 vs 245.1–260.0 mg). The proximate composition of male and female *A. domestica* specimens was determined in lyophilised samples and recalculated on a DM basis. The DM of the tested fresh samples before lyophilisation was in the range of 31.9–37.9 g/100 g, and females contained lesser water than the males did ($p = 0.0001$). Regarding basic nutrients, males contained significantly higher protein (66.3–69.6 vs 61.2–64.9 g/100 g, $p = 0.0001$) and lower lipid (12.9–16.1 vs 18.3–21.7 g/100 g, $p = 0.0001$) content than females (Table 1). In contrast, no significant sex effects were found in the quality of these nutrients represented as the amino acid and fatty acid profiles and expressed as EAAI (72.3–77.1, $p = 0.6149$), IA ($p = 0.1170$). The IT ranged was 1.2–1.5, and a difference was found only between males and females in the third trial (Tables 2 and 3, $p = 0.0003$). Methionine and cysteine (1.09–1.15 and 1.02–1.27 g/100 g DM, respectively) were determined to be the limiting amino acids in all the samples. The fatty acid linoleic, oleic, palmitic, and stearic fatty acids were detected as dominant in both males and females. For the other proximate components, sex did not influence ash ($p = 0.4314$) and NFE ($p = 0.4871$) levels. Males contained more chitin ($p = 0.0015$) with more nitrogen chains than females ($p = 0.0003$). Finally, females showed slightly higher energy value than the males did (18.7–19.2 vs 17.4–17.6 MJ/kg DM, $p = 0.0001$). Moreover, the experiment showed some differences among the tested batches (1, 2 and 3), which varied in levels of protein ($p_{1,2} = 0.0001$; $p_{1,3} = 0.0001$), lipid ($p_{1,2} = 0.0001$, $p_{1,3} = 0.0001$), IT ($p_{1,2} = 0.0001$; $p_{1,3} = 0.0001$) and IA ($p_{1,3} = 0.0002$; $p_{2,3} = 0.0007$). On the other hand, no differences of ash, NFE and EAAI were observed Table 4.

Table 2
Gas chromatography-mass spectrometry determination of fatty acid profile of males and females of the house cricket (*Acheta domestica*).

Fatty acid	Trial 1		Trial 2		Trial 3	
	Females	Males	Females	Males	Females	Males
	% of total fatty acids					
C12:0	0.12	0.07	0.15	0.08	0.21	0.08
C14:0	0.90	0.95	0.80	0.75	1.13	1.11
C15:0	0.24	0.22	0.19	0.16	0.22	0.20
C16:0	26.45	26.38	26.76	27.66	26.05	26.28
C16:1 (cis-9)	1.35	1.13	1.16	0.79	1.44	1.03
C17:0	0.50	0.44	0.46	0.43	0.58	0.50
C18:0	13.58	14.10	14.01	15.25	17.24	17.35
C18:1 (cis-9)	24.72	24.39	24.12	22.43	22.52	21.67
C18:2 (trans-9,12)	0.02	0.09	0.09	0.12	0.11	0.11
C18:2 (cis-9,12)	28.84	29.04	29.37	30.13	26.26	26.74
C20:0	0.79	0.70	0.61	0.69	0.87	0.76
C20:1 (cis-11)	0.27	0.20	0.05	0.29	0.14	0.36
C18:3 (cis-9,12,15)	1.14	1.35	1.57	1.08	1.54	1.21
C20:2 (cis-11.14)	0.13	0.14	0.08	0.12	0.11	0.13
C22:0	0.19	0.15	0.36	0.00	0.20	0.12
C20:4 (cis-5,8,11,14)	0.09	0.15	0.17	0.02	0.34	0.51
C20:5 (cis-5,8,11,14,17)	0.47	0.49	0.00	0.00	0.52	0.54
ΣSFA	42.88	43.02	43.34	45.03	46.50	46.39
ΣMUFA	26.34	25.73	25.33	23.51	24.62	24.36
ΣPUFA	31.09	31.25	31.28	31.34	28.88	29.25
AI	0.53	0.53	0.53	0.56	0.58	0.57
TI	1.22	1.25	1.29	1.45	1.39	1.43

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA polyunsaturated fatty acids; IA – index of atherogenicity; IT – index of thrombogenicity
Results are presented as arithmetic means (n = 4).

Table 1
Proximate composition of adult males and females of house cricket (*Acheta domestica*).

Trial	Sample	Weight per fresh insect	DM	CP	TFC	Ash	Chitin	CHCP	DPC	NFE	Energy
		mg	g/100 g fresh weight	g/100 g DM							MJ/kg DM
1	Females	408.7 ± 101.0	31.9 ± 1.2	63.1 ± 0.4	21.7 ± 0.6	4.0 ± 0.2	5.4 ± 0.4	1.9 ± 0.5	61.2 ± 0.8	5.7 ± 0.3	19.2 ± 0.1
	Males	248.5 ± 38.6	33.0 ± 1.1	69.9 ± 0.4	16.1 ± 0.4	4.3 ± 0.1	6.2 ± 0.2	3.6 ± 1.1	66.3 ± 1.2	3.6 ± 0.2	17.6 ± 0.2
2	Females	403.3 ± 65.8	35.0 ± 0.8	66.6 ± 0.5	18.5 ± 0.2	3.9 ± 0.3	5.5 ± 0.3	1.6 ± 0.1	64.9 ± 0.5	5.6 ± 0.6	18.8 ± 0.1
	Males	260.0 ± 39.5	31.3 ± 1.5	70.8 ± 0.6	13.2 ± 0.5	3.5 ± 0.8	6.1 ± 0.3	3.4 ± 1.0	67.5 ± 0.9	6.4 ± 1.2	17.4 ± 0.2
3	Females	354.5 ± 90.2	37.9 ± 0.2	65.7 ± 0.1	18.3 ± 0.2	4.3 ± 0.1	5.5 ± 0.1	1.8 ± 0.1	63.9 ± 0.1	6.2 ± 0.4	18.7 ± 0.1
	Males	245.1 ± 36.4	31.7 ± 0.2	71.9 ± 0.3	12.9 ± 0.1	3.9 ± 0.2	6.0 ± 0.7	2.4 ± 0.6	69.6 ± 0.7	5.7 ± 0.8	17.5 ± 0.1

DM – dry matter; TFC – total fat content; CP – crude protein (N × 6.25); CHCP – crude protein in chitin (chitin-bound nitrogen content × 6.25), DPC – digestable protein content (CP – CHCP), NFE – nitrogen free extract.
Results are presented as arithmetic means (n = 4). Methods used: CP and CHCP– Kjeldahl method, TFC – Soxhlet method, ash – mineralisation, chitin – hydrolysis, NFE were calculated as NFE = 100 − (CP + TFC + ash + chitin), energy was calculated as E = [(DCP × 17) + (TFC × 37) + (NFE × 17)]/100.

4. Discussion

Although the house crickets analysed in this study were obtained from the same source during a 1-year period, the nutrient contents varied slightly in the repeat experiments. Some significant differences were observed among the three test groups for many of the investigated chemical parameters including the protein, lipids, IA, and IT. Even though conditions in large scale commercial farming were expected to be more or less constant, specific changes in the breeding program such as temperature, age of harvested adults or diet (used vegetable could vary seasonally) probably caused these differences. For instance,

Table 3
Non-essential amino acid content in males and females of the house cricket (*Acheta domestica*) analysed by Amino Acid Analyser.

Trial	Sample	Asp	Ser	Glu	Pro	Gly	Ala	His	Tyr	Arg	Cys
		g/100 g of N × 6.25									
1	Females	5.95 ± 0.12	4.02 ± 0.13	5.46 ± 0.11	4.25 ± 0.15	5.60 ± 0.08	8.43 ± 0.07	1.55 ± 0.08	2.48 ± 0.16	3.22 ± 0.08	1.03 ± 0.04
	Males	5.88 ± 0.18	3.57 ± 0.14	5.88 ± 0.47	4.89 ± 0.21	6.95 ± 0.43	10.13 ± 0.32	1.60 ± 0.05	2.37 ± 0.02	3.44 ± 0.03	1.16 ± 0.11
2	Females	5.76 ± 1.08	4.04 ± 0.70	5.17 ± 0.73	4.39 ± 0.95	5.60 ± 0.76	8.34 ± 1.18	1.52 ± 0.32	2.32 ± 0.45	2.93 ± 0.73	0.96 ± 0.07
	Males	4.61 ± 0.70	3.02 ± 0.45	4.73 ± 0.55	4.30 ± 0.62	5.94 ± 0.60	8.77 ± 0.92	1.35 ± 0.20	1.94 ± 0.31	2.68 ± 0.37	1.14 ± 0.04
3	Females	6.37 ± 0.99	4.45 ± 0.68	5.48 ± 0.71	4.93 ± 0.98	5.88 ± 0.73	8.78 ± 0.23	1.68 ± 0.32	2.52 ± 0.45	3.35 ± 0.72	0.99 ± 0.04
	Males	5.68 ± 0.58	3.39 ± 0.28	5.78 ± 0.56	5.48 ± 0.60	6.88 ± 0.45	9.98 ± 0.52	1.70 ± 0.15	2.39 ± 0.26	3.54 ± 0.37	1.20 ± 0.14

Results are presented as arithmetic means ± SD (n = 4).

Table 4
Essential amino acid content in males and females of the house cricket (*Acheta domestica*) analysed by Amino Acid Analyser.

Trial	Sample	Thr	Ile	Leu	Phe	Val	Lys	Met	EAAI %
		g/100 g of N × 6.25							
1	Female	2.73 ± 0.03	2.84 ± 0.12	4.76 ± 0.13	1.91 ± 0.08	4.61 ± 0.16	3.62 ± 0.08	1.06 ± 0.18	74.1 ± 2.44
	Male	2.84 ± 0.01	2.91 ± 0.15	4.96 ± 0.26	1.84 ± 0.04	4.83 ± 0.23	3.68 ± 0.20	1.03 ± 0.07	75.9 ± 2.38
2	Female	2.75 ± 0.08	2.80 ± 0.07	4.54 ± 0.28	2.00 ± 0.25	4.50 ± 0.12	3.62 ± 0.32	1.05 ± 0.05	73.6 ± 2.98
	Male	2.88 ± 0.12	2.89 ± 0.10	4.63 ± 0.06	1.72 ± 0.18	4.75 ± 0.14	3.12 ± 0.34	1.07 ± 0.02	72.3 ± 2.87
3	Female	2.75 ± 0.08	2.78 ± 0.07	4.54 ± 0.28	2.15 ± 0.28	4.49 ± 0.12	3.73 ± 0.32	1.10 ± 0.03	75.1 ± 3.72
	Male	3.01 ± 0.09	3.16 ± 0.14	4.99 ± 0.16	1.93 ± 0.10	4.56 ± 0.41	3.62 ± 0.15	1.08 ± 0.04	77.1 ± 2.28

EAAI – essential amino acid index.
Results are presented as arithmetic means ± SD (n = 4).

Oonincx and van der Poel (2011) proved, that inclusion of wheat bran and carrot into the diet of migratory locusts increased fat content and decreased protein level. An influence of the diet on the fatty acid profile of insects is also known, where it most likely reflects the fatty acid composition of their feed (Barroso et al., 2014). This finding indicates that the nutritional value of insects from the same producer may not be consistent and should be considered when calculating and making claims about the nutritional information of edible insect products. In contrast, the EAAI and NFE values did not vary significantly.

The nutrient analysis confirmed that both male and female house crickets are a potentially excellent source of protein and lipids. Furthermore, sexual dimorphism and assumption of gravidity enable the females to reached significantly higher weight than the males did. The average weight of 349 mg per insect reported by Finke (2002) is very similar to that obtained in this investigation (320 mg) and not affected by gender. However, the differences between both groups were evident where the fresh weight of only very few sampled crickets was close to the arithmetic mean of the entire population.

The nutritional value of the house crickets in this study was in accordance with those determined by other studies (Barroso et al., 2014; Bosch et al., 2014; Finke, 2002). The protein and lipid contents (20–25 and 4–7 g/100 g fresh weight, respectively) were also comparable to those of conventional animal sources such as beef (beef, production meat, H2, raw: protein and fat, 20.0 and 6.8 g/100 g, respectively) or chicken (chicken, breast trimmings: protein and fat, 20.6 and 5.6 g/100 g, respectively) (CFCD, 2018; Velisek, 2013). These results indicate that both protein and lipid concentrations were significantly affected by sex. In all the experimental repetitions, the sampled male house crickets contained significantly more protein and fewer lipids than the female did. This effect may be attributed to the differences in their nutritional requirements for optimal performance and fitness maximisation, which are known to be sex-specific (Reddiex, Gosden, Bonduriansky, & Chenoweth, 2013). In crickets, the fecundity of the females depends mainly on ingestion of protein, which is necessary for egg development. Thus, adult females prefer protein-rich diets, whereas males prefer carbohydrate-rich diet to meet the energy demands of their vigorous courtship behaviour (Maklakov et al., 2008).

As quality is the decisive factor for utilisation of proteins and lipids, this study aimed to describe the amino and fatty acid composition of male and female house crickets. The proteins and lipids showed no significant differences in their quality. The only significant sex-related differences were found in the non-essential amino acid profiles. These findings also differed from the results of other studies such as those of Yi et al. (2013), who reported phenyl-alanine and tyrosine complex contents that were almost two times higher than our present results. Furthermore, aspartic acid and alanine were also detected as major amino acids (in males and females). However, because non-essential amino acids are hardly influenced by the diet composition, their values are very likely related to differences in food preferences and provided feed.

In the essential amino acid profiles, tryptophan seems to be a minor amino acid in all insects including the house cricket (Yi et al., 2013). However, since we did not analyse tryptophan in this study because of the methodology used, the sulphur-containing amino acid methionine was identified as the limiting amino acid as seen in studies by Ghosh et al. (2017) as well. This finding agrees with the data previously reported in the literature, where lack of tryptophan analysis was obviously not unusual (Sánchez-Murós et al., 2014). Leucine was then determined to be the major essential amino acid. Additionally, high lysine, valine, and arginine contents were also discovered (Table 2). These findings are in line with the published profiles of house cricket available in the literature (Barroso et al., 2014; Bosch et al., 2014; Finke, 2002). Moreover, the EAAI was calculated for all the samples and based on this index it was obvious that the protein quality was not sex-dependent. Furthermore, the results (EAAI ranged 72.3–77.1) indicate that the protein quality of both male and female house crickets was slightly lower than that of traditional livestock products, where the index is approximately 80% (chicken, 78%; fish, 82%; pork, 84%; and beef, 82%). However, the protein composition of the house crickets could be considered comparable to or even better than that of some conventional plant protein sources, such as rice (72%) or legumes (68%) (Velisek, 2013). This finding is in agreement with that of Yi et al.

(2013), who also consider insect protein quality to be better than that of soybean, but lower than that of casein.

Generally, proteins and fats are two major components of insects. Compared with conventional animal lipids such as lard, tallow, and milk fat, insects have a higher proportion of unsaturated fatty acids including essential polyunsaturated fatty acids (PUFA). The results of the fatty acid analysis of the tested samples are in line with those of Oonincx et al. (2015) who also reported linoleic, oleic, palmitic, and stearic acids as major fatty acids in *A. domestica*. The same dominant fatty acids were determined in other Orthoptera by Chakravorty et al. (2014) as well. Studies on the fat and fatty acids in *A. domestica* (Tzompa-Sosa, Yi, van Valenberg, van Boekel, & Lakemond, 2014) were not focused on sex-related differences in the fat content and fatty acid profile. Our results show that although there were differences between males and females in total fat content, the proportions of fatty acids in both sexes were identical.

Indices based on the functional properties of different fatty acids provide a better evaluation of the nutritional quality of foods, than only the sums of all SFA, MUFA and PUFA and their ration. On the other hand, due to best of author's knowledge, this is the first determination of IA and IT in insects, these values have been unknown for any of insect species since and comparison is thus not possible. The IA, which indicates the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated fatty acids, was within the range 0.51–0.60, which is comparable to that of pork lard (0.55) or fish oil (0.52). The IT, which shows the tendency to induce clot formation in the blood vessels, was calculated as 1.17–2.66, which is comparable to that of pork lard (1.42), palm oil (1.98), and beef tallow (2.26) The IA and IT, which were calculated based on the fatty acids profiles reported by Woodgate and van der Veen (2014), are considered indices of cardiovascular disease risks and, thus, low values are desirable.

Owing to the extremely diverse life cycles of various insects, their nutritional value is undoubtedly species-dependent (Sánchez-Murós et al., 2014). Currently, diet is also considered to be an important factor, which can influence both quantity and quality of the insecťs nutrients (Barroso et al., 2014; Oonincx and Van der Poel, 2011; Oonincx et al., 2015). Additionally, the developmental stage is known to affect the nutritional quality although this has not been clearly defined. According to the results published by Finke (2002), differences between the chemical composition of juvenile and adult house crickets indicate the possible effect of developmental stage on protein, fat, amino acid, and finally, fatty acid composition. Furthermore, Kulma et al. (2016) obtained similar results of lipid and protein quality, unlike their very different quantity compared with the nutritional values of subadult and adult roaches. Unfortunately, these results were not statistically evaluated and, so, the accurate effect of the developmental stage has still not been demonstrated. Similarly, the effect of sex, which is generally regarded as a factor affecting the nutritional value of insects, has not been previously evaluated. The present results indicate that protein and lipid amounts in adult crickets are sex-dependent, whereas the other profiles did not vary between males and females.

Among the proximate components, the ash level was not significantly different between males and female house crickets. NFE is probably formed mainly by feed residues and, thus, was expectedly not affected by sex. Chitin is the major carbohydrate present in insects and its level was clearly affected by sex, with males containing significantly higher amounts than females. Considering significant differences found in their weight, this could be simply explained by the presence of more males in 1 kg DM. Although Paoletti, Norberto, Damini, & Musumeci (2007) demonstrated, that humans are able to digest chitin, it is generally regarded as indigestible for the majority of the global population. On the other hand, its benefits to the immune system and antioxidative, hypocholesterolaemic, and prebiotic effects are known (Halder et al., 2013; Van Huis et al., 2013). Therefore, higher chitin content should be strictly not regarded as a negative attribute. Since chitin is a nitrogenous polysaccharide and protein content is usually calculated from total nitrogen using 6.25 as the nitrogen to protein conversion factor, values could be overestimated and misleading. Hence, Janssen, Vincken, van den Broek, Fogliano, & Lakemond (2017) proposed calculating protein amounts using species-dependent factors such as 4.75, 4.86, and 4.67 for the mealworm, lesser mealworm, and black soldier fly, respectively. In this study, the chitin nitrogen content was determined to be slightly higher in the males than in females, but it did not exceed 1 g/100 g DM in all the samples. Therefore, other novel or unknown factors could lead to total crude protein underestimation. This observation is in line with that of Finke et al. (2015) who estimated that chitin nitrogen represents only a fairly small fraction, and thus the 6.25 factor might not be unrealistic. However, further research focusing on this topic including the possible effects of other nitrogen-containing complexes such as excretion products, need to be conducted.

Considering that the present investigated sex-related effect probably occurs in the entire Insecta class, these results and novel approach to determining the nutritional value of insects could be applicable to other species of insects that are collected, captured, prepared, and eaten individually (especially in species showing significant sexual dimorphism). However, this phenomenon also could play an important role in assessing the nutrient content of harvested insects, when the proportion of males and females would influence nutritional composition of the biomass.

5. Conclusion

The results of the present study showed significant differences in the proximate composition of male and female house crickets, and thus confirmed our hypothesis that sex should be included among factors that can influence the nutritional values of insects. Females proved to be of a higher energy value and contained significantly more lipids and less protein than males did. However, the quality of nutrients as expressed by the EAAI, IT, and IA were similar. These findings may contribute to improving the evaluation of the nutritional value of insects as a novel food.

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[Nutriční hodnota jedlého hmyzu z řádu Coleoptera (*Tenebrio molitor, Zophobas morio* a *Alphitobius diaperinus)* odchovaného v České republice]

Jedním z faktorů, které ovlivňují nutriční hodnotu, je výživa hmyzu. Vzhledem k tomu, že zdroje pro krmení hmyzu se mezi jednotlivými státy liší, dá se předpokládat, že i místo, kde byl hmyz odchován bude hrát určitou roli ve výsledné nutriční hodnotě. Tato publikace popisuje nutriční hodnotu larev tří nejčastěji chovaných krmných brouků, odkrmených na zeleninové dietě skládající se z mrkve, zelí, čínského zelí, rajčat a brambor. Larvy potemníka moučného (*Tenebrio molitor*) a potemníka stájového (*Alphitobius diaperinus*) byly dobrým zdrojem bílkovin (630 g/kg sušiny a 600 g/kg sušiny respektive) a nijak výrazně se nelišily od nutričních hodnot zjištěných v jiných částech světa. Naopak larvy potemníka brazilského (*Zophobas morio*) byly oproti dostupné literatuře výrazně tučnější s menším obsahem bílkovin. Co se týče profilu mastných kyselin, nejpříznivější poměr n-6 : n-3 byl zjištěn u *Z. morio*.

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NUTRITIONAL VALUES OF EDIBLE *COLEOPTERA* (*TENEBRIO MOLITOR*, *ZOPHOBAS MORIO* AND *ALPHITOBIUS DIAPERINUS)* REARED IN THE CZECH REPUBLIC

Anna Adámková, Lenka Kouřimská, Marie Borkovcová, Martin Kulma, Jiří Mlček

ABSTRACT

Edible insects have gained the status of highly nutritious food with high protein and fat content. However, nutritional value of insects is not constant. It could be affected by species, developmental stage, rearing technology, nutrition or sex. This study's goal is to determine the protein and fat contents of three edible beetle species (giant mealworm – larvae of *Zophobas morio*, mealworm – larvae of *Tenebrio molitor* and, lesser mealworm – larvae of *Alphitobius diaperinus*) bred in the Czech Republic. Based on the obtained results, all investigated species could be considered as a reasonable source of lipids and two of them (mealworm and lesser mealworm) are also an excellent source of protein. Crude protein content of mealworm (630 g. kg^{-1} DM) was found to be higher than in other studies. The investigated species of lesser mealworm contained 600 g of crude protein/kg DM, which was equal to the results of other authors. Most authors report a higher content of nitrogen in the giant mealworm than were the values measured by this experiment (390 g.kg^{-1} DM). The lipid content in the tested samples was found in a range of 170 – 390 g.kg^{-1} DM. The highest lipid content was found in the larvae of giant mealworm and the lowest lipid content was found in the larvae of mealworm. The determined fat content of lesser mealworms was 290 g.kg^{-1}. The fatty acid profiles of all samples were also determined.

Keywords: edible insects; nutrition; protein, fat; fatty acid; *Coleoptera*

INTRODUCTION

Edible insects form a common part of the human diet in many parts of the world **(van Huis et al., 2013; Vantomme et al., 2012)**. They are also being considered an extra food source in countries where people have limited access to sufficient, safe and nutritious food to maintain a healthy and active life **(Kampmeier and Irwin, 2009; van Huis et al., 2013; Vantomme et al., 2012)**.

Edible insects are seen as an interesting alternative source of proteins and lipids **(Zielińska et al., 2015)**. They are also believed to be an ideal option for the space agriculture **(Katayama et al., 2008)**. In the developing countries, edible insects may serve as a potential animal protein source because of its better digestibility and utilization than vegetable protein **(Hoffman and Falvo, 2004)**. They could also help the children suffering from malnutrition **(Brázdová, 2011)**.

Some species of insects could serve as an important source of lipids. Fatty profile of insects varies among different species as well as among the developmental stages within one species **(Finke, 2004)**. It may also be easily affected by the feed composition **(Schaefer, 1968; Bukkens, 1997; Mariod, Abdel-Whab and Ain, 2011).** Fatty acid composition of insect is reported to be similar to that of poultry or fish **(DeFoliart, 1992)**.

Entomophagy is not very common in Europe. Insects are usually considered a delicacy or a means to diversify one's diet. Although the amount of information about the insects' nutritional composition and the potential risks has been recently increased, insects are still not considered a standard human food. A list of edible insects (including mealworm, giant mealworm, and lesser mealworm) was published by **EFSA (2015)** together with the risk related to production and consumption of insects as food and feed. Although entomophagy is considered to be safe due to its long history, manipulation with and consumption of edible insect may involve some risks **(EFSA, 2015)**. These risks are usually represented by collecting the insects in dangerous areas without protective equipment, consuming inappropriate developmental stages or inadequate culinary treatment **(Ramos-Elorduy, 2005; Belluco et al., 2013; Mlček et al., 2014)**. The toxic substances content or allergic reactions (mostly to chitin) are among other potential risks of edible insect consumption **(Park, Kim and Yang, 2009)**.

Available data about nutritional values of insect species bred in Europe are not sufficient. The mealworm is probably the most-studied species **(Bernard, Allen and Ullrey, 1997; Oonincx and Dierenfeld, 2012; Bednářová et al., 2013** and **van Broekhoven et al., 2015)**. It could be considered as a good source of protein and lipids, although the nutritional composition varies among individual developmental stages. The highest protein content (637.0 – 676.5 g.kg^{-1} DM) and the lowest fat content (148.8 – 184.0 g.kg^{-1} DM) were found in adult beetles. However, adult beetles are not very suitable for human consumption because of the high anti-nutritional substances content (wings, exoskeleton, legs etc.).

From the nutritional point of view the larvae (protein: 477.6 – 527.0 .kg^{-1} DM, fat 189.0 – 382.9 .kg^{-1} DM) and pupae (protein: 531.3 – 546.0 .kg^{-1} DM, fat

308.0 –366.5 g.kg^{-1} DM) seem to be more interesting. The nutrient content of hormonally modified mealworm form ("super mealworm") is known as well. These mealworms with artificially delayed pupation have the protein content comparable to other mealworms (471.8 g.kg^{-1} DM) but the fat content differs significantly (430.8 g.kg^{-1} DM) (calculated from **Finke, 2002**).

Giant mealworm, whose larvae could reach 55 mm **(Friedrich and Volland, 2004)**, is also considered to be a good source of quality protein and lipids. The nutritional composition of this species was determined by various authors **(Barker, Fitzpatrick and Dierenfeld, 1998; Finke, 2002; Bednářová et al., 2013; Yi et al., 2013; Bosch et al., 2014; van Broekhoven et al., 2015)**. The protein content of giant mealworm larvae was 431.3 – 516.2 g.kg^{-1} DM, the fat content was 328.0 – 435.4 g.kg^{-1} DM. **Oonincx and Dierenfeld (2012)** evaluated the nutrient content of giant mealworms adults and determined the protein level to be 680.5 g.kg^{-1} DM and lipid content 142.5 g.kg^{-1} DM.

The nutrient content of lesser mealworm is only available for larvae stages. **Bosch et al. (2014)** reported 648 g.kg^{-1} DM of protein and 222 g.kg^{-1} DM of fat. **Yi et al. (2013)** determined 580.3 g.kg^{-1} DM of protein and 239.5 g.kg^{-1} DM of fat. **Van Broekhoven et al. (2015)** found protein content to be 617 – 650 g.kg^{-1} DM and fat content 134 – 243 g.kg^{-1} DM.

Besides the factor of the above-mentioned development stage, the nutrient content of insects is also affected by feed composition, microclimate, environment, sex and other factors **(Oonincx and van der Poel, 2011)**. **Van Broekhoven et al. (2015)** reported that the feeding mixture change caused differences in content of both fat and protein (by 8 % and 11 % respectively). This research is therefore focused on the determination of basic nutrient contents of three edible insect species reared under defined farming conditions in the Czech Republic and the comparison of the obtained data with results from other countries and wild species.

MATERIAL AND METHODOLOGY

Material

The insect samples tested for the purposes of this study were larvae of darkling beetles (*Zophobas morio,* Fabricius, 1776), which are known by the common name superworm or giant mealworm, mealworm (larvae of *Tenebrio molitor*, Linnaeus, 1758) and lesser mealworm (larvae of *Alphitobius diaperinus*, Panzer, 1797). All of them are common warehouse pests and can be easily kept and bred in the European climate conditions. The samples were purchased in the ultimate or penultimate instars (most suitable to culinary purposes) from a private company Radek Frýželka, Brno. The insect species were fed by a mixture of plant material (carrots, cabbage, Chinese cabbage, tomatoes, and potatoes). Prior to the analysis, the insects were fasted for 48 hours to minimize the effects of food retained in the gut, then killed in boiling water (100 °C) and finally dried at 105 °C for 12 h. The obtained samples were then homogenized for 1 minute by the coffee grinder Scarlett Silver Line SL-1545 (ARIMA, UK) and stored at 4 – 7 °C. All sample analyses were done at least in triplicate.

The used chemicals were of the p.a. grade and were purchased from the Sigma Aldrich company.

Methods

Nitrogen and crude protein content determination

The nitrogen and crude protein were analysed using the Kjeldahl's method **(ISO 1871:2009)**. The samples (1 g) and blank runs were mineralised at 420 °C for 105 min. The distillation was performed on Kjeltec™ 2200 (FOSS, Denmark) for 4 minutes. The protein content was calculated using nitrogen-to-protein conversion factor of 6.25.

Fat content determination

The fat content determination was performed by extraction using Soxhlet method **(Soxhlet, 1879)** on the Gerhardt Soxtherm SOX414 (C. Gerhardt GmbH & Co. KG, Germany). Approximately 5 g of dried and homogenized samples (with the accuracy of 0.0001 g) were put into extraction thimbles and extracted by 150 ml of petroleum ether via cold water extraction (program: 70 °C for 120 minutes). The extraction flask was then dried at 103 °C and weighed until a constant sample weight was attained.

Fatty acid profile determination

The esterification of lipids extracted form samples of insects via the Soxhlet extraction was performed according to the **ISO 12966-2:2011** standard using 0.25 M methanolic KOH (test weight of fat for esterification was 0.5 g). Methyl esters of fatty acids were analysed by GC Agilent 7890 (Agilent Technologies, USA) with a flame ionization detector (detector temperature: 250 °C) equipped with a RestekRt®-2560 column (100 m × 0.25 mm ID × 0.2 µm film) from Restek Corporation. Hexane was used as a solvent and the sample volume of 1 μL was injected in split mode (ratio 20:1) into the injector heated to 225 °C. The initial oven temperature was 70 °C (hold 2 min), ramp1 to 225 °C at 5 °C/min (hold 9 min), ramp2 to 240 °C at 5 °C/min (hold 15 min). Helium was used as carrier gas with the flow rate of 1.2 mL/min. The methylated fatty acids were identified using a Restek Food Industry FAME mix (cat#35077). Real chromatogram of Restek Food Industry FAME mix is shown in Figure 1. The proportions of fatty acids were calculated using the area normalisation method.

Statistical analysis

The data were analysed using Excel 2013 (Microsoft Corporation, USA) and the results were expressed by means ±standard deviations.

RESULTS AND DISCUSSION

Crude protein and fat contents of the three investigated edible insect species are shown in Table 1. The obtained values of crude protein in tested insects ranged from 390 to 630 g.kg^{-1} DM. The protein content of *Tenebrio molitor* was found to be higher than in the studies of **Bernard, Allen and Ullrey (1997); Finke (2002); Ramos-Elorduy (2006); Oonincx and Dierenfeld (2012); Yi et al. (2013)** or **van Broekhoven et al. (2015)**. It was also higher than the levels reported by **Bednářová et al. (2013)** who measured the nutrient content of insects bought from a local Czech supplier. The protein content of *Alphitobius diaperinus*is found in this study were consistent with the results reported by **Yi et al. (2013); Bosch et al. (2014)** and **van Broekhoven et al. (2015)**.

Table 1 Lipid and crude protein contents of three edible insect species.

species	**crude protein**	**lipids**
	$g.kg^{-1}$ DM ±SD	
Giant mealworm *(Zophobas morio)*	390 ±1	390 ±4
Mealworm *(Tenebrio molitor)*	630 ±4	170 ±1
Lesser mealworm *(Alphitobius diaperinus)*	600 ±5	290 ±3

Table 2 Fatty acid profile of analysed samples.

Fatty acid composition	TM (%)	ZM (%)	AD (%)
	SFA		
C8:0	<0.1	1.8	<0.1
C10:0	<0.1	0.4	<0.1
C12:0	0.2	0.1	0.1
C13:0	0.1	<0.1	<0.1
C14:0	3.5	1.7	1.4
C15:0	0.2	0.4	0.3
C16:0	18.4	30.2	26.4
C17:0	0.3	0.7	0.7
C18:0	6.6	8.8	10.9
C19:0	0.1	0.1	0.2
C20:0	0.3	0.2	0.6
C22:0	0.1	0.1	<0.1
Sum of SFA	29.7	44.6	40.6
	MUFA		
C14:1, cis - 11	0.1	<0.1	<0.1
C16:1, trans - 11	0.1	<0.1	<0.1
C16:1, cis - 9	1.4	0.7	1.1
C17:1, cis - 10	0.1	0.2	0.2
C18:1, trans - 9	<0.1	<0.1	0.2
C18:1, cis - 9	36.5	31.1	35.9
C20:1, cis - 11	0.1	0.1	0.4
Sum of MUFA	38.4	32.1	37.8
	PUFA		
C16:2, trans - 7,10	0.3	1.1	0.3
C 18:2, trans - 9,12	<0.1	<0.1	0.2
C18:2, cis -9,12	30.5	21.2	20.2
C 20:2, cis - 11,14	<0.1	<0.1	0.2
C18:3, cis - 9,12,15	1.1	0.9	0.4
C 20:4, cis - 5,8,11,14	<0.1	<0.1	0.4
Sum of PUFA	31.8	23.2	21.6

Note: TM - larvae of *Tenebrio molitor*, ZM - larvae of *Zophobas morio*, AD - larvae of *Alphitobius diaperinus*.

Figure 5 Giant mealworm *(Zophobas morio)* **(Karwath, 2005)**.

Figure 6 Mealworm *(Tenebrio molitor)* **(Halasz, 2008)**.

Figure 7 Lesser mealworm *(Alphitobius diaperinus)* **(USDA-ARS-GMPRC, 2016)**.

Information about this species reared in the Czech Republic is not available. Compared to traditional protein sources in human nutrition, the content of proteins of both above mentioned species *T. molitor* and *A. diaperinus* are comparable to beef loin (640 g.kg^{-1} DM) or beef flank (640 g.kg^{-1} DM) **(Pipek, 1995; Steinhauser, 1995)**. Crude protein content of the giant mealworm (390 g.kg^{-1} DM) was lower than the contents reported by **Barker, Fitzpatrick and Dierenfeld (1998); Finke (2002); Yi et al. (2013); Bosch et al. (2014)** and also the only known Czech author dealing with this issue **Bednářová et al. (2013)**. On the other hand, the protein content was similar to that determined by **van Broekhoven et al. (2015)**. In comparison with the conventional food, this species could be considered similar to roast pork (410 g.kg^{-1} DM) **(Pipek, 1995; Steinhauser, 1995)**. The differences between obtained results and other studies could probably be caused by using different feeding mixtures or analysing different developmental stages of the sampled larvae.

While the protein contents of the investigated insects varied significantly, the lipid content (Table 1) was found in a range of 170 – 390 g.kg^{-1} DM. The highest lipid content was found in the larvae of the giant mealworm. The analysed lipid content of the giant mealworm was similar to the findings of other papers **(Finke, 2004; Bednářová et al., 2013; van Broekhoven et al., 2015)**. The fat content of *T. molitor* was lower than previously published works suggested. Higher values were reported by **Finke (2004); Bednářová et al. (2013); Yi et al. (2013)** and **van Broekhoven et al. (2015)**. The samples of lesser mealworm contained about fifty grams more lipids than **van Broekhoven et al. (2015)** reported. They discovered the possibility of changes in fat content (up to 10 %) to be caused by feed mixture changes. Therefore, the differences between results of this study and other reported values could be caused by the variety of used feed. In terms of lipid content, all tested species are comparable to a number of traditional foods such as eel meat (300 g.kg^{-1} DM), pork rump (320 g.kg^{-1} DM) or young goose meat (360 g.kg^{-1} DM) **(Pipek, 1995; Steinhauser, 1995)**.

From a nutritional point of view, fatty acid content is very important. Our results in Table 2 show the fatty acid profiles of the fat extracted from the giant mealworm – larvae of *Zophobas morio*, mealworm - larvae of *Tenebrio molitor* and lesser mealworm - larvae of *Alphitobius diaperinus*. Real chromatogram samples of fatty acids composition for all selected insect species are shown in Figure 2, Figure 3 and Figure 4. The recommended ratio of fatty acids for human nutrition is SFA : MUFA : PUFA 1.25 : 1.5 : 1, but the ratio found in *Zophobas morio* is 1.9 : 1.4 : 1. The determined MUFA : PUFA ratio meets the requirements for human consumption (1.4 : 1), but the amount of SFA is significantly higher. Similar ratio is reported by **Bednářová (2013) and Jabir (2012)** – 2.2: 1.9 : 1 and 2.1 : 1.1 : 1. However, **Barroso (2014)** described a lower ratio. Higher SFA content was also determined in the case of lesser mealworms. On the other hand, **Tzompa-Sosa (2014)** presents a lower ratio (1.4 : 1.6 : 1) in case of these species. In contrast to these species, the amount of SFA in mealworm was significantly lower. Similar results were published by **Zielinska (2015) and Barroso (2014)**. **Tzompa-Sosa (2014)** reported a significant content of MUFA (1.1 : 2.3 : 1), but on the contrary **Bednářová (2013)** measured a greater amount of PUFA (0.7 : 0.8 : 1).

These differences can be caused by a different type of feed and breeding conditions, which were not fully specified by the authors.

Professional and general public pays considerable attention to the ratio of fatty acids n-6 : n-3, which WHO recommends to be 5 : 1 for human nutrition **(Dostálová, Dlouhý and Tláskal, 2012)**. This ratio has a protective effect against non-infectious civilization diseases. The content of n-6 fatty acids in all species, that we analysed, was significantly higher (the ratios of n-6 : n-3 were 26 : 1 for TM, 22 : 1 for ZM, and 53 : 1 for AD). The giant mealworms were the closest to this requirement. On the other hand, lesser mealworms had the highest ratio from the analysed samples. Therefore, the lesser mealworm does not seem to be a perfect primary nutritional source for a long-term human consumption. However, the fatty acids proportions could be affected by changing the insects' feed composition. Balanced diet of people eating insects is also important.

A higher content of unsaturated (n-9) oleic acid was measured in all samples. The amount of this acid is comparable to the traditional sources such as beef tallow (26 – 50 %), and sheep tallow (30 – 42 %), but **DeFoliart (1992)** reported that composition of fatty acids is similar to poultry and fish. The second most represented fatty acid in giant mealworm and lesser mealworm was palmitic acid (31.1 % and 26.4 %). A similar content of this acid is to be found in rabbit lard (32 %) **(Velíšek, 2002)**. Linoleic acid was the third most abundant fatty acid in these samples (21.2 % and 20.2 %). However, in case of mealworm, the second most represented acid was the essential linoleic acid (30.5 %) and the third was palmitic acid (18.4 %). The highest content of essential α-linolenic acid was also measured in mealworm. Therefore, mealworm could be the most suitable insect of the analysed species for human consumption.

The descending order of the first four minor fatty acids for giant mealworm is the same as reported by **Bednářová et al. (2013)**. However, the ratios found differed slightly. Also, some fatty acids not detected by **Bednářová et al. (2013)** were determined. An example of such is the α-linolenic acid, whose content was measured to be twice the amount of the arachidic acid. Unfortunately, **Bednářová (2013)** did not mention the composition of the feed mixture. Our results are also in line with the data reported by **van Broekhoven et al. (2015)**. The order of the first four acids is identical, while the mutual ratio varied in dependence on feed (i.e. when insects were fed by feed with high starch and low protein content, the ratio between linoleic and oleic acid was 0.22 : 1; when they were fed by high-protein and low-starch feed this ratio changed to 0.94 : 1).

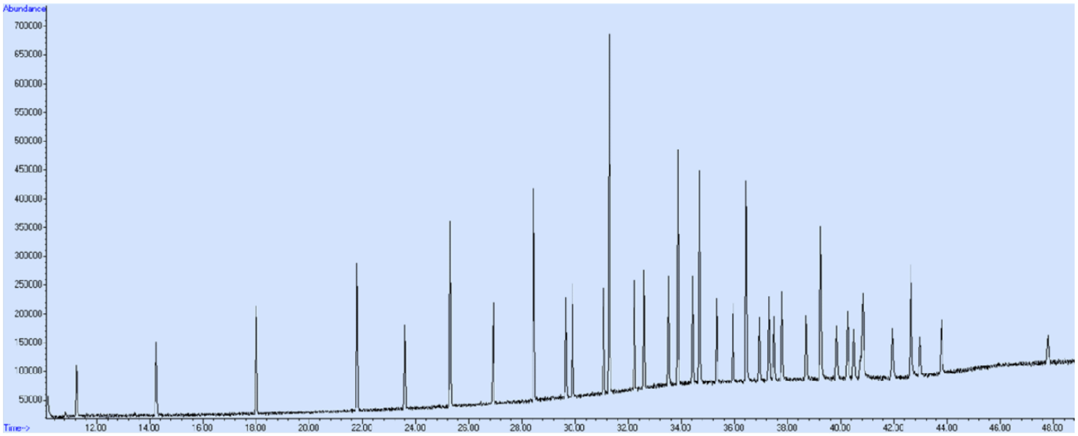

Figure 1 Chromatogram - fatty acids composition of Restek Food Industry FAME mix (cat#35077).

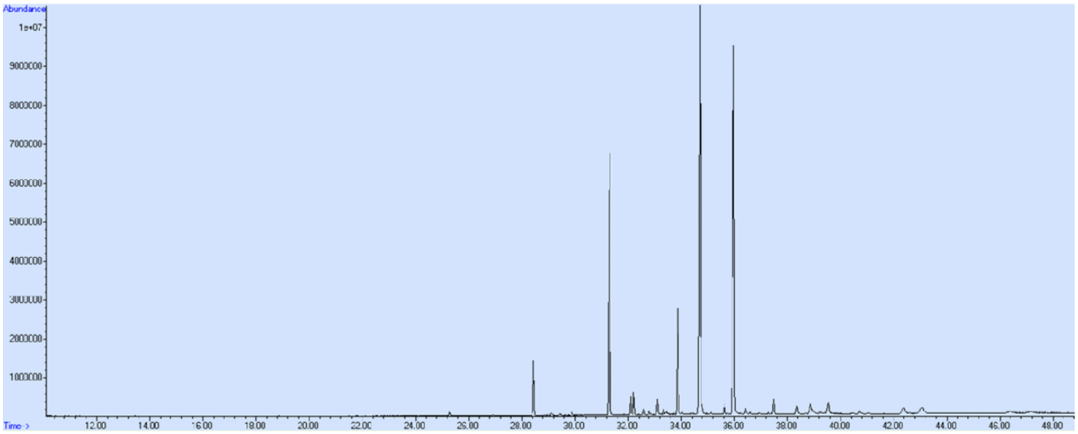

Figure 2 Chromatogram – fatty acids composition of mealworm – larvae (*Tenebrio molitor*) reared in the Czech Republic.

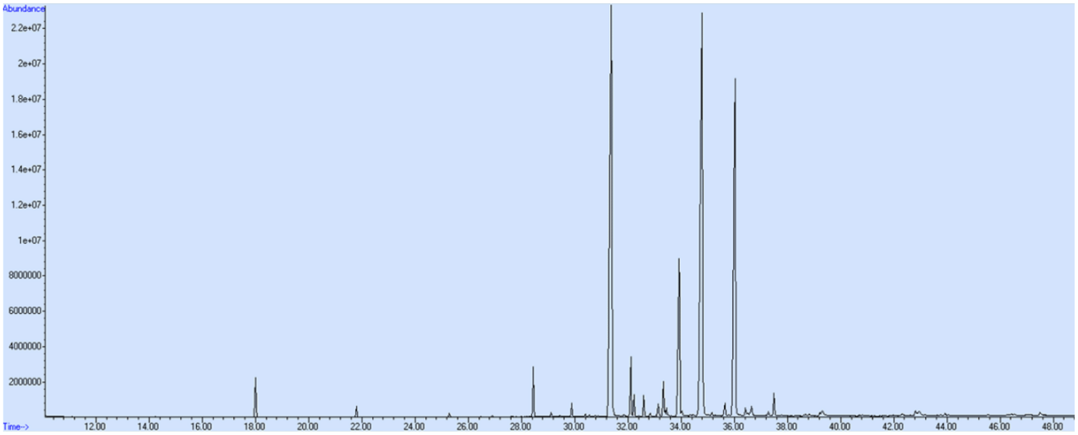

Figure 3 Chromatogram – fatty acids composition of giant mealworm – larvae (*Zophobas morio*) reared in the Czech Republic.

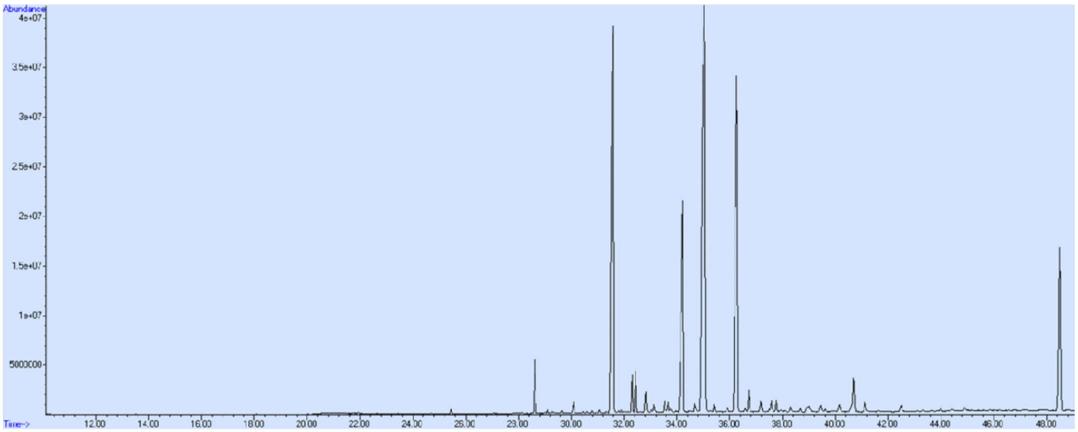

Figure 4 Chromatogram – fatty acids composition of lesser mealworm – larvae (*Alphitobius diaperinus*) reared in the Czech Republic.

CONCLUSION

This work was focused on the nutritional composition determination of three edible insect species reared in the Czech Republic. Based on the obtained results, all investigated species (*Zophobas morio*, *Tenebrio molitor* and *Alphitobius diaperinus*) could be considered as a reasonable source of lipids and two of them (mealworm and lesser meal worm) are also an excellent source of proteins. The results of fatty acids profile of the giant mealworm and lesser mealworm showed that they are not very suitable as the main food ingredient due to a high SFA content and an inappropriate n-6 and n-3 ratio. Out of all measured samples, mealworm has the highest content of linoleic and α-linolenic acid, which are among essential components of human nutrition.

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5 Diskuze

Globální biodiverzita čelí na prahu 21. století několika vážným hrozbám. Hmyz je největší skupinou živočichů a jeho činnost je pro fungování celého ekosystému klíčová (Balvanera et al. 2006). V posledních letech se početnost hmyzu v Evropě dramaticky snižuje (Hambler & Speigh 2004) a hledání strategie k zvrácení tohoto negativního trendu je aktuálním problémem pro celou planetu. Základním požadavkem pro účinnou ochranu organismů, včetně hmyzu, je komplexní porozumění mechanismu ztráty biodiverzity, potřebám jednotlivých druhů (Purvis & Hector 2000) a také tomu, jak tyto druhy reagují na nastávající změny (Gilman et al. 2010).

5.1 Ochrana stanoviště

Za předpokladu znalosti výše uvedených předpokladů lze následně pomocí různých managementových opatření pozitivně působit na populace cílových druhů. Obecně se ochranářské managementy zaměřují na zachování co nejvyšší možné heterogenity krajiny, snížení kontrastu mezi stanovištěm a okolní krajinou, úsporu využití půdy mimo chráněná území, simulaci přírodních jevů či disturbancí a propojování stanovišť podobného typu (Samways 2007). Toto schéma specifikuje a rozvíjí studie shrnující vhodné typy managementů pro ohrožené druhy evropských motýlů, prezentovaných v Bubová et al. (2015). Nejvhodnějším managementem je mozaiková (rotační) seč (celkem vhodná pro 27 ohrožených druhů motýlů) a pastva (41 druhů ohrožených motýlů). Oba tyto typy managementu svým způsobem napodobují tradiční hospodaření, po jehož opuštění došlo k poklesu početnosti druhů vázaných na extenzivně využívané louky, jako jsou například modrásci rodu *Phengaris* (Thomas et al. 2009), žluťásek barvoměnný *Colias myrmidone* (Konvička et al. 2008) a mnoho dalších. Aby však byl tento typ úprav pro motýly opravdu prospěšný, musí být dodržena určitá pravidla vycházející z požadavků cílových organismů. Seč musí být provedena ve vhodném termínu tak, aby nedošlo k poškození zakladených živných rostlin. Pastva by neměla být příliš intenzivní, aby vegetace včetně živných rostlin stihla dorůstat. Podle studií zaměřených na tuto problematiku u různých druhů motýlů jako např. *Colias myrmidone* (Konvička et al. 2008), *Carcharodus flocciferus* (Dolek & Geyer 1997), *Euphydryas desfontainii* (Pennekamp et al. 2013) či *Parnassius apollo* (Schmeller et al. 2011) lze usuzovat, že maximální zatížení pastvou by nemělo přesáhnout 0.5 dobytčích jednotek na hektar. Důležité je též načasování pastvy a druh pasených zvířat. Pastva by, stejně jako v případě seče, neměla být provozována v období pozdního jara a léta, aby nedošlo ke ztrátě zdrojů pro larvální stádia i dospělce motýlů. Podzim a časné jaro (duben) je pak naopak období pro pastvu za tímto účelem velice vhodné (Beneš et al. 2002). Pastva ovcí se ukázala být nevhodná pro modrásky stepní (*Polyommatus damon*) (NT) (Dolek & Geyer 2002), protože ovce cíleně vyžírají živnou rostlinu druhu. Pro jiné druhy jako je např. modrásek *Pseudophilotes vicrama* je pastva ovcí a koz doporučená (Beneš et al. 2002).

Aplikace vhodného managementu na populace modrásků *P. nausithous* a *P. teleius* byla experimentálně ověřována na lokalitě Dolní Labe. Velikost populací na sledovaných plochách s vhodným managementem se během 4 let monitoringu významně nezvětšila (Bubová et al. 2016a). Neprokázání vhodného vlivu pravděpodobně souvisí s populační fluktuací, která byla u těchto motýlů při dlouhodobém MRR monitoringu v minulosti prokázána (Nowicki & Vrabec 2011). Naopak (Kőrösi et al. 2014) pozorovali u *P. teleius* během tří let signifikantní populační nárůst. Co se týče ploch bez managementu (nebo s managementem nevhodným) na lokalitě Dolní Labe, jejich populace během monitoringu na rozdíl od udržovaných ploch poklesly, což je v souladu s autory (Bergman et al. 2004; Kőrösi et al. 2014), kteří popisují, že upuštění od managementu má pro metapopulace modrásků fatální následky.

Fatální následky pro *P. teleius* a *P. nausithous* má, vzhledem k jejich nízkým migračním schopnostem, také ztráta stanoviště. Takovýmto případem se teoreticky zabývají Vrabec et al. (2017). V rámci stavby plavebního kanálu určeného ke splavnění řeky Labe do Pardubic by bylo částečně ohroženo či úplně zničeno několik luk s modrásky včetně těch s největší početností. Zničení části lokality může způsobit nejprve drastické snížení abundance motýlů a následně vlivem stochastických změn také kolaps celé metapopulace (Bouhours & Lewis 2016). Během monitoringu v okolí plánované stavby byly zjištěny další populace na opačném břehu Labe. Díky nálezům těchto populací vypadá situace pro přežití *P. teleius* a *P. nausithous* lépe, protože studie prokázala jejich propojení přelety motýlů v jediné metapopulaci. V okolí byly také nalezeny další vlhké louky s potenciálem pro osídlení studovanými motýly. V současné době jsou ale tyto louky koseny povětšinou intenzivně a během léta. Proto je, navzdory přítomnosti hostitelských rostlin i mravenců, jejich kolonizace modrásky rodu *Phengaris* nízká či vůbec žádná. Pokud by opravdu došlo k stavbě kanálu, bylo by tedy vhodné nastavit na těchto loukách vhodný management, podpořit tak celou populaci a do budoucna jimi nahradit poničené lokality. Na příkladu modrásků se potvrzuje, že ztráta stanoviště, ať už je příčinou zánik v důsledku jiného využití, sukcese nebo intenzivního hospodaření, může mít pro hmyz fatální důsledky, ale v populačním systému ji lze do značné míry predikovat a případně v určitém časovém období kompenzovat. Potvrdilo se také, že aplikace ochranářského managementu na stanovištích má vliv na stabilizaci populace, nicméně k dlouhodobému zvýšení početnosti je zapotřebí opakovaná intervence ve prospěch chráněných druhů v delším časovém období (v řádu několika let).

Aby bylo možné účinně hmyz chránit, je třeba znát atributy, které některé druhy činí zranitelnějšími ve srovnání s druhy jinými. Nejvíce chránění jsou živočichové, jejichž početnost je nejnižší nebo se vyskytují na velmi omezeném areálu, který může být snadno postižen. Další důležité faktory z tohoto pohledu jsou pak například persistence a četnost (unikátnost) či úbytek habitatu (Abellán et al. 2005). V publikaci Bubová et al. (2016b) jsme se zaměřili na vztah mezi délkou letového období druhů a časovým rozpětím života dospělců u evropských ohrožených motýlů a jejich ochranářským statusem. Ze souboru dat shromážděných studií zpětného odchytu značených jedinců týkajících se 50 ohrožených druhů motýlů dle evropského červeného listu, vyšel statisticky významný vztah mezi délkou života v dospělosti a statusem ochrany, kdy více ohrožení jsou ti motýli, kteří jako dospělci žijí kratší dobu. Tento fenomén lze vysvětlit například tím, že samice takových druhů motýlů mají kratší čas na vyhledání partnera a snůšku vajíček (Haeler et al. 2014). Disperzní schopnosti resp. mobilita těchto druhů jsou navíc vzhledem ke krátkému přežití dospělce omezené, a tudíž jsou takové druhy náchylnější k ústupu v důsledku fragmentace stanovišť a hůře se vyrovnávají s následky ztráty jakéhokoliv stanoviště (Hanski et al. 2000; Schmeller et al. 2011). Na rozdíl od předchozích studií autorů Komonen et al. (2004) a Franzén & Johannesson (2007), prezentované výsledky neprokazují hypotézu, že délka letového období motýlů přímo souvisí s jejich rizikem vyhynutí. Jev může být vysvětlen tím, že dlouhá i krátká letová období mají svá pozitivní i negativní specifika. Během dlouhé letové sezóny je vyšší riziko fragmentace a izolace. Na druhou stranu se na životě motýlů tolik nepodepisují stochastické faktory typu nepřízně počasí či nevhodných zákroků managementu (Cormont et al. 2011). Dosažené výsledky zdůrazňují nutnost zvážit délku života motýlů, potažmo jiného druhu hmyzu, v rámci zařazování do ochranářských programů a přiřazování ochranných statusů.

5.2 Biologické invaze

Dalším faktorem, který může způsobit imbalanci původních společenstev i přes zdánlivě pozitivní podmínky na stanovišti, jsou biologické invaze. Během čtyř let sbírání informací a monitoringu výskytu a šíření invazních druhů na území České republiky byl v létě 2016 zaznamenán první výskyt kudlanky *Ameles spallanzania* (Kulma et al. 2016a), která byla zřejmě dovezena za účelem hobby chovu. Druh se sice aktuálně vyskytuje hojně hlavně v subtropické oblasti Evropy, nicméně vzhledem k prokázané schopnosti přezimovat v severní části Itálie (Pezzi 2011), má do budoucna potenciál usídlit se i ve střední Evropě.

Podařilo se také zaznamenat další šíření komára tygrovaného (*Ae. albopictus*) automobilovou dopravou po Čechách (Kulma & Rettich 2018; Rettich & Kulma 2018). Mimo invaze hmyzu bylo autorem práce na území České republiky také zaznamenáno šíření dalších subtropických členovců jako např. strašníka dalmatského na podzim roku 2017 (*Scutigera coleoptrata*) (Kulma & Rettich 2017) či tropického klíštěte *Hyalomma marginatum* (Kulma, nepublikováno) v Rakovníku v létě

2018. Tyto práce nejsou součástí disertační práce, nicméně jsou k ní přiloženy. Dalším druhem, jehož populace se podařilo v České republice lokalizovat jsou rybenky *Ctenolepisma longicaudata* (Kulma et al. 2018), které se tak staly pátým českým zástupcem řádu Zygentoma (Štys & Rozkošný 1996; Král & Davidová-Vilímová 2001). Kromě prvního nálezu v rámci České republiky se jedná také o jednu z prvních zmínek o tom, že tento druh se pozvolna přemisťuje z muzeí a knihoven, kde byl v minulosti vážnou hrozbou pro vystavené či uložené exponáty (Mallis et al. 1958; Smith 2017), do skladů a společně s papírovými krabicemi pak i do domácností. Její potravou může být jak škrob či celulóza (Van der Weijden et al. 2007), tak v podstatě jakákoli jiná hmota organického původu (Goddard et al. 2016). Navíc tento živočich vydrží velmi dlouho hladovět (Lindsay 1940) a má tak potenciál stát se dominantním synantropním druhem i v našich podmínkách. První nález této rybenky byl učiněn v areálu firmy, která se zabývá prodejem elektroniky. Rybenky se nacházely ostrůvkovitě po celé ploše, největší infestace byla pak pozorována ve skladu v kartonových krabicích. Vzhledem k přítomnosti všech stádií na velké ploše byla populace prohlášena za etablovanou a doporučena dezinsekce areálu, aby se předešlo dalšímu šíření. V létě 2018 byla autorem práce rybenka *C. longicaudata* identifikována jako původce škod na exponátech v Národní galerii v Praze (Kulma 2018). Stejně jako v prvním případě, byla doporučena eliminace škůdců. Bohužel, přestože v obou případech došlo k insekticidnímu postřiku, rybenky se dle tázaných zaměstnanců v obou areálech objevují stále a mohou tak být zdrojem dalšího šíření v rámci České republiky i mimo ni.

5.3 Hmyz jako alternativní zdroj živin

Hmyz je považován za alternativní zdroj kvalitního proteinu, který by v porovnání s intenzivním zemědělstvím byl šetrnější k životnímu prostředí. Klíčovou znalostí v tomto ohledu je nutriční hodnota, která je nejen informativní hodnotou pro spotřebitele, ale také vypovídá o potřebách živin pro hmyz a má tudíž význam z hlediska výživy hmyzu za účelem optimalizace technologie chovu. Ve studii Kulma et al. (2016b) byl definován obsah živin u tří druhů švábů, které jsou běžně komerčně dostupné v České republice, prozatím hlavně za účelem krmiva pro terarijní zvířata. Přestože švábi jsou v evropské kultuře obecně považovaní spíše za škůdce či symbol nečistoty, v Číně se tradičně chovají jako komponent pro biomedicínu (Li et al. 2018), v Brazílii byla moučka vyrobená ze švábů *Nauphotea cinerea* využita v pečivu (De Oliveira et al. 2017) a Niaz et al. (2018) považují tekutinu vylučovanou samicemi rusa tečkovaného *Diplotera functata,* někdy označovanou jako „entomléko“, dokonce za potenciální superpotravinu budoucnosti. Recentně jsou známy i první pokusy o začlenění švábů *Blatta (=Shellfordella) lateralis* do výživy psů (Kierończyk et al. 2018) a zkrmování diety s příměsí švábů amerických (*Periplaneta americana*) potkanům (Boateng et al. 2018). Znalost nutriční hodnoty je tak esenciální z hlediska dalšího možného použití této skupiny hmyzu v potravinářství či krmivářství. Publikované výsledky (Kulma et al. 2016b) ukazují, že testované druhy švábů obsahují vysoké množství bílkovin (až 652 g/kg sušiny) a tuku (až 363 g/kg sušiny), což prakticky odpovídá dalším druhům švábů, jejich nutriční hodnota je známa (Oonincx & Dierenfeld 2012; Bosch et al. 2014; De Oliveira et al. 2017) a víceméně odpovídá i schématu této hodnoty u ostatních skupin hmyzu (Makkar et al. 2014; Sánchez-Muros et al. 2014; Payne et al. 2016). Co se týče kvality bílkovin, index esenciálních aminokyselin se hlavně díky vysoké hladině lysinu pohyboval od 0,4 – 0,9 což potvrzuje, že se jedná o komponent lepší či srovnatelný se sójou, ale o něco málo horší než živočišná bílkovina (Yi et al. 2013). Za limitující aminokyseliny bylo možné označit methionin a cystein. Také toto měření je v souladu s literaturou. Například Bosch et al. (2014) zjistili u švábů argentinských (*B. dubia*) vysokou hladinu valinu, leucinu, lysinu a argininu, což odpovídá stanovení v prezentované práci. Stejně tak Kierończyk et al. (2018) uvádí pro *B. lateralis* stejných pět dominantních aminokyselin, jako byly pro tyto šváby detekovány v této práci. Kvalita tuku též odpovídala zjištěním jiných autorů. Nejvyšší hladiny mastných kyselin byly zjištěny pro kyselinu olejovou, stejně jako uvádí pro šváby *B. lateralis* a *B. dubia* (Finke 2002; Van Broekhoven et al. 2015). Dominantní kyseliny olejová, palmitová a linolenová jsou pak s výjimkami shodné i s ostatními druhy hmyzu (Finke 2002; Barroso et al. 2014). Stejně jako u ostatních živočišných tuků, vysoké hodnoty nasycených a mononenasycených kyselin vypovídají o horší kvalitě tuku z pohledu lidského zdraví.

Vzhledem k tomu, že pokus byl proveden na dospělcích a subadultech od každého druhu, bylo též možné provést určité srovnání nutričních hodnot v závislosti na vývojovém stádiu. Všichni dospělci obsahovali více bílkovin a méně tuku než subadultní švábi stejného druhu. Kvalita tuku a bílkovin se však lišila minimálně. Stejné rozdíly v obsahu základních živin mezi různými stádii zaznamenal u švábů *B. dubia* také Yee et al. (2018). Naopak Oonincx & Dierenfeld (2012) sice našli rozdíly mezi jednotlivými stádii švábů, nicméně bez pozorovatelné pravidelnosti. Výsledky této práce tudíž indikují nutnost přiřadit vývojové stádium k dalším faktorům, které mají prokazatelný a významný vliv na nutriční hodnotu, jako je např. druh hmyzu (Payne et al. 2016; Nongonierma & FitzGerald 2017) či krmná směs (Oonincx & Van der Poel 2011; Barroso et al. 2014).

Dalším atributem, který by mohl ovlivnit nutriční hodnotu je pohlaví hmyzu. Na tento jev se zaměřil Kulma et al. (2019), podle kterého byly obě skupiny cvrčků (*Acheta domestica*) dobrým zdrojem bílkovin a tuků. V tom se publikace shoduje s ostatními publikacemi (Finke 2002; Barroso et al. 2014; Bosch et al. 2014). Samci cvrčka domácího obsahovali v porovnání se samicemi stejného druhu více bílkovin a méně tuku. Tyto rozdíly mohou být způsobeny rozdíly v příjmu potravy a potravních preferencích, které jsou v dospělosti rozdílné (Reddiex et al. 2013). Zatímco dospělé samice se specializují na stravu s vysokým obsahem bílkovin a tuku, aby mohli investovat do potomstva, samci potřebují dostatek energie na stridulaci a páření, prot dávájí přednost potravě bohaté na cukry (Maklakov et al. 2008). Z pohledu kvality hlavních živin, bílkovin a tuků, se faktor pohlaví projevil pouze u neesenciálních aminokyselin, což může souviset s rozdílnými fyziologickými požadavky mezi samci a samicemi (Reddiex et al. 2013). Na druhou stranu, rozdíl v kvalitě bílkovin a tuků, vyjádřený indexy esenciálních aminokyselin, thrombogenicity a atherogenecity, nebyl v závislosti na pohlaví potvrzen. Index esenciálních aminokyselin byl díky vysokému obsahu leucinu, lysinu, argininu a valinu vyšší než v sóji (Yi et al. 2013), srovnatelné s kuřecím masem, a dokonce jenom o něco málo nižší než u vepřového, hovězího a rybího masa (Velisek 2013). Očekávaná využitelnost bílkovin se ale očekává nižší než u jiných zdrojů a to z důvodu, že limitující aminokyselinou byl určen methionin, což je společně s tryptofanem (tryptofan v publikacích prezentovaných v této práci nebyl stanoven z metodologických důvodů) nejčastěji zmiňovaná minoritní aminokyselina (Sánchez-Muros et al. 2014; Ghosh et al. 2017). Co se týče kvality tuků, cvrčci měli v porovnání s konvenčními potravinami typu sádla či mléka vysoké množství polynenasycených mastných kyselin (Velíšek 2013). Jako majoritní byly určeny linoleová, olejová, pamitová a stearová kyselina. Což je shodné s hodnotami, které pro *A. domestica* publikoval (van Broekhoven et al. 2015) i s jinými druhy rovnokřídlého hmyzu (Chakravorty et al. 2014; Paul et al. 2017).

Jako dobrý zdroj proteinu a tuku se dle očekávání ukázali také potemníkovití brouci *Tenebrio molitor* a *Alphitobius diaperinus,* kteří dříve byli považováni hlavně za rychle se množící skladištní škůdce, a právě proto jsou s touto problematikou od začátku spojováni. Stanovení jejich nutriční hodnoty bylo publikováno v článku Adámková et al. (2016). Má-li se hmyz stát alternativním zdrojem proteinu, je vhodné jej odchovávat na lokálních, dobře dostupných surovinách. Protože nutriční hodnota je úzce spjata s potravou hmyzu, lze tak předpokládat, že nutriční hodnota hmyzu se bude pro stejný druh lišit v návaznosti na jeho geografický původ. To se potvrdilo i v této studii, kdy v době publikace obsah bílkovin byl u *T. molitor* vyšší a obsah tuku naopak nižší než v publikacích některých autorů (např. Barker et al. 1998; Finke 2002; Yi et al. 2013; Bosch et al. 2014). Intenzivní výzkum v posledních letech, zapříčiněný hlavně povolením hmyzu jako krmiva v akvakultuře, ovšem přinesl velké množství dalších hodnot a rozpětí známého obsahu bílkovin pro larvy potemníka moučného se pohybuje od 58 až do 65 % (Rumbos et al. 2018). Naopak hodnoty proteinu a tuku pro menšího z potemníků *A. diaperinus* nebyly již v době pokusu výrazně odlišné od zahraniční literatury (Bosch et al. 2014; Van Broekhoven et al. 2015).

Všechny druhy hmyzu, u kterých v rámci této práce byla stanovena nutriční hodnota, snad s výjimkou velkého a tučného potemníka brazilského, lze považovat za dobrý zdroj bílkovin, které obyčejně vycházejí z měření celkového dusíku a jeho vynásobení indexem 6,25 (bílkoviny obsahují

16 % dusíku). V případě hmyzu je ovšem část dusíku vázána v chitinu, který je pro většinu monogastrů nestravitelný. Proto se Janssen et al. (2017) pokusili navrhnout nové, druhově specifické indexy, které by měly zpřesnit výpočet (např. 4,75 pro moučné červy), což by celkovou hodnotu bílkovin ponížilo o více než 10 %. Proto jsme v publikacích Kulma et al. (2016b, 2019) stanovili množství dusíku, vázaného v chitinu a tento pak odečetli od celkového množství proteinu. Takto stanovená hodnota proteinu vázaného v chitinu se pohybovala v rozmezí od 2 do 4 %. Z pohledu toho, že nutriční hodnota je lehce ovlivnitelná mnoha faktory, navrhujeme tuto metodu jako vhodnější a přesnější alternativu k výše uvedeným indexům, které se z pohledu našich výsledků zdají být naopak výrazně podhodnocené a paradoxně více odchýlené od skutečnosti než původní faktor 6,25.

6 Závěry

Předložená disertační práce představuje soubor zveřejněných studií zaměřených na tři témata, která spolu úzce souvisí, a kterým se na začátku 21. století v souvislosti s hmyzem dostává největší pozornosti. Pokles biodiverzity v kontextu globálních změn krajiny je potvrzeným fenoménem, jenž by v budoucnosti mohl ohrozit fungování celého světového ekosystému. Je proto potřebné přijmout určitá opatření, která povedou k odvrácení tohoto trendu. Prvním takovým opatřením je ochrana stanovišť hmyzu. Je akutně zapotřebí definovat a rozpracovat nejvhodnější managementy lokalit nutných pro přežití bezobratlých, v případě disertace byli motýli použiti jakožto modelové druhy. Následně je potřeba v praxi ověřovat, že aplikace zvolené údržby stanovišť má na velikost populace motýlů požadovaný vliv. Prokázali jsme, že vliv se nemusí projevit v krátkodobém časovém horizontu. V rámci studie jsme také zjistili, že motýlí druhy s krátkou dobou života jsou náchylnější k vyhynutí, zatímco délka letového období tento faktor nijak neovlivňuje, přestože se výrazně liší. Dalším atributem, který musí přispívat ke stabilizaci biodiverzity, je včasná detekce invazních druhů a jejich regulace. Z hlediska šíření invazních druhů tak bylo v rámci doktorského studia či disertace zdokumentováno šíření několika invazních druhů hmyzu na území České republiky. V neposlední řadě je nutné přistupovat ke všem přírodním zdrojům jako k alternativně využitelným v budoucnosti a v rámci principu předběžné opatrnosti o nich shromažďovat co nejúplnější informace. Vhodným příkladem je hledání alternativních zdrojů potravy a krmiva. V poslední části disertace se tak potvrdilo, že i hmyz, běžně dostupný v České republice může být z nutričního pohledu považován za alternativní zdroj živin, srovnatelný s konvenčními potravinami či krmivy pro zvířata. Bylo také potvrzeno, že se obsah živin mění v závislosti na pohlaví, vývojovém stádiu a předkládaném krmivu. Tyto poznatky jsou klíčové k vytvoření optimální technologie chovu hmyzu, která by mohla být alternativou k současným zdrojům a která by mohla zajistit do budoucna dostatečné potravinové zabezpečení lidské populace bez následků v podobě ztráty biologicky cenných habitatů.

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