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Ekologie opaskovců: variabilita druhové skladby, habitatové preference a odpovědi na gradienty prostředí máloštětinatců a pijavic

Habilitační práce

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Poděkování

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Obsah

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1. Struktura a cíle habilitační práce

Předkládaná habilitační práce se skládá z 12 vědeckých prací opublikovaných v ISI časopisech a jednoho rukopisu přijatého do ISI časopisu. Všechny jsou zaměřeny na studium ekologie dvou největších skupin opaskovců (Clitellata): máloštětinatých opaskovců „Oligochaeta“ a pijavic Hirudinida. Jde o významné zástupce bentické fauny obývající v našich podmínkách bahnité i kamenité substráty všech vodních ekosystémů od pramenišť, toků, rybníků, jezer a nádrží až po hyporeické vody. Široký rozsah citlivosti ke znečištění předurčuje tuto skupinu k využití jak v terénních výzkumech, tak v laboratorních pokusech. Výsledky studií máloštětinatých opaskovců a pijavic jsou v předkládané práci souhrnně představeny a doplněny současným fylogenetickým postavením studovaných skupin a úvodem k jejich biologii. Práce se zabývají odpovědí opaskovců na gradienty prostředí a pro konkrétní druhy vyhodnocují jejich ekologické valence. Rozšíření znalostí o této skupině přispívá k jejich základnímu poznání a posouvá hranice možností bioindikace stavu sladkovodních ekosystémů na základě jejich ekologických preferencí a umožňuje i lépe chránit druhy vzácné.

Hlavní cíle habilitační práce:

1. Popsat diverzitu opaskovců na území České republiky a sestavit aktuální check-listy pro máloštětinaté opaskovce a pijavice.
2. Popsat variabilitu druhové skladby máloštětinatců a pijavic v různých akvatických biotopech, především v tekoucích vodách.
3. Najít hlavní gradienty prostředí, které ovlivňují tuto variabilitu a vyhodnotit indikační potenciál skupiny.
4. Zjistit odpověď společenstva máloštětinatců na antropogenní ovlivnění lokalit
5. Vyhodnotit habitatové preference vybraných druhů máloštětinatců a pijavic

2. Shrnující komentář

2.1 Úvod do problematiky

Nejznámější skupiny opaskovců – máloštětinatci a pijavice nepatří mezi bezobratlými živočichy ke skupinám oblíbeným laickou veřejností, snad vyjma rybářů. Žížaly mohou vzbuzovat nepříjemné pocity, neboť vylučují na povrchu sliz a pijavic se lidé podvědomě bojí, neboť jim evoluční zkušenost říká, že by je mohly napadnout a přenášet choroby. Přesto představují fascinující skupiny kroužkovců (Annelida), které osídlily jak akvatické (sladkovodní a mořské), tak suchozemské biotopy a dosáhly zde vysoké diverzity.

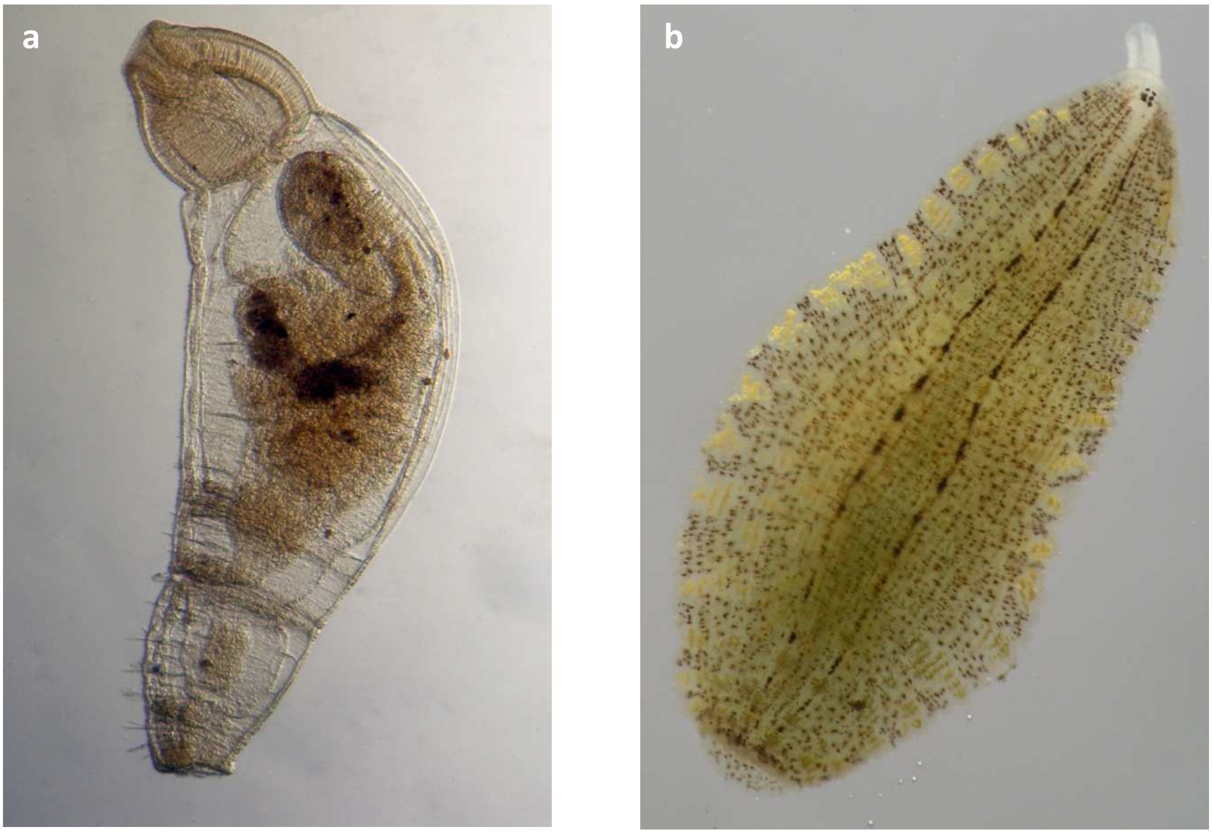
Skupina opaskovci (Clitellata) představuje druhově bohatý taxon, zahrnující téměř třetinu z 15000 druhů kroužkovců (Annelida) (Erséus 2005). Název opaskovci je odvozen od žláznaté epidermální struktury ve tvaru kruhu nebo sedla kolem těla nazývané opasek (clitellum), který se vytváří v době rozmnožování a napomáhá přenosu pohlavních buněk a později tvorbě kokonů. Jeho přítomnost je jedním z morfologických znaků potvrzujících monofylii tohoto taxonu kroužkovců (Ferraguti & Erséus 1999, Purschke 1999, Westheide *et al.* 1999), prokázanou i na základě sekvencí DNA (McHugh 1997, Siddall *et al.* 2001 a Erséus & Källersjö 2004). Pozice opaskovců uvnitř kroužkovců (Annelida) zůstává stále vysoce neurčitá, pravděpodobně leží někde mezi „mnohoštětinatci“ a stále není zcela jasná ani její sesterská skupina (Jördens *et al.* 2004, Rousset *et al.* 2006). Také fylogenetické vztahy uvnitř opaskovců jsou složitější, než by ukazovalo tradiční dělení opaskovců (Clitellata) na základě morfologických znaků na Oligochaeta a Hirudinea, které se dále dělily na pravé pijavice (Euhirudinea) a na pijavicím podobné („leech-like“) taxony štětinovky (Acanthobdellida) a potočnice (Branchiobdellida) (Sawyer 1986, Brusca & Brusca 1990, Rouse & Fauchald 1995). V současnosti, přesto že každá další molekulární analýza přináší odlišnosti v detailech, nastala zásadní shoda ve zjištění, že Hirudinida (pravé pijavice), Branchiobdellida (komenzálové nebo parazité raků) a Acanthobdellida (skupina představovaná jediným reliktním druhem *Acanthobdella peledina* Grube, 1851 parazitujícím na lososovitých rybách) představují samostatnou větev opaskovců, jejímž společným předkem byl máloštětinatý červ blízce příbuzný žížalicovitým Lumbriculidae (Martin 2001, Erséus 2005, Marotta *et al.* 2008). Složitost situace dokresluje, že existuje podpora i pro monofyletický taxon zařazující Lumbriculidae dovnitř tohoto taxonu společně s Acanthobdellida, Branchiobdellida a Hirudinida (Rousset *et al.* 2008). Nicméně máloštětinatci jako validní taxon definitivně zanikají a pro tuto parafyletickou část opaskovců

Clitellata se zavádí označení máloštětinatí opaskovci – „oligochaetous Clitellata“ či „Oligochaeta“ s použitím uvozovek.

Studium evoluce habitatových preferencí skupiny Clitellata ukázalo, že společný předek obýval původně akvatické bitopy (Erséus & Källersjö 2004), přesněji řečeno sladkovodní biotopy (Rousset *et al.* 2008). Zástupci čeledi Naididae (sensu Erséus *et al.* 2008) s asi 800 popsanými druhy obývali nejprve sladké vody a sekundárně se pak rozšířili také do brakických a mořských. I o monofylii této skupiny dnes zahrnující podčeledi Naidinae, Pristininae, Tubificinae, Rhyacodrilinae (a Telmatodrilinae, Phallodrilinae a Limnodriloidinae, které se u nás nevyskytují) ovšem existují určité pochybnosti (Rousset *et al.* 2008). Část z nich pak opustila typický život v sedimentech dna a naučila se obývat jejich povrch a povrch vodních rostlin (podčeledi Naidinae a Pristininae). K takovému životnímu stylu jim napomáhá častá přítomnost očních skvrn a schopnost plavat. Někteří z nich (rody *Dero* a *Ripistes*) si navíc staví na povrchu substrátu tenké trubičky, kterými se chrání. Ve stojatých vodách s množstvím drobných bezobratlých se pak některé druhy z podčeledi Naidinae uchýlily k získávání potravy predací, jako např. rod *Chaetogaster* (Obr. 1a).

Další skupina dnes především terestrických máloštětinatců skupiny Crassiclitellata zahrnující 3000 validních druhů, u nás zastoupených čeledí žížalovití Lumbricidae, měla pravděpodobně naopak předka terestrického. Jejich trávicí soustava je přizpůsobena ke zpracování organického materiálu pohlceného současně s částicemi půdy přítomností baktérií, hub a prvoků, které umožňují trávení celulózy a rozklad organické hmoty. Podle ekologie je můžeme rozdělit na epigeické (žijící na povrchu půdy a v listovém opadu), endogeické (žijí v půdě v horizontálních trubičkách) a anektické (žijící ve vertikálních trubičkách, do kterých si zatahují listy, které zpracovávají) (Bouché 1977). Druhá dnes převážně půdní skupina roupicovití Enchytraeidae čítající asi 650 druhů pak pochází z vodního prostředí a k osídlení půdy došlo až sekundární invazí (Rousset *et al.* 2008). Dnes obývají většinou svrchní vrstvu půdy, i když i mezi nimi najdeme sladkovodní (např. rod *Cognettia*) i mořské zástupce (např. někteří zástupci rodů *Marionina* a *Lumbricillus*).

Pijavicím podobné skupiny (Acanthobdellida, Branchiobdellida a Hirudinida), ať už s čeledí Lumbriculidae nebo bez ní, dnes obývají sladkovodní biotopy stejně jako jejich předek (či předkové) (Rousset *et al.* 2008). Také v čeledi žížalicovití (Lumbriculidae) se objevily druhy, které osídlily rostlinné habitaty místo sedimentů dna (např. *Lumbriculus variegatus* (Müller, 1774) a i u nich se pak vyvinula schopnost plavat. Pijavicím podobné skupiny se naopak přizpůsobily karnivornímu způsobu života. Vznikla u nich zadní přísavka, sloužící k přichycení k substrátu či kořisti, došlo k redukci štětin, vytvořil se svalnatý hltan a různý počet očních skvrn na přídě (předním konci těla), které umožňují orientaci (Obr. 1b).



Obrázek 1. Příklad volně žijícího máloštětinatého opaskovce *Chaetogaster diastrophus* (Gruithuisen, 1828) (a), velikost 3 mm (preparát v Kanadském balzámu) a volně žijící pijavice *Glossiphonia complanata* (Linnaeus, 1758) (b), velikost 15 mm (živý jedinec). Foto J. Schenková

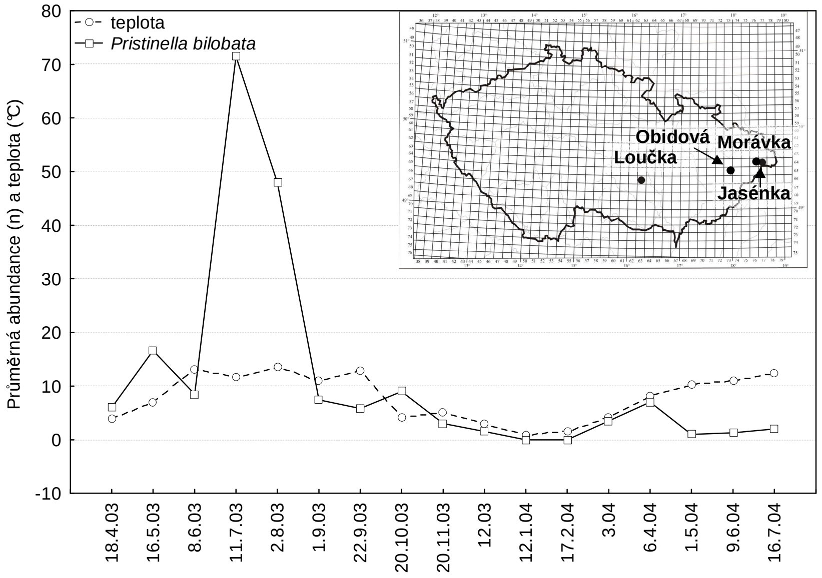
2.2 Biologie sladkovodních opaskovců (Clitellata)

Přesto, že některé znaky skupiny Clitellata jako přítomnost opasku, nepřítomnost parapodií, omezení počtu gonád jen na několik článků a přímý vývoj juvenilních jedinců po uvolnění z kokonů jsou pro celou skupinu shodné, celková biologie dvou hlavních skupin – máloštětinatců a pijavic je natolik rozdílná, že si každá zaslouží samostatné pojednání.

2.2.1. **Biologie máloštětinatých opaskovců („Oligochaeta“)**

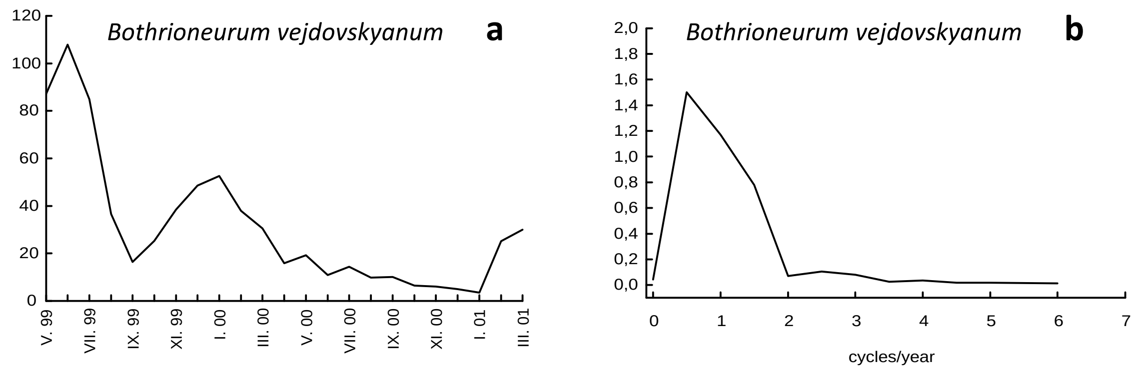
Stavba těla máloštětinatých opaskovců skupiny „Oligochaeta“ se podobá typickému tělnímu plánu kroužkovců s homonomní segmentací těla. Odlišuje se jen první článek (peristomium) s ústním otvorem a čelním lalokem (prostomium) na předním konci těla, který se také nazývaná příďa, a pygidium (poslední článek tvořený jedním nebo srůstem více článků) na jeho zadním konci. Počet tělních článků je variabilní od 7 po asi 200, pro jednotlivé druhy je pak relativně stálý. Každý tělní článek kromě prvního je opatřen primárně 4 svazky chitinózních štětin, které napomáhají při pohybu. Máloštětinatci se vrtají v substrátu či pohybují po jeho povrchu peristaltickými kontrakcemi podkožní svaloviny s oporou o štětiny. Některé druhy mohou plavat (např. z rodu *Stylaria* a *Pristina*) hadovitým pohybem těla. „Oligochaeta“ se živí primárně jako neselektivní detritofágové, zpracovávají detrit spolu s bakteriemi a organickými zbytky, anorganické částice pak nestrávené odchází z těla análním otvorem. Ovšem mýtus, že máloštětinatci se živí jakoukoli potravou, se kterou se setkají, byl vyvrácen laboratorními pokusy na druhu *Tubifex tubifex* (Müller, 1774), který si dokáže selektivně vybírat potravu s částicemi menšími než 25 µm a z bahnitého substrátu vyžírá ten, který obsahuje nejvíce organické hmoty (Rodriguez *et al.* 2001). Některé druhy se živí na uhynulých tělech ryb (zástupci čeledi Enchytraeidae). Drobní máloštětinatci z podčeledí Naidinae a Pristininae žijící na rostlinách se živí detritem a nárostovými řasami, zejména rozsivkami. Pomocí svaloviny hltanu vysouvaného z dutiny ústní přichytávají podtlakem potravu a jejím zatažením zpět ji odtrhují od substrátu (Sperber 1948), takto dokážou do trávicího traktu vtáhnout i vláknitou řasu. Velmi drobní zástupci rodu *Chaetogaster* se živí jako predátoři, široce roztaženou přídou vychytávají z vody prvoky a drobné bezobratlé živočichy – vířníky, korýše a larvy hmyzu, v jejich trávicím traktu se nachází i zástupci „své skupiny“ tedy drobní máloštětinatci (Obr. 1a, v tělní dutině je vidět kořist, máloštětinatý opaskovec z podčeledi Naidinae). V České republice velmi vzácný druh *Ripistes parasita* (Schmidt, 1847) se živí jako pasivní filtrátor, který vychytává potravu z proudu vody pomocí svazku dlouhých a hustých štětin v VI.–VIII. segmentu. V pravidelných intervalech pak otáčí přídu k zadnímu konci těla a protaženým prostomiem vybírá nachytané částice potravy. Vzácně se vyvinul i parazitický způsob života: druh *Chaetogaster limnaei* Baer, 1827 žije na povrchu ulit a v plášťové dutině plovatek, kde se živí nejen drobnými prvoky a bezobratlými, ale může ožírat i epitel plášťové dutiny hostitele.

Skupina máloštětinatí opaskovci („Oligochaeta“) vyvinula velmi úspěšné reprodukční strategie. U podčeledí Naidinae a Pristininae probíhá nepohlavní rozmnožování formou tzv. paratomie, kde mateřský jedinec na zadním konci tvoří řetízky dceřiných jedinců, tzv. zoidů, kteří ještě před oddělením mají plně vyvinuté prostomium, včetně např. chobotku (proboscis). Místa budoucího dělení jsou pevně dána a typická pro každý druh. Toto rozmnožování probíhá především v jarním období. U poměrně vzácného druhu *Pristina bilobata* (Bretscher, 1903), který žije spíše v podhorských oblastech, se pak asexuální reprodukce posouvá do letního období (Obr. 2) a k sexuálnímu nemusí docházet vůbec, jak bylo pozorováno na řece Morávce (Schenková & Kroča 2007). Paratomické rozmnožování se může střídat s pohlavní reprodukcí, většinou před zimním obdobím.



Obrázek 2. Sezonní změny abundance u druhu *Pristina bilobata*, reprodukujícího se paratomií. Mapa jeho nálezů v České republice. Více viz Schenková & Kroča (2007), odkud byl obrázek převzat a upraven; do mapy byly doplněny dva současné nálezy.

Druhým typem asexuální reprodukce je architomické dělení, kdy nejprve dochází k fragmentaci dospělého jedince a teprve pak k diferenciaci koncových částí těla. Vyskytuje se např. u podčeledi Tubificinae, u rodu *Aulodrilus*, podčeledi Rhyacodrilinae, u druhu *Bothrioneurum vejdovskyanum* Štolc, 1886 a čeledi Lumbriculidae, kde je typické pro druh *Lumbriculus variegatus*. Nepohlavní architomické rozmnožování probíhá opět nejčastěji v jarním a na začátku letního období. Např. u druhu *B. vejdovskyanum* se mateřský jedinec s asi 140 segmenty rozpadá v průměru na šest částí v období od poloviny dubna a rozmnožování probíhá po celé letní období (Hrabě 1935). Podobně i v případové studii na řece Rokytné byl zaznamenán vývojový cyklus se zvyšováním denzity v průběhu května a



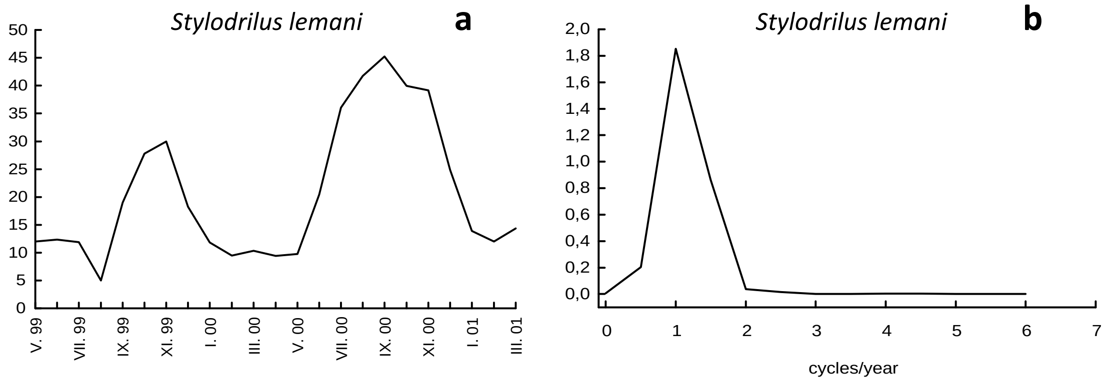
Obrázek 3. Analýza časové řady průměrných denzit (a) a peridiogram, tj. graf počtu cyklů za rok (b) architomicky se množícího druhu *Bothrioneurum vejdovskyanum*. Blíže viz Schenková *et al.* 2006, odkud byly obrázky převzaty.

maximem v červnu, ovšem jen v prvním roce pozorování (Obr. 3a), dvouleté období pozorování bylo pravděpodobně nedostatečné (Schenková *et al.* 2006). Pohlavně dospělí jedinci se vyskytovali jen zcela sporadicky; celkově byl u tohoto druhu vyhodnocen jednoletý vývojový cyklus (Obr. 3b) (Schenková *et al.* 2006). Architomické rozmnožování se opakuje pravidelně a nebylo prokázáno, že by jej ovlivňovaly některé z parametrů prostředí jako teplota vody, průtok, rozpuštěný kyslík nebo množství organické hmoty v toku (Schenková *et al.* 2006).

U některých druhů se také vyvinulo partenogenetické rozmnožování, kdy juvenilní jedinci vznikají z neoplodněných vajíček, např. u čeledi Lumbricidae (Sims & Gerard 1999).

Nejvýznamnější způsob reprodukce máloštětinatých opaskovců ovšem představuje rozmnožování pohlavní. Zástupci této skupiny jsou hermafrodité s přímým vývojem. Kopulace probíhá spojením dvou jedinců proti sobě v opačném směru, tj. přída jednoho ke kaudální části druhého. Opasky obou jedinců jsou přiloženy k sobě a sliz, který produkují, umožňuje přenos pohlavních buněk. Vývod chámovodu jednoho jedince ústí do chámových schránek (receptaculum seminis) druhého jedince (a naopak). Jedinci si předávají shluky spermií (spermatozeugma) nebo spermatofory (shluky spermií s obalovou vrstvou). Přenos je u některých druhů usnadněn přítomností různě dlouhých penisů, které mohou být vyztuženy peniálními trubkami, jejichž tvar je druhově specifický a představuje dobrý determinační znak. Obdobně štětiny, které mohou být v oblasti opasku u některých druhů upravené jako spermatekální, jsou často lopatkovitě zakončené a slouží k přenosu spermatozeugmat při kopulaci. K oplodnění vajíček dochází v hlenovém pouzdře, produkovaném buňkami opasku, do něhož se vajíčka uvolňují a posunují směrem k přednímu konci jedince, při průchodu kolem vývodu chámových schránek jsou oplodněna. Hlenové pouzdro s oplodněnými vajíčky se svléká přes přídu jedince a vytváří kokon, jehož tvar, struktura a počet vajíček uvnitř je specifický pro jednotlivé druhy/rody máloštětinatých opaskovců.

V současnosti je problematika pohlavního rozmnožování mnoha druhů zejména z podčeledi Tubificinae, které se vyskytují ve znečištěných vodách, velmi dobře prostudována, např. *Tubifex tubifex*, *Limnodrilus hoffmeisteri* Claparède, 1862 a *L. udekemianus* Claparède, 1862, (Ladle 1971, Bonomi 1979, 1980, Pfannkuche 1981, Lazim & Learner 1986, Lazim *et al.* 1989). Také další běžné druhy z této podčeledi jsou z hlediska sezonní dynamiky dobře probádané: *Potamothrix hammoniensis* (Michaelsen, 1901) (Thorhauge 1976, Risnoveanu & Vadineanu 2002), *Psammoryctides barbatus* (Grube, 1861) and *Spirosperma ferox* Eisen, 1879 (Adreani *et al.* 1984). Podčeledi Naidinae a Prisitininae jsou dobře zpracovány např. v rozsáhlé studii (Learner *et al.* 1978). Méně známé jsou reprodukční cykly u druhů, které se vyskytují buď vzácně, nebo je jejich determinace spojena s určitými obtížemi. U čeledi Lumbriculidae, kde je dobře prostudovaná sezonní dynamika u druhů *Stylodrilus heringianus* Claparède, 1862 a *S. parvus* (Hrabě-Černosvitov, 1927) (Dumnicka & Kukuła 1990, Petto & Humpesch 1992) najdeme i druhy z tohoto hlediska velmi málo prostudované, jako např. *Stylodrilus lemani* (Grube, 1879). Tento druh se množí pohlavně; v případové studii na řece Rokytné probíhal vývoj v jednoletém cyklu, kdy v jarním období vzrůstající teplota stimulovala vývoj pohlavních orgánů a následně kopulaci jedinců, v období srpna až listopadu narůstal počet juvenilních jedinců (Obr. 4) (Schenková *et al.* 2006). Byla nalezena pozitivní korelace abundance tohoto druhu s teplotou vody s dvou měsíčním posunem (lagem) a rozdílné habitatové preference juvenilních jedinců (příbřežní partie) a dospělců (proudnice toku) (Schenková *et al.* 2006).



Obrázek 4. Analýza časové řady průměrných denzit (a) a peridiogram, tj. graf počtu cyklů za rok (b) pohlavně se množícího druhu *Stylodrilus lemani*. Blíže viz Schenková *et al.* 2006, odkud byly obrázky převzaty.

2.2.2 **Biologie pijavic (Hirudinida)**

Tělo pijavic je dorzoventrálně zploštělé, výrazně uniformní, tvořené vždy 34 články, na předním konci je opatřeno menší přísavkou s ústním otvorem, na zadním konci větší přísavkou, vzniklou splynutím sedmi článků, sloužící k pohybu a přichycení k podkladu. Stavba těla pijavic odráží jejich dravý či parazitický způsob života. Měkké tělo máloštětinatých opaskovců, jehož oporou je hlavně hydrostatická kostra, je u této specializované skupiny zpevněno mohutným svalovým vakem, který spolu s parenchymatickou tkání zatlačil tělní dutinu. Pijavice ztratily přepážky mezi segmenty, štětiny a segmentovanou célomovou dutinu se změnou způsobu pohybu od peristaltického vrtání v substrátu k pohybu lezením a plaváním. Mohutná svalovina slouží ke kontrakci těla při pohybu po podkladu, který se děje píďalkovitým pohybem pomocí obou přísavek, a při plavání, které probíhá dorzoventrální undulací. Na rozdíl od máloštětinatých opaskovců, kde jsou oční skvrny přítomny jen u podčeledi Naidinae a nemají podstatný vliv na nacházení potravy, pijavice mají vždy na přídě několik párů očí sloužících k vyhledávání kořisti či hostitele.

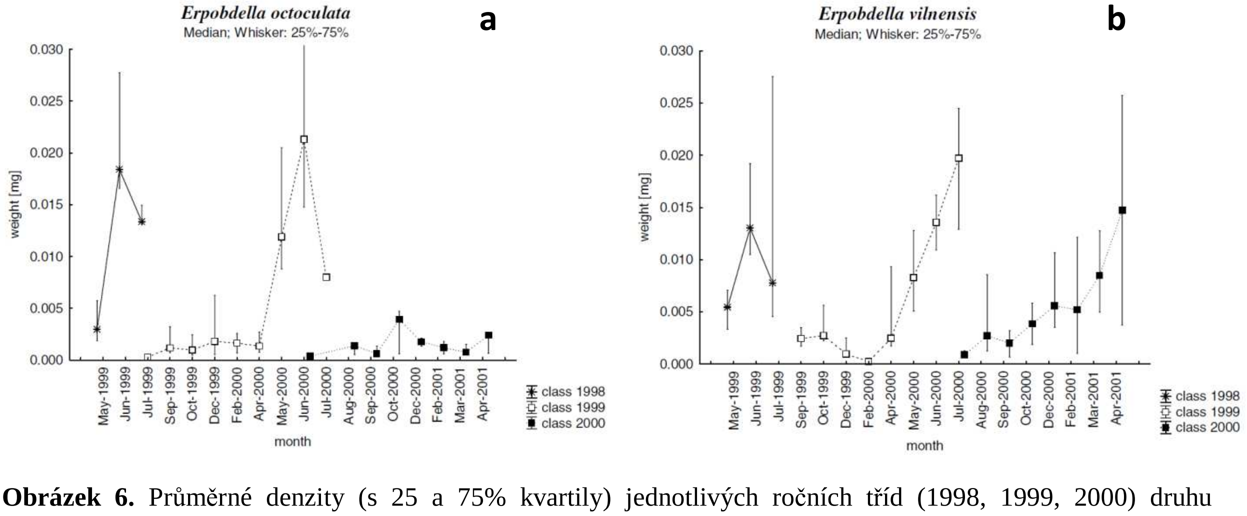
Pijavice se živí jako krevsající (hematofágní) parazité a predátoři. Ty, které se živí krví, mají buď kutikulární čelisti se zoubky, jako např. pijavka lékařská (*Hirudo medicinalis* Linnaeus, 1758) (Obr. 5) z řádu čelistnatky (Gnathobdellida) nebo sají krev z hostitele pomocí chobotku jako zástupci řádu chobotnatky (Rhynchobdellida). *H. medicinalis*, která se v České republice řadí ke kriticky ohroženým druhům (Schenková & Košel 2005), se živí krví obratlovců. Sání krve umožňuje pijavkám přítomnost protisrážlivého enzymu hirudinu. Drobné juvenilní pijavky nejsou schopny prokousnout kůži savců, proto se živí na obojživelnících (Sawyer 1986). Dospělci sají krev obojživelníků, ptáků, případně ryb, ale dominantním zdrojem potravy je savčí krev, kterou potřebují jakožto energeticky nejbohatší, aby mohly dosáhnout pohlavní dospělosti (Mann 1955). Pijavky se zdržují na hostiteli jen v době sání, kdy se jejich hmotnost zvýší až 9x, protože bez další potravy mohou vydržet až jeden rok (Davies & McLoughlin 1996). Z čeledi Piscicolidae se parazitické druhy náležících do rodů *Piscicola* a *Caspiobdella* živí krví ryb, kterou sají pomocí vysouvatelného chobotku. Zatímco zástupci rodu *Piscicola* tráví celý život přichycení na hostiteli, zástupci rodu *Caspiobdella* žijí na dně tekoucích vod a rybího hostitele navštěvují jen kvůli sání. V čeledi Glossiphoniidae se parazitické druhy živí ektoparaziticky sáním krve ryb – *Hemiclepsis marginata* (O. F. Müller, 1774) nebo ptáků – *Theromyzon tessulatum* (O. F. Müller, 1774), který parazituje v nosní dutině kachen.

Obrázek 5. *Hirudo medicinalis*, adultní a juvenilní jedinec, Foto J. Schenková.

Ostatní zástupci pijavic naší fauny se živí jako predátoři bezobratlých. *Haemopis sanguisuga* (Linnaeus, 1758) z řádu Gnathobdellida se živí vodními bezobratlými živočichy, ale pomocí kutikulárních čelistí je schopna ulovit i rybí plůdek (osobní pozorování).

Neparazitičtí zástupci čeledi Glossiphoniidae se živí likvidosomatofágní predací (sají tělní tekutiny a měkké části těl) především měkkýšů (Mollusca). Zástupci řádu hltanovky (Pharyngobdellida), u nás jen z čeledi Erpobdellidae se živí makrofágní predací, kořist polykají celou, což jim umožňuje svalnatý hltan opatřený třemi lištami k drcení potravy. Jejich kořistí jsou především zástupci máloštětinatých opaskovců („Oligochaeta“) a pakomárovitých (Chironomidae), přičemž velikost kořisti je přímo úměrná velikosti predátora (Schenková *et al.* 2007). Selektivní výběr potravy pro zástupce čel. Glossiphoniidae (Mollusca) ani pro zástupce Erpobdellidae („Oligochaeta“ a Chironomidae) však nebyl potvrzen na velké škále (studie na 109 lokalitách); na lokalitách s vyšším zastoupením měkkýšů, máloštětinatců a pakomárovitých nebyly signifikantně více zastoupeny pijavice, které se jimi živí (Kubová *et al.* 2013).

Reprodukce pijavic probíhá výhradně pohlavně, kopulací, a nejen, že se nejsou schopny množit asexuálně, ale ani regenerovat poškozené články, což je u kroužkovců netypické. Pijavice jsou protandričtí (varlata se vyvíjejí dříve než vaječníky) hermafrodité, při kopulaci nalepují poblíž samičích pohlavních otvorů spermatofory. Vajíčka jsou odkládána v kokonech a u čeledi Glossiphoniiidae byla zaznamenána péče o potomstvo (kokony v tenkém obalu a následně juvenilní jedinci jsou nošeni na ventrální straně mateřského jedince). Většina pijavic má jedno až dvouletý vývojový cyklus začínající kopulací v jarním či letním období, líhnutím juvenilů během léta a dospíváním během dalšího roku (Ruppert *et al.* 2004). Reprodukční strategie koexistujících druhů pak může být využita i k dělení nik. Například sympatricky žijící druhy *Erpobdella octoculata* (Linnaeus, 1758) a *Erpobdella vilnensis* (Liskiewicz,



Obrázek 6. Průměrné denzity (s 25 a 75% kvartily) jednotlivých ročních tříd (1998, 1999, 2000) druhu *Erpobdella octoculata* (a) a *Erpobdlella vilnensis* (b). Blíže viz Schenková *et al.* 2007, odkud byly obrázky převzaty.

1925) snižují vzájemný boj o potravu rozdílnou rychlostí vývojových cyklů (Obr. 6), kdy ačkoli se líhnou z kokonů téměř ve stejnou dobu, pomalejší nárůst váhy u *E. octoculata* umožní rozdělení potravních zdrojů, jak bylo zjištěno v případové studii na řece Rokytné (Schenková *et al.* 2007).

2.3 Druhová diverzita opaskovců a její změny podél gradientů prostředí

2.3.1 Druhová diverzita opaskovců (Clitellata)

2.3.1.1 Máloštětinatí opaskovci („Oligochaeta“) na území České republiky

Pro území České republiky je v aktuálním check-listu uvedeno 95 vodních, případně semiakvatických, druhů máloštětinatých opaskovců (Schenková *et al.* 2010). Check-list byl sestaven z publikovaných dat, především významných monografií Hraběte (1954, 1981), publikovaných nálezů druhů (Schenková 2005, Schenková & Kroča 2007) a pro čeleď žížalovití (Lumbricidae) byly zařazeny vodní a semiakvatické druhy z monografie Pižl (2002). V neposlední řadě pak zahrnuje současné nálezy všech spoluautorů. V České republice žije 12 druhů z čeledi žížalicovitých (Lumbriculidae), 72 druhů z čeledi Naididae sensu Erséus *et al.* (2008), které zahrnují 36 druhů z podčeledi naidkovití (Naidinae), osm druhů z podčeledi Pristininae, 22 druhů z podčeledi nitěnkovití (Tubificinae), šest druhů z podčeledi Rhyacodrilinae, po jednom druhu z čeledí Propappidae, kryodrilovití (Criodrilidae) a pastrunovcovití (Haplotaxidae) a osm vodních/semiakvatických druhů žížalovitých (Lumbricidae). Přesto, že je již známo, že někteří zástupci čeledi roupicovitých (Enchytraeidae) žijí spíše akvatickým způsobem života (např. rod *Cognettia*), nebyla tato čeleď vzhledem k obtížné dostupnosti ekologických údajů o druzích a celkově odlišné metodice determinace do check-listu vodních máloštětinatců České republiky zařazena.

Většina máloštětinatých opaskovců představuje široce rozšířené euryvalentní druhy schopné vyrovnat se s různými podmínkami prostředí. Jde především o zástupce podčeledi Tubificinae, často až extrémně odolné k organickému znečištění. Do této kategorie náleží z naší fauny 44 druhů (Schenková *et al.* 2010). Řadíme sem i některé nepůvodní druhy jako *Potamothrix moldaviensis* Vejdovský & Mrázek, 1903 a *Potamothrix bavaricus* (Oschman, 1913) (Drake 2009). Ovšem vzhledem k jejich historickým nálezům, *P. moldaviensis* zaznamenal Hrabě na začátku 20. století ve Vltavě a *P. bavaricus* ve 30. letech v Labi (Hrabě

1939), by tyto druhy mohly být i na našem území původní. Třicet osm druhů naší fauny se jeví jako druhy vzácné (Schenková *et al.* 2010), neznamená to ovšem, že všechny jsou ohrožené, protože na svých specifických habitatech mohou dosahovat vysokých abundancí, jako např. *Stylodrilus lemani*, který dominuje v několika eutrofizovaných tocích jižní Moravy, např. v řece Rokytné (Schenková & Helešic 2006), jinde se s ním ale nesetkáme. Naopak nepůvodní teplomilný obyvatel velkých řek *Branchiura sowerbyi* Beddard, 1892, se zvolna šíří Evropou pravděpodobně s lodní dopravou (Lišková 1964, Gruszka 1999), avšak od prvního nálezu (Lišková 1964) byly získány pouze čtyři další. Třináct druhů historicky náležejících do fauny České republiky bohužel nebylo od roku 1996, kdy se autoři check-listu začali věnovat máloštětinatým opaskovcům, vůbec zaznamenáno (Schenková *et al.* 2010). Jde o druhy specifických habitatů, které nespadají do běžných výzkumů, jako např. jeskynní druh *Stylodrilus absoloni* (Hrabě, 1970), druh podzemních vod *Trichodrilus pragensis* Vejdovský, 1876 nebo parazit plžů (plovatek) *Chaetogaster limnaei*. Zaměření českých hydrobiologů na tekoucí vody, především jejich monitoring pak také snižuje pravděpodobnost nalezení některých vzácnějších druhů typických pro stojaté či dokonce vysychající vodní habitaty. Takto nám zmizel například specialista na temporální tůně *Lamprodilus mrazeki* Hrabě, 1929 a některé druhy stojatých vod, jako *Dero dorsalis* Ferronière, 1899, *Dero nivea* Aiyer, 1929, *Dero obtusa* Udekem, 1855 a *Chaetogaster setosus* Světlov, 1925. Jiné druhy zaznamenané jen jedenkrát jsou pravděpodobně nesmírně vzácné nebo vyhynulé: *Pristina amphibiotica* Lastočkin, 1927, *Haber speciosus* (Hrabě, 1931), *Peipsidrilus pusillus* Timm, 1977, *Potamothrix bedoti* (Piguet, 1913) a *Tubifex nerthus* Michaelsen, 1908.

Tabulka 1. Seznam druhů máloštětinatých opaskovců navržených pro Červený seznam. Blíže viz Schenková *et al.* 2010, odkud byla tabulka převzata a upravena. Jednotlivé kategorie dle (IUCN 1994).

ohrožený druh (EN)		**zranitelný druh (VU)**	
Arcteonais lomondi	(Martin, 1907)	*Amphichaeta leydigi*	Tauber, 1879
Criodrilus lacuum	Hoffmeister, 1845	*Aporrectodea limicola*	(Michaelsen, 1890)
Haber speciosus	(Hrabě, 1931)	*Pristina aequiseta*	Bourne, 1891
Lamprodrilus mrazeki	Hrabě, 1929	*Pristina bilobata*	(Bretscher, 1903)
Peipsidrilus pusillus	Timm, 1977	*Pristina jenkinae*	(Stephenson, 1931)
Piguetiella blanci	(Piguet, 1906)	*Pristina longiseta*	Ehrenberg, 1828
Stylodrilus absoloni	(Hrabě, 1970)	*Pristina osborni*	(Walton, 1906)
Trichodrilus pragensis	Vejdovský, 1876	*Nais behningi*	Michaelsen, 1923
Trichodrilus strandi	Hrabě, 1936	*Nais christinae*	Kasprzak, 1973
		Nais stolci	Hrabě, 1981
		Rhyacodrilus subterraneus	Hrabě, 1963
		Specaria josinae	(Vejdovský, 1884)
téměř ohrožený druh (NT)		*Stylodrilus lemani*	(Grube, 1879)
Apporrectodea georgii	(Michaelsen, 1890)	*Uncinais uncinata*	(Ørsted, 1842)

Přesto, že se jedná o skupinu vodních bezobratlých, u nichž je nesmírně obtížné zjistit, z jakých příčin je její výskyt ojedinělý, některé druhy zasluhují ochranu. Tu je u máloštětinatých opaskovců možné realizovat pouze pomocí ochrany jejich stanovišť, proto byly některé druhy byly navrženy pro zařazení do Červeného seznamu, aby došlo k ochraně biotopů, na nichž se vyskytují. Dosud publikovaný seznam chráněných máloštětinatých opaskovců (Schenková 2005, Pižl 2005) byl modifikován a rozšířen (Tab. 1) (Schenková *et al.* 2010), ale brzká doba jistě přinese další změny.

2.3.1.2 Pijavice (Hirudinida) na území České republiky

Z taxonu pijavice (Hirudinida) je pro území České republiky v současnosti uváděno 24 druhů (Schenková *et al.* 2009). Data pro check-list byla získána z historických údajů, především z prací Hrabě 1954, Koubková & Vojtková 1973, Lucký & Dyk 1964 a Košel 1998, 1999a, dále z údajů z projektů monitoringu tekoucích vod a vlastních nálezů všech autorů. Nejbohatěji je v České republice zastoupena čeleď Glossiphoniidae, zahrnující 10 druhů převážně ze stojatých vod, většina z nich jsou predátoři, dva druhy jsou ektoparazité. Čeleď Erpobdellidae je druhá nejpočetnější se sedmi druhy, kteří jsou predátoři tekoucích i stojatých vod. V čeledi Piscicolidae, kde jsou všichni zástupci ektoparazité, je známo pět druhů, v čeledi Haemopidae jeden druh (predátor) a Hirudinidae jeden parazitický druh.

Území České republiky je zajímavé tím, že jeho středoevropská poloha zahrnuje povodí tří významných evropských toků – Labe, Dunaje a Odry, po nichž se některé pijavice mohou šířit napříč Evropou. Z hlediska síly populací a původnosti pro naše území můžeme pijavice žijící u nás rozdělit do tří skupin: druhy původní se stabilními populacemi, druhy původní, jejichž populace jsou slabé a roztroušené a invazní druhy, nově se šířící našem území (Schenková *et al.* 2009).

Do první skupiny řadíme evropské palearktické druhy, které historicky osidlují nejrůznější vodní tělesa na našem území s vysokou frekvencí i abundancemi – *Alboglossiphonia heteroclita* (Linnaeus, 1761), *Glossiphonia complanata*, *Helobdella stagnalis* (Linnaeus, 1758), *Hemiclepsis marginata*, *Theromyzon tessulatum*, *Piscicola geometra* (Linnaeus, 1758), *Haemopis sanguisuga*, *Erpobdella octoculata* a *E. vilnensis*. Tři další druhy, považované dříve za vzácné – *Glossiphonia concolor* (Apáthy, 1888), *G. nebulosa* Kalbe, 1964 a *Erpobdella nigricollis* (Brandes, 1900), byly pak do této skupiny přiřazeny nově vzhledem k vysoké četnosti současných nálezů. U prvních dvou druhů mohlo v minulosti docházet k záměně s *G. complanata*. Celkem je u nás 12 druhů původních pijavic se stálými populacemi (Schenková *et al.* 2009).

Druhá skupina, do které řadíme druhy se vzácným výskytem, nezahrnuje jen skutečně ohrožené druhy, ale i druhy, které jsou vzhledem k malé velikosti a nenápadnému vzhledu častěji přehlíženy, jako *Alboglossiphonia hyalina* (O. F. Müller, 1774) a *Alboglossiphonia striata* (Apáthy, 1888), které jsou velmi drobné (v prvním případě dokonce průsvitné) a obývají stojaté vody. Navíc jako druhy byly poměrně nedávno odděleny od druhu *A. heteroclita* (Trontelj 1997, Neubert & Nesemann 1999). K nejzáhadnějším druhům naší fauny patří *Glossiphonia slovaca* (Košel, 1973), zranitelný druh (VU) dle Schenková & Košel (2005), který byl nalezen pouze na území jižní Moravy v řece Dyji (Košel 1995, 1999b) a později již nebyl zaznamenán. Ovšem odlišení tohoto druhu od ostatních zástupců rodu pouze na základě počtu varlat a tvaru pohlavních vývodů činí determinaci velmi náročnou a přesto, že velmi podobná pijavice byla z jediné známé lokality výskytu opět nalezena (leg. L. Mikl, 2015, det. J. Schenková), nebylo možné ji spolehlivě určit. Další zajímavý druh pro naši faunu je *Dina lineata* (O. F. Müller, 1774), která žije sporadicky v drobných stojatých vodách, prameništích a potocích v nížinách. Tyto biotopy jsou citlivé k různým zásahům a malý počet nálezů vedl k návrhu zařadit tento druh mezi zranitelné VU (Schenková *et al.* 2009). Naprosto unikátní je také situace kolem našeho největšího druhu *Trochaeta cylindrica* Örley, 1886, jehož lokality jsou jak v tůních jižní Moravy, tak v prameništích a potocích v Beskydech, takže je i vzhledem ke zjištěným morfologickým rozdílům velká pravděpodobnost, že se zde mohou ukrývat druhy dva (Bílková & Schenková 2015).

Nejvíce pozornosti ovšem přitahuje kriticky ohrožený druh CR *Hirudo medicinalis* (Schenková & Košel 2005), neboť se jedná o krevsajícího ektoparazita s možností lékařského využití, který měl rozsáhlý areál výskytu v povodí řeky Moravy i Labe (Hrabě 1936). Díky přítomnosti protisrážlivého enzymu hirudinu a řady dalších enzymů, které vypouští do rány při sání krve, byl v historických dobách využíván k „pouštění žilou“, dnes prožívá jeho příbuzný laboratorně chovaný druh *Hirudo verbana* Carena, 1820 renesanci a je používán v mikrochirurgii, k léčbě onemocnění lymfatického systému a řadě dalších chorob. Typickým biotopem *H. medicinalis* jsou mělké rozsáhlé mokřady v zátopovém území řek pravidelně zaplavované při jarních povodních, bohatě zarostlé makrovegetací, hojně osídlené obojživelníky (zdroj potravy pro juvenilní jedince) a neosídlené žádnou nebo jen minimální rybí obsádkou (Glombová & Schenková 2015). Takové biotopy jsou už dnes vzhledem k regulaci říční soustavy a intenzivnímu zemědělskému využití krajiny v povodí velkých řek velmi vzácné a většina z 18 současných lokalit výskytu (15 lokalit uvedeno v Glombová & Schenková 2015, nově v roce 2015 Lesní tůň u Lanžhota, ústní sdělení J. Glombová a tůně Lesní herda a Háček u Nejdku, ústní sdělení E. Lorencová) leží v oblastech, které jsou do různé míry chráněné.

Dalšími vzácně nacházenými druhy jsou rybí parazité *Piscicola respirans* Troschel, 1850 a *Piscicola fasciata* Kollar, 1842 (jediný záznam Lucký & Dyk 1964) žijící trvale přichycené na rybím hostiteli, což znemožňuje jejich nalezení během hydrobiologických výzkumů. Také jediný nález druhu *Erpobdella testacea* (Savigny, 1822) svědčí o jeho velké vzácnosti (Košel 1999b). Opět se jedná o druh obývající u nás ohrožené biotopy, jako bažiny v litorální zóně stojatých vod a velkých řek, který je navíc snadno zaměnitelný s *E. nigricollis*.

Poslední skupina zahrnuje druhy, které se u nás úspěšně šíří. Pro rybí ektoparazity jako je druh ponto-kaspického původu *Caspiobdella fadejewi* (Epshtein, 1961), který se k nám rozšiřuje z řeky Dunaje, je rychlé šíření umožněno na těle hostitelských ryb. Od prvního výskytu v řece Moravě na soutoku s Dyjí (Košel 1998, 1999a) v devadesátých letech do dneška osídlil celé území České republiky, a dostal se do povodí řek Moravy, Odry a Labe. Z dalších druhů, jejichž počet nálezů postupně narůstá, lze uvést i druh *Piscola haranti* Jarry, 1960, který byl zaznamenán nejprve na třech (Schenková *et al.* 2009), následně na dalších dvou lokalitách (Kubová & Schenková 2014). Vzdálenost jednotlivých lokalit a jejich příslušnost k různým povodím (Labe, Morava) vedou ovšem i k alternativní hypotéze, že spíš než pomalé šíření tohoto parazitického druhu do České republiky se jedná o jeho stálý vzácný výskyt, který dříve nebyl zaznamenán vzhledem k jeho relativně nedávnému popisu a jeho snadné záměně s *Piscicola geometra*. Velmi řídké jsou také nálezy dalšího šířícího se druhu *Dina punctata* Johansson, 1927. Šíří se k nám z povodí Dunaje, kde je běžný, a takto se dostal proti proudu Dyje až do Vranova. Jeho další nálezy v jižních Čechách v povodí Vltavy jsou však těžko interpretovatelné. Vzhledem k tomu, že jde o druh semiakvatický, lze spekulovat o přenosu vodním ptactvem.

Současný výzkum ukazuje, že oblast největší diverzity této skupiny se nachází na jižní Moravě, především v oblasti soutoku řeky Moravy a Dyje. Většina ohrožených druhů pijavic obývá nížinné mokřady v povodí velkých řek zarostlých vegetací a právě tyto jsou nejohroženější negativními dopady lidské činnosti. Stejně jako u jiných bezobratlých živočichů je péče o zachování přirozených habitatů a jejich zákonná ochrana jediným účinným mechanismem k zachování vzácných druhů. Proto si habitaty hostící silné populace

Tabulka 2. Seznam druhů pijavic navržených pro Červený seznam. Blíže viz Schenková *et al.* 2009, odkud byla data převzata a upravena. Jednotlivé kategorie dle (IUCN 1994).

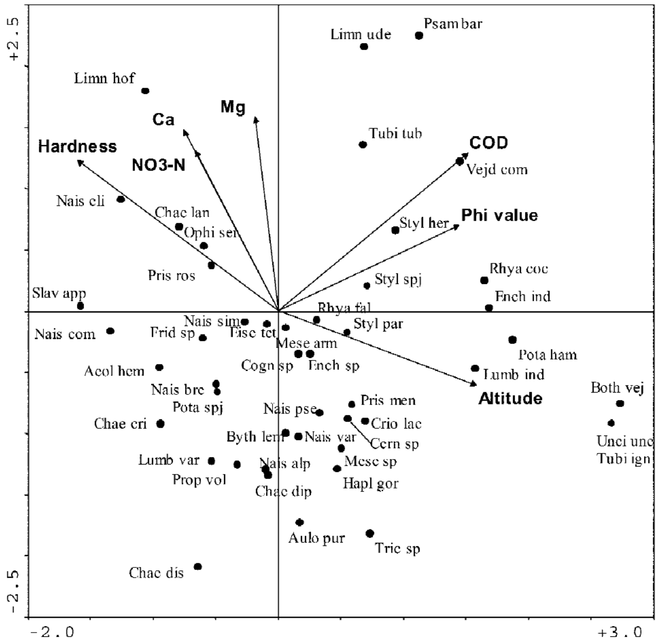
kriticky ohrožený druh (CR)		**zranitelný druh (VU)**	
Hirudo medicinalis	Linnaeus, 1758	*Alboglossiphonia hyalina*	(O. F. Müller, 1774)
		Dina lineata	(O.F. Müller, 1774)
ohrožený druh (EN)		*Glossiphonia slovaca*	(Košel, 1973)
Alboglossiphonia striata	(Apáthy, 1888)	*Piscicola respirans*	Troschel, 1850
Erpobdella testacea	(Savigny, 1822)	*Trocheta cylindrica*	Örley, 1886

vzácných druhů, např. *H. medicinalis*, zasluhují přísnou a dobře promyšlenou ochranu. Na základě těchto zjištění byl proto seznam ohrožených druhů z roku 2005 (Schenková & Košel 2005) rozšířen o další druhy, takže určitá úroveň ochrany náleží celkem deseti druhům a potažmo i jejich biotopům (Tab. 2, Schenková *et al.* 2009).

2.3.2 Variabilita druhové skladby máloštětinatých opaskovců v tocích

Máloštětinaté opaskovce nacházíme téměř ve všech akvatických biotopech, od pramenišť po veletoky, stojaté i podzemní vody nevyjímaje, od čistých oligotrofních až po vysoce eutrofizované. Tato skupina má vzhledem ke svému širokému zastoupení, vysokým denzitám a velkému rozsahu tolerance ke znečištění u některých druhů, významný potenciál z hlediska hodnocení kvality vod a jejich managementu, zejména vod tekoucích. Zatímco v minulosti se zájem soustředil na hodnocení různých biotických indexů (např. Sládeček 1973, Chapman *et al.* 1982, Milbrink 1983, Uzunov *et al.* 1988) se zaměřením především na vody znečištěné, dnes se studium rozšiřuje o poznání všech faktorů, které podmiňují ekologii této skupiny, o zjištění geografické distribuce, habitatových preferencí a požadavků na kvalitu vody nejen u běžných, ale i vzácných a ojediněle se vyskytujících druhů.

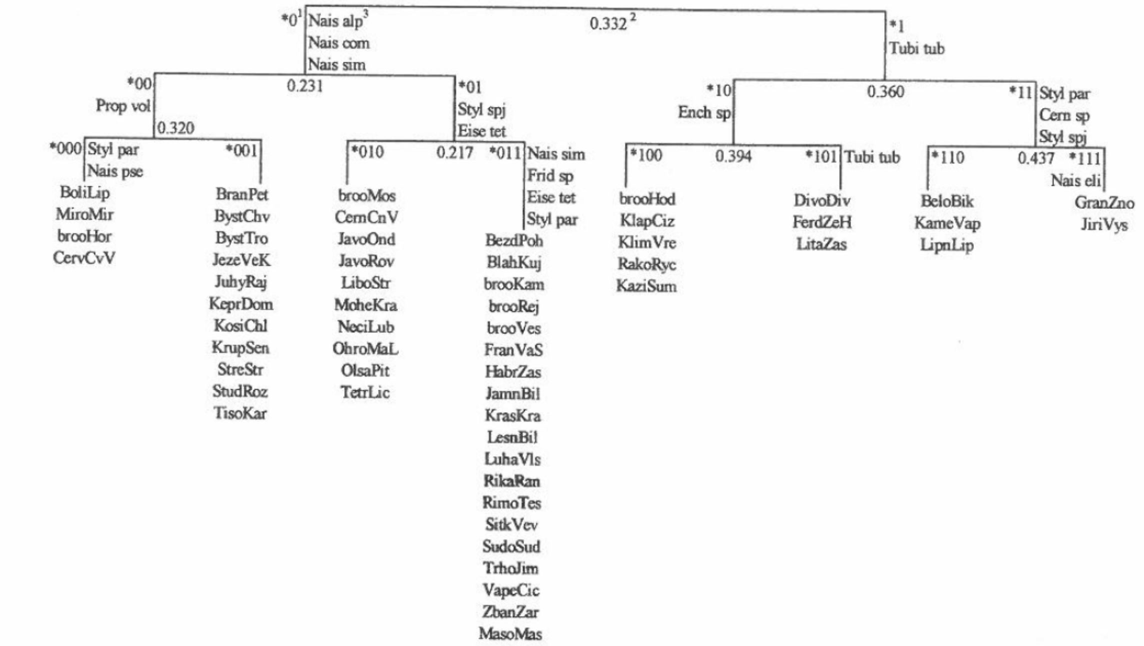
Z hlediska významu pro člověka a zastoupení na našem území jsou středně velké tekoucí vody převažujícím biotopem, proto byla jejich studiu věnována největší pozornost. Nicméně v evropském měřítku nejsou studie máloštětinatých opaskovců tekoucích vod na velké škále (tedy z mnoha toků pokrývající větší geografické území) příliš frekventované (Martínez-Ansemil & Collado 1996, Nijboer *et al.* 2004 a Verdonschot 2006). Na území České republiky byl výzkum zaměřen na menší, pokud možno neznečištěné toky z povodí řek Moravy a Odry (Schenková & Komárek 1999, Schenková *et al.* 2001a, Schenková *et al.* 2001b). Hlavním gradientem strukturujícím taxocen máloštětinatých opaskovců v tomto typu toků v České republice je gradient nadmořské výšky úzce svázaný s charakterem substrátu: skladba taxocenu máloštětinatých opaskovců se mění spolu se snižující se drsností dna (přechod od kamenitého k bahnitému substrátu), které doprovází klesající nadmořskou výšku (Schenková *et al.* 2001a). Druhý gradient, který strukturuje skladbu a abundance máloštětinatých opaskovců, má komplexní charakter, neboť je představován koncentrací vápenatých a hořečnatých iontů, tedy chemismem a současně gradientem množství organické hmoty vyjádřené chemickou spotřebou kyslíku COD (Obr. 7, Schenková *et al.* 2001a). Velmi podobné výsledky s nejvýznamnějším gradientem přestavovaným substrátem a rychlostí proudění, následovaným gradientem mineralizace vody pro máloštětinaté opaskovce potvrzuje i práce z oblasti Pyrenejského poloostrova (Martínez-Ansemil & Collado 1996). Parametry vázané na řád toku jako např. průtok jsou rozhodující proměnné pro strukturu taxocenu máloštětinatí opaskovci, i v rovinatých oblastech Holandska, kde také reprezentují nejvýznamnější gradient, charakterizovaný hloubkou a šířkou toku, jeho stálostí (stálý/intermitentní); druhý gradient z hlediska vysvětlené variability představuje pH a další chemické proměnné (Nijboer *et al.* 2004). Proměnná průtok, která u českých řek nebyla měřena, byla zastoupena drsností dna a nadmořskou výškou, které klesají směrem od pramene a byly prokázány jako signifikantní, význam zaznamenané hloubky a šířky toku prokázán nebyl (Schenková *et al.* 2001a).



Obrázek 7. Ordinační diagram kanonické korespondenční analýzy (CCA) první a druhé ordinační osy s pozicí všech druhů a proměnných prostředí vybraných v postupném výběru testovaným Monte Carlo permutačním testem na hladině významnosti P = 0,01 (999 permutací). Zkratky názvů druhů a další detaily viz Schenková *et al.* 2001a, odkud byl obrázek převzat.

Získaná databáze výskytu máloštětinatých opaskovců na 57 téměř neznečištěných tocích spolu s fyzikálně-chemickými parametry byla využita i k vyhodnocení kvality vody a nalezení indikátorových druhů, které ji v malých tocích charakterizují. Na základě analýzy hierarchické divizivní klasifikace TWINSPAN (Hill 1979) byly studované lokality rozděleny do dvou velkých skupin zahrnující lokality zcela neznečištěných toků s indikátorovými druhy

Nais alpina Sperber, 1948, *N. communis* Piguet, 1906 a *N. simplex* Piguet, 1906 a toky se sníženou kvalitou vody, indikované druhem *Tubifex tubifex*. Skupinu čistých vod indikují druhy spásající řasové nárosty a obývající kamenitá dna čistých toků, toky s více organickou hmotou dobře indikoval nejodolnější euryvalentní druh *T. tubifex* (Obr. 8, Schenková *et al.* 2001b). Vodní máloštětinatí opaskovci pak mohou indikovat celkem čtyři skupiny čistých toků (z leva): čisté meandrující se štěrkovým dnem (*Stylodrilus parvus*, *Nais pseudoobtusa* Piguet, 1906), rychle tekoucí toky s vyšším sklonem (bez indikátoru), toky se štěrkopísčitým dnem (bez indikátoru) a chladné pomalu tekoucí toky v oblasti Hercynika (*Nais simplex*, *Eiseniella tetraedra* (Savigny, 1826), *Stylodrilus parvus*). A dále čtyři skupiny s různou mírou znečištění: zemědělsky ovlivněné toky (bez indikátoru), toky na flyšovém podloží s přirozeně vyšší konduktivitou (*Tubifex tubifex*), toky s vyšším obsahem organických látek COD (bez indikátoru) a toky s nejnižší kvalitou vody v rámci studovaného souboru (*Nais elinguis* Müller, 1774).



Obrázek 8. Divizivní hierarchická klasifikace lokalit TWINSPAN s indikátorovými druhy. Zkratky lokalit a druhů a další detaily viz Schenková *et al.* 2001b, odkud byl obrázek převzat.

Indikace malých čistých až mírně znečištěných toků pomocí máloštětinatých opaskovců je možná, přičemž úspěšnost klasifikace toků stoupá s mírou jejich znečištění. Avšak indikátorové druhy jsou často běžné a je známo, že stejné druhy jako např. *Spirosperma ferox*, *Stylodrilus heringianus* i *Tubifex tubifex* mohou charakterizovat jak oligotrofní podmínky, tak eutrofizaci např. jezer, záleží jen na jejich denzitách (Milbrink & Timm 2002).

2.3.3 Variabilita druhové skladby máloštětinatých opaskovců na prameništních slatiništích

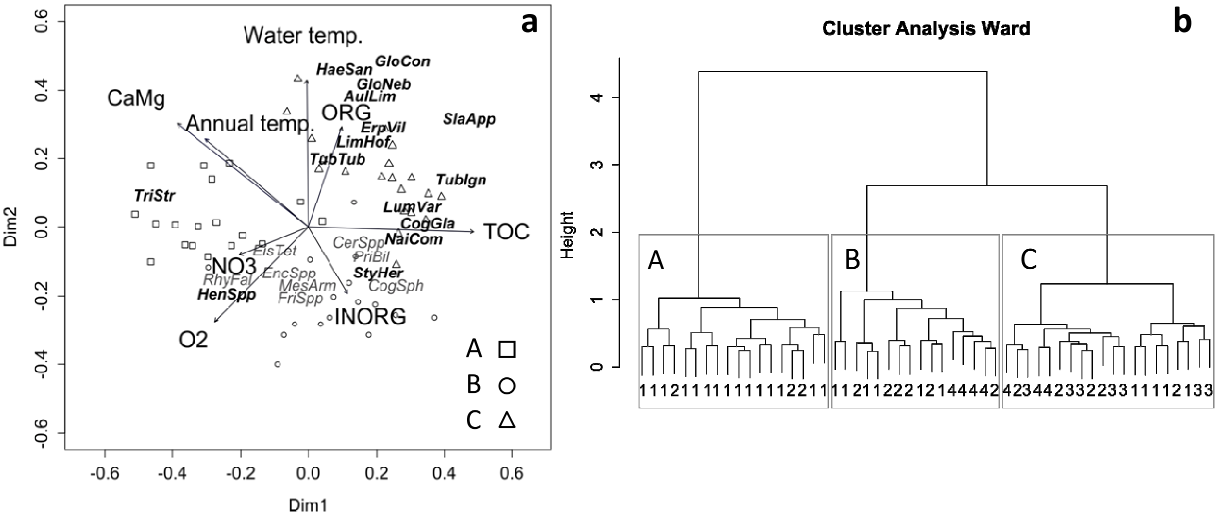
Dalším biotopem tekoucích vod, jehož studiu jsme se věnovali, byla prameništní slatiniště. Jedná se o jeden z nejohroženějších biotopů v Evropě, k jehož vymizení přispívají především antropogenní vlivy, jako je odvodňování, eutrofizace a změny managementu vedoucí k zarůstání a postupnému vysychání. Prameništní slatiniště představují roztroušené mokřady, které se nacházejí v bezlesých místech, jsou syceny podzemní vodu a hostí živinami limitovanou vegetaci. Většinou vyvěrají ve svažitém terénu, na našem území nejsou příliš rozsáhlá a vzájemně jsou izolovaná. Mnoho zachovalých prameništ se nachází v oblasti Západních Karpat, proto byly naše výzkumy rozšířeny na Slovensko, kde se většina tohoto pohoří nachází. Studované území zahrnovalo tři oblasti: Moravsko-slovenské pomezí na flyšovém podloží (charakteristické střídajícími se vrstvami pískovce a jílovce), oblast Oravy, která představuje východní výběžek flyšové zóny a oblast vnitřních Západních Karpat, jejíž geologická struktura je složitější, tvořená jádrem paleozoických a krystalinních břidlic překrytých mezozoickými pískovci a karbonátovými litofaciemi (odlišitelnými částmi vrstevního sledu sedimentů).

Vzhledem k různému podloží je chemismus prameništních slatinišť značně variabilní a vytváří tak gradient minerální bohatosti, který je pro ně typický, a byl popsán jako tzv. poor-rich gradient (Malmer 1986, Hájek *et al.* 2006). Na základě změny chemismu vyvěrající vody a typu vegetace pak bylo možné klasifikovat pět základní typů prameništních slatinišť: typ 1 – vápnitá pěnovcová, v nichž dochází ke srážení uhličitanu vápenatého ve formě pěnovce a dominuje mech *Cratoneuron commutatum*; typ 2 – vápnitá bez srážení pěnovce, kde se v chladnějších oblastech pěnovec již nesráží, na povrchu je rašelina, obsah živin je nízký, dominují ostřice, suchopýry, mechy, zejména *Drepanocladus revolvens* agg.; typ 3 – mírně vápnitá mechová, kde je koncentrace vápníku nižší a reakce vody není zásaditá nebo je příjem vápníku snížen velkým obsahem železa, což umožňuje uchycení kalcitolerantních rašeliníků (*Sphagnum warnstorfii*); typ 4 – kyselá přechodová rašeliniště, kde na prameništi je voda spíše kyselá a mohou se zde vyskytnout i další druhy rašeliníků; typ 5 – druhově chudá kyselá slatiniště s rašeliníky rodu *Sphagnum* (podrobněji viz Hájek *et al.* 2006).

Gradient minerální bohatosti, který významně strukturuje složení rostlinných společenstev, ovlivňuje i další skupiny organismů, jako druhovou skladbu a bohatost měkkýšů (Horsák & Hájek 2003), řas (Poulíčková *et al.* 2003, Fránková *et al.* 2009) nebo krytenek (Opravilová & Hájek 2006). Situace u hmyzu je složitější, zatímco některé studie ukazují jen slabý vztah hmyzích skupin (Virtanen *et al.* 2009) nebo pošvatek (Bojková & Helešic 2009) k chemismu vody v silně mineralizovaných prameništích, u pakomárovitých na celém poor-rich gradientu prokázán byl (Omelková *et al.* 2013). Tato situace nabízela dosud nestudovanou otázku, zda skupina máloštětinatých opaskovců, která náleží k permanentní fauně a žije v nejtěsnějším spojení se substrátem, je také ovlivňována měnícím se chemismem vody.

Odpověď opaskovců na hlavní gradienty prostředí v tomto částečně zjednodušeném systému (např. relativně malé kolísání průtoku a teploty vody) je však komplexnější. Studujeme-li lokality v oblasti moravsko-slovenského pomezí, kde se nachází všechny prameništní typy a jsou až na výjimky neovlivněné eutrofizací (17 lokalit), ukazuje se, že je taxocen Clitellata strukturován mírou mineralizace pramenící vody a charakterem substrátu (Bojková *et al.* 2011). Výměna druhů podél tohoto gradientu souvisí s měnícími se vlastnostmi („traits“) dominantních druhů: na minerálně bohatých slatiništích dominuje vzácný stygofilní (např. dle Dumnicka 2001) druh *Trichodrilus strandi*, který přímo preferuje slatiniště, kde dochází ke srážení pěnovce. S klesající minerální bohatostí nastupuje čeleď Enchytraeidae, která většinou zahrnuje půdní druhy schopné využívat i hlubších vrstev sedimentů a tolerovat jeho vysychání, ale i vodní druhy jako např. *Cognettia sphagnetorum* (Vejdovský, 1878) s širokou nikou, neboť se vyskytuje jak v minerotrofních rašeliništích (např. van Duinen *et al.* 2006), tak v jehličnatých lesích (např. Graefe & Schmelz 1999). Podobně vodní máloštětinatec *Rhyacodrilus falciformis* Bretscher, 1901 se kromě vodních habitatů může vyskytnout i na loukách a v listnatých lesích (Graefe & Schmelz 1999, Schlaghamerský & Kobetičová 2005). Minerálně chudá rašeliniště ležící na opačném konci gradientu jsou osídlena běžnými euryvalentními druhy, jako *Limnodrilus hoffmeisteri*, *Tubifex tubifex*, *Stylodrilus heringianus* a pijavicí *Erpobdella vilnensis*, která vyžaduje chladnější vodu, ale je velmi tolerantní k organickému znečištění (Kubová & Schenková 2014). Druhová bohatost opaskovců narůstá od minerálně bohatých směrem ke kyselým slatiništím, která obsahují větší množství nerozložené organické hmoty, tedy v opačném směru než u cévnatých rostlin, rozsivek nebo měkkýšů (Hájek *et al.* 2002, Horsák & Hájek 2003, Fránková *et al.* 2009). Ovšem odpověď opaskovců bude pravděpodobně spíše unimodální, neboť na ombrotrofních vrchovištích, která nebyla předmětem našeho zkoumání, nalézáme v nejkyselejších rašeliništích jen jediný acidotolerantní druh (van Duinen *et al.* 2006). Druhý nejvýznamnější gradient strukturující opaskovce představuje gradient hydrologického režimu spojený s produktivitou lokalit (více živin na jedné straně, více vlhka a nižší produktivita vegetace na straně druhé) (Bojková *et al.* 2011).

Jak ale reaguje tato skupina na minerálně trofický gradient na velké škále, zahrnující širokou geografickou oblast, s jednotlivými typy pramenišť v oblastech s různým podložím, různou mírou eutrofizace a různého stáří? Pro takovou studii bylo vybráno 54 lokalit v oblasti Moravsko-slovenského pomezí, Oravy a vnitřních Západních Karpat (Schenková *et al.*, accepted). Výběr lokalit byl podmíněn zjištěním, že opaskovci, zejména specialisté z této skupiny, jsou silně ovlivňováni omezenou možností disperze na nové lokality (Rádková *et al.* 2014a), která probíhá jen pasivním způsobem, a proto nový soubor zahrnoval více než polovinu lokalit starších než 2 500 let (Horsák *et al.* 2015), aby všichni potenciální obyvatelé daného typu prameniště měli šanci ho osídlit. Hlavní gradient, který strukturuje taxocen opaskovců na této škále, je opět gradient minerální bohatosti (analýza hlavních koordinát PCoA, Obr. 9a). Skladba opaskovců se mění od lokalit minerálně chudých, kyselých, s množstvím nerozložené organické hmoty (vysoké koncentrace celkového organického uhlíku – TOC), až po lokality s vysokými koncentracemi Ca a Mg a vysokou vodivostí vyvěrající vody. Nicméně shluková analýza skupiny opaskovců (Schenková *et al.*, accepted,



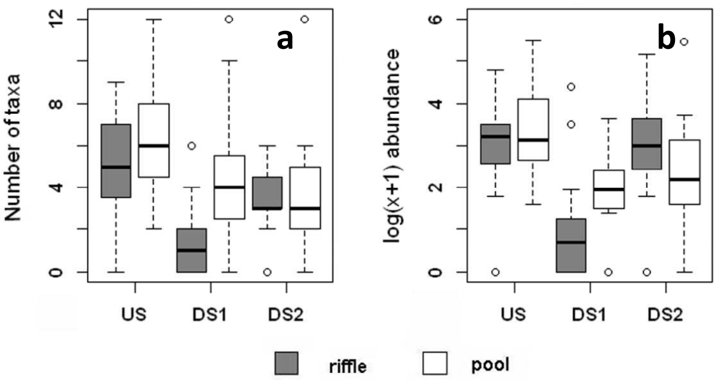
Obrázek 9. (a) Analýza hlavních koordinát PCoA 54 lokalit s osmi proměnnými prostředí pasivně proloženými do prostoru 1. a 2. osy, fit P < 0,05. Zobrazeno 15 indikátorových druhů pro tři skupiny shlukové analýzy a druhy frekventované (na 15ti lokalitách a více). Symboly lokalit podle shlukové analýzy: čtverce, skupina A; kolečka, skupina B; trojúhelníky, skupina C. (b) Shluková analýza Wardovou metodou na Bray-Curtis vzdálenostech. Čísla lokalit označují typ prameništního slatiniště na základě vegetace a chemismu (Hájek *et al.* 2006). Zkratky názvů druhů a další detaily viz Schenková *et al.*, accepted, odkud byly obrázky převzaty.

Obr. 9b) nám jednotlivé lokality uspořádala částečně odlišně od čtyř základních typů prameništních slatinišť (oproti původnímu dělení na 5 typů, zde jsme použili dva nejkyselejší sloučené v jednu skupinu) vytvořených na základě vegetace a chemismu (Hájek *et al.* 2006). Za tímto rozdílem stojí právě vliv substrátu ovlivňující druhovou skladbu podle přítomnosti organické hmoty nebo naopak převážně anorganického substrátu. Substrát se spolu s teplotou vody podílí na druhém nejvýznamnějším směru variability v datech. Tři skupiny opaskovců nalezené shlukovou analýzou můžeme popsat podle indikátorových druhů (Analýza indikátorových druhů, Dufréne & Legendre 1997): skupina A s indikátorovým druhem *T. strandi* zahrnující minerálně bohaté lokality většinou se srážením pěnovce (typ 1), skupina B s indikátorovým druhem *Stylodrilus heringianus*, vodním máloštětinatcem indikujícím kamenitý substrát a vysychavost toků (Nijboer *et al.* 2004) a skupina C, s velkým počtem euryvalentních indikátorových druhů (*Erpobdella vilnensis*, *Limnodrilus hoffmeisteri*, *Lumbriculus variegatus*, *Tubifex tubifex*, *Cognettia glandulosa* (Michaelsen, 1888), *Tubifex ignotus* (Štolc, 1886), zahrnující teplejší lokality s dostatkem organické hmoty (Schenková *et al.*, accepted). Skupiny B a C zahrnují směs všech typů pameništních slatinišť a skladba skupiny opaskovci zde odráží jen charakter substrátu, organický vs. anorganický, vlhký vs. vysychavý a teplotu, nikoli chemismus vody. Signifikantní vliv množství organického substrátu na skladbu opaskovců zcela nezávislý na chemismu potvrdili také Rádková *et al.* (2014b). Druhý gradient, který strukturuje společenstvo opaskovců, je podobně jako ve studii na malé škále (Bojková *et al.* 2011) představován hydrologickým režimem, avšak podstatnou měrou je navíc ovlivňován přítomností rozkládající se organické hmoty, která je zdrojem potravy studované skupiny a rozhoduje o jejich výskytu bez ohledu na typ prameništního slatiniště dle vegetace. Tak je možné přísunem organické hmoty, např. vlivem pastvy koní, nevratně přeměnit jedinečné pěnovcové slatiniště s dominantním vzácným druhem *T. strandi* na místo osídlené akvaristickými nitěnkami *T. tubifex* a *L. hoffmeisteri*, jejichž kvantita indikuje znečištění lokalit (Hrabě 1954).

2.3.4 Změny společenstva opaskovců v oteplenén toku

Výjimečné vlastnosti skupiny opaskovci (máloštětinatí i pijavice), která jak se potvrdilo, zahrnuje citlivé druhy indikující čistotu a neovlivněnost stanoviště i druhy, které ukazují na velkou míru dotace organické hmoty uváděné např. v Brinkhurst & Cook (1974), Uzunov *et al.* (1988), ale i v našich výzkumech (Schenková *et al.* 2001b, Bojková *et al.* 2011), vedly k možnosti studovat i ekosystémy lidskou činností silně pozměněné, jako jsou např. oteplené důlní vody. Jedinečnou příležitost ke studiu vlivu změny teplotního režimu představuje řeka Nedvědička protékající Českomoravskou vrchovinou, která je recipientem oteplených důlních vod z posledního uranového dolu ve střední Evropě v Rožné. Tato řeka je vhodným modelovým systémem, neboť teplá důlní voda nezvyšuje zásadním způsobem průtok a nemění tak hydrologické poměry a neobsahuje ani významně zvýšené množství radioaktivních látek. Nejen voda v řece Nedvědičce pod přítokem důlních vod s obsahem uranu $U_{\text{nat}} = 0{,}010$ mg l^{-1}, ale i sama důlní voda s hodnotou $U_{\text{nat}} = 3{,}4$ mg l^{-1} splňuje limity evropských norem a je pod hodnotou 24 mg l^{-1} vyžadovanou směrnicí „Directive 2008/105/EC of the European Parliament“ (Hudcová *et al.* 2012). Důlní voda se ovšem vyznačuje nejen zvýšenou teplotou, ale i vodivostí (vysoká koncentrace síranů), pH a sníženým obsahem kyslíku a živin (odstraněny v čističce odpadních vod), takže celý systém je více komplexní (podrobněji viz Růžičková *et al.* 2014).

Pro případovou studii byla vybrána tři odběrová místa na řece Nedvědičce: nad přítokem oteplené důlní vody (US), těsně pod přítokem (DS1) a níže po toku (DS2) a jako pozadí jedno odběrové místo v přítoku oteplené důlní vody (MW); na každém z těchto čtyř míst byly studovány dva habitaty: peřej a tůň (podrobněji v Růžičková *et al.* 2014). Silný dopad na společenstvo opaskovců byl potvrzen; jak druhová diverzita, tak abundance opaskovců pod přítokem oteplené důlní vody signifikantně poklesla v habitatech tůň i peřej (Obr. 10). Přesto, že nebylo možné odlišit vliv chemických proměnných od vlivu změny teploty, je vysoce pravděpodobné, že absence typického sezonního jevu, jako je zamrzání toku, také ovlivnila skladbu opaskovců. Jedna z nemnoha studií vlivu změny teplotního režimu zahrnující i skupinu Clitellata prokázala v industriální oblasti po oteplení vody jiný výsledek, a to nárůst abundance máloštětinatců, ovšem při signifikantně zvýšeném průtoku (Nedeau *et al.* 2003). Ochuzení společenstva opaskovců na řece Nedvědičce však mělo jiné příčiny. První je vliv zvýšené vodivosti, k níž jsou různé druhy do různé míry citlivé (Generlich & Giere 1996), např. výskyt druhu *Propappus volki* Michaelsen, 1916 je silně negativně korelován s vodivostí (Dumnicka & Pasternak 1978), druhý je vliv zvýšené teploty, neboť v řece Nedvědičce se za normálních okolností vyskytují i stenotermní druhy, např. *Haplotaxis*



Obrázek 10. Krabicové grafy druhové diverzity (a) a abundance (b) opaskovců na vzorkovacích místech na řece Nedvědičce: nad přítokem oteplené důlní vody (US), těsně pod přítokem (DS1) a níže po toku (DS2); hodnoceno odděleně pro peřej (tmavá) a tůň (bílá). Další detaily viz Růžičková *et al.* 2014, odkud byl obrázek převzat.

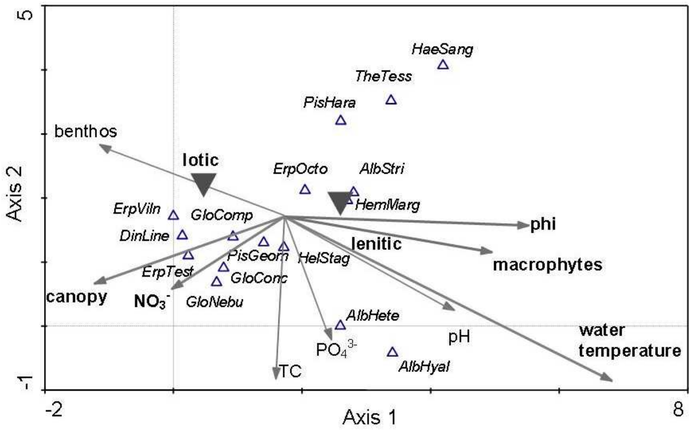
gordioides Hartmann, 1821 a *Stylodrilus heringianus* (Dumnicka 2000), které se nedokážou vyrovnat s oteplenou a v zimním období nezamrzající vodou. Tyto vlivy pak jsou různě intenzivní v prokysličených peřejích, kde pod přítokem důlních vod chybí šest taxonů, a v hlubších tůních, kde chybí tři. Peřeje jsou obývány rheofilními taxony, typickými pro horní toky s méně živinami a vyšší koncentrací rozpuštěného kyslíku (Schwank 1981) a citlivějšími na pokles kvality vody (Verdonschot 2006).

Změny v teplotním režimu vedou nejen k úbytku, ale i k výměně druhů (Živić *et al.* 2006), kdy právě na druhové úrovni je možné proniknout do podstaty změn v druhové diverzitě a změn v abundancích taxocenu Clitellata. Oligostenotermní druhy, mezi něž patří obyvatel podzemních vod a pramenišť *Haplotaxis gordioides* (Schwank 1981, Dumnicka 2000), se už pod přítokem oteplené vody neobjevují. Zástupci chladnomilného rodu *Stylodrilus* reagují poklesem početnosti a následným návratem do původního stavu vzhledem k jeho široké ekologické plasticitě (Syrovátka *et al.* 2009). Stejně tak reaguje *Lumbriculus variegatus*, ovšem z jiného důvodu – vzhledem ke své vazbě na nižší hodnoty pH (Nijboer *et al.* 2004) se přesouvá z místa přítoku důlní vody do oblasti s pro něj vhodnějším pH. Nenáročné druhy rodu *Limnodrilus* tolerující různé teploty, množství kyslíku i různou vodivost (např. Korn 1963, Dumnicka & Galas 2006), zůstávají v početnostech nad i pod přítokem stejné. Byl nalezen i druh *Aulodrilus japonicus*, který na oteplení vody reaguje nárůstem početnosti. Celkově lze shrnout, že i tato skupina permanentní fauny vodních bezobratlých je vstupem oteplených důlních vod do toku do různé míry ohrožena, přičemž pro některé druhy je tento vliv limitující.

2.3.5 Variabilita druhové skladby pijavic v tekoucích a stojatých vodách

Pijavice (Hirudinida) představují úžeji specializovanou skupinu parazitů a predátorů, kteří se odlišují nejen morfologicky, ale i celou svojí biologií, proto obvyklou metodikou pro studium makrozoobentosu, vhodnou pro vzorkování máloštětinatých opaskovců, zachytíme jen malou část jejich diverzity (Koperski 2011). K podrobnějšímu studiu ekologických parametrů stojících za strukturováním skupiny pijavic je zapotřebí použití odlišných vzorkovacích metod (Kubová & Schenková 2012) a její hodnocení samostatně. Pijavice obývají jak tekoucí, tak stojaté vody, přesto je většina prací věnována buď jednomu, nebo druhému habitatu (např. stojatým vodám se věnují Young & Ironmonger 1981, Spelling & Young 1987, tekoucím např. Grosser *et al.* 2001, Koperski 2005, 2010). Omezení existujících souhrnných prací z tekoucích i stojatých vod spočívá ve spíše popisném zaměření na distribuci druhů, charakteristiku preferovaných habitatů, případně vztah ke znečištění (např. Bennike 1943, Elliott & Mann 1979, Košel 1988, 1989, van Haaren 2004, Schenková *et al.* 2009) nebo naopak jen na jejich vztah k chemismu vody (Mann 1955, Grosser *et al.* 2001). Proto je velmi zajímavá otázka studia vlivu nabídky potravy i fyzikálně-chemických parametrů současně se zohledněním rozdílných podmínek v tekoucích a stojatých vodách.

První předpoklad zjištěný také již v pracích autorů Mann (1953) a Koperski (2006) o odlišnosti společenstev obývajících tekoucí a stojaté vody, se potvrdil. Nejsilnější gradient strukturující taxocen pijavic představuje rozdíl v typu habitatu: tekoucí versus stojaté vody. DCA analýza ukázala spolu s ním další významné morfologické a fyzikální proměnné, které tyto habitaty odlišují: tekoucí – vyšší zastínění, stojaté – nižší drsnot dna, větší množství makrofyt a vyšší teplota vody (Obr. 11, Kubová *et al.* 2013). Při porovnání variability vysvětlené jednotlivými proměnnými prostředí v přímých analýzách tří datových souborů – oba habitaty, jen tekoucí a jen stojaté vody – vykazují výsledky celkového souboru mnohem větší podobnost s habitatem tekoucích vod. Gradienty proměnných prostředí v tekoucích vodách jsou pro strukturování společenstva pijavic zahrnující oba habitaty rozhodující. Zcela novým zjištěním, dosud pouze odhadovaným bez statistických důkazů, je význam dostupnosti kořisti, který je prediktorem výskytu pijavic a to jak v tekoucích, tak ve stojatých vodách, tak v souboru zahrnujícím oba typy (Kubová *et al.* 2013). Dosud studované zástupné proměnné charakterizující úživnost ekosystému a vykazující signifikantní vliv na strukturu společenstva pijavic jsou např. alkalinita, vodivost, koncentrace fosforečnanů nebo biotické indexy charakterizující trofický stav (Bennike 1943, Mann 1955, Herrmann 1970, Koperski 2005, 2010). Přímý vliv těchto proměnných na společenstvo pijavic je silně nepravděpodobný, celková úživnost systému vede k vyšším abundancím makrozoobentosu, jímž se pak pijavice živí. Jiným neočekávaným výsledkem je význam zastínění pro výskyt pijavic, vysvětlující



Obrázek 11. Ordinační diagram DCA společenstva pijavic na 109 lokalitách v prostoru 1. a 2. ordinační osy. Všechny nalezené druhy kromě odlehlé hodnoty pro *Hirudo medicinalis* jsou znázorněny. V diagramu jsou pasivně proložené proměnné prostředí, které signifikantně korelovaly s 1. až 4. ordinační osou; signifikantní i po Bonferronniho korekci jsou tučně ($P < 0,0026$). Zkratky názvů druhů a proměnných prostředí a další detaily Kubová *et al.* (2013), odkud byl obrázek převzat.

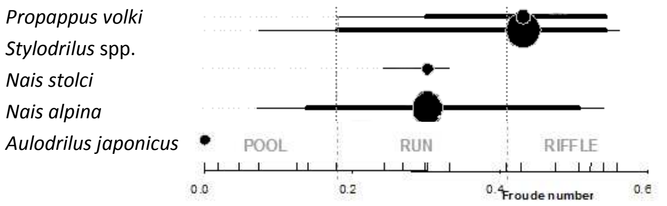
vysoký podíl variability jak v tekoucích tak stojatých vodách, jejich vliv je však protichůdný (Kubová *et al.* 2013). V tekoucích vodách narůstá druhová diverzita i abundance s vyšším zastíněním, neboť pijavice jsou zde lépe chráněné před fluktuacemi teploty, a množství kyslíku. Zastíněná místa představují pro chladnomilné druhy refugia (Aguiar *et al.* 2002, Ebersole *et al.* 2003). Naopak ve stojatých vodách – v rybnících a nádržích – s nižším zastíněním narůstá diverzita teplomilných druhů. Také přímo teplota vody a průměrná roční teplota vzduchu jsou pro pijavice významným parametrem. Druhová bohatost ve stojatých vodách vykazuje podél gradientu průměrné roční teploty unimodální průběh (Kubová *et al.* 2013), neboť ani nížinné lokality s vysokou zemědělskou produkcí na jednom konci gradientu ani chladné podhorské rybníky a nádrže na druhém konci nepodporují diverzitu teplomilných druhů žijících ve stojatých vodách, jak bylo obdobně pozorováno v britských jezerech (Young & Ironmonger 1981).

Zajímavé je srovnání výsledku z tekoucích a stojatých vod u nás, kdy mezi stojatými a tekoucími vodami nebyl nalezen rozdíl v abundancích (Kubová *et al.* 2013), avšak druhová diverzita byla vyšší v tekoucích vodách, s polskými lokalitami, kde nejvyšší diverzita byla v jezerech a nižší v řekách a rybnících (Koperski 2006). Relativně čistá jezera v Polsku, jaká se na našem území bohužel nenacházejí (vyjma horských, která jsou tuto skupinu příliš chladná), představují pro pijavice unikátní kombinaci ekologických parametrů s vysokými počty vodních bezobratlých (makrozoobentosu) jako kořisti při relativně nízké trofii.

2.4 Odpovědi vybraných druhů opaskovců na různé proměnné prostředí – malá a velká škála

Odpověď celého společenstva na různé gradienty prostředí je vždy souhrnem odpovědí jednotlivých druhů, jejich prezencí či absencí a změnami jejich abundancí. Zatímco na malé škále ve smyslu mezohabitatů (Armitage *et al.* 1995) se jedná především o hydraulické a substrátové preference, na velké škále jsou druhy vodních bezobratlých determinovány hydromorfologií toků, klimatickými podmínkami, chemismem a množstvím potravy (např. Hynes 1970, Cummins *et al.* 1984).

Na malé škále na úrovni habitatů podmiňuje výskyt máloštětinatých opaskovců charakter substrátu: velikost zrna podmiňující jeho drsnost, přítomnost makrofyt a přítomnost hydraulicky klidných míst umožňujících akumulaci organické hmoty, která tvoří jejich hlavní složku potravy (Verdonschot 2001, Křoupalová *et al.* 2011). Detailní studium prostorové distribuce jednotlivých druhů na úrovni mezohabitatů lze nejlépe studovat srovnáním různých mezohabitatů lišících se typem substrátu, hydraulicky i množstvím akumulované organické hmoty v rámci jednoho toku. Tento náročný přístup ovšem omezuje počet studovaných druhů na druhy dostatečně frekventované a vyskytující se ve vysokých abundancích. Takto byly ve studii věnované skupinám „Oligochaeta“ a Chironomidae na řece Svratce zjištěny hydraulické preference pro pět druhů máloštětinatých opaskovců a další druhy pakomárovitých (Obr. 12, Syrovátka *et al.* 2009, upraveno). Dva taxony preferují velmi úzké rozpětí hydraulických podmínek - *Aulodrilus japonicus* Yamaguchi, 1953 se vyskytuje pouze v tůních s nízkou hodnotou Froudova čísla (výpočet zahrnuje rychlost proudění, hloubku a gravitační zrychlení) a *Nais stolci* preferuje středně rychle proudící úseky bez turbulencí. Další druhy nevykazují tak úzké preference, *Nais alpina* se optimálně vyskytuje v úsecích se středním prouděním, ale v nízkých denzitách se vyskytuje i v tůních s nejnižší rychlostí proudění a peřejích s nejvyšší rychlostí. Rod *Stylodrilus* preferuje peřeje, ale obývá i středně proudivé úseky a druh *Propappus volki* silně preferuje peřeje, obývá i proudivé úseky, ale tůním se vyhýbá (Syrovátka *et al.* 2009). Oba tyto druhy jsou pro obývání peřejí dobře adaptovány, *Stylodrilus* si staví hlenové trubičky, které ochraňují při pohybu substrátu (Mrázek 1926), *P. volki*, který je známým obyvatelem pohyblivých písků a intersticiálních prostor se přilepuje k substrátu pomocí adhezivní látky (Bird 1982, Petto & Humpesch 1992).



Obrázek 12. Hydraulické preference pěti frekventovaných druhů máloštětinatých opaskovců. Kolečka znázorňují medián, silné linky horní a dolní kvartil, tenké 10% a 90% kvantily Froudova čísla váženého abundancí taxonu. Převzato a upraveno pouze pro „Oligochaeta“ ze Syrovátka *et al.* (2009), kde jsou uvedeny další detaily.

Stanovení habitatových preferencí pro jednotlivé druhy opaskovců na velké prostorové škále vyžaduje rozsáhlý datový soubor s vysokým počtem lokalit postihující celou škálu proměnných prostředí měřených na všech lokalitách, což velmi limituje počet takových studií v tekoucích vodách. Takto precizně byl stanoven vztah jednotlivých druhů k saprobitě (Sládeček & Košel 1984, Uzunov *et al.* 1988), tekoucím vs. tůňovým habitatům a minerálnímu substrátu vs. vegetaci (Martínez-Ansemil & Collado 1996), typu vodního tělesa (Koperski 2006), eutrofizaci, nadmořské výšce a substrátu (Verdonschot 2006) a některým chemickým proměnným (Mann 1955, Timm *et al.* 2001).

Bohatý datový soubor opaskovců (většinou máloštětinatých) z 54 prameništních slatinišť spolu s proměřením celkem 17 proměnných prostředí na každé lokalitě představoval možnost stanovit enviromnentální prediktory jejich výskytu. Po redukci počtu druhů na dvanáct dostatečně početných a proměnných prostředí na osm vzájemně nekorelovaných, vybraných na základě předchozích studií opaskovců (Martínez-Ansemil & Collado 1996, Timm *et al.* 2001, Verdonschot 2006, Bojková *et al.* 2011) byly spočítány zobecněné lineární modely (GLM, McCullagh & Nedler 1989). Tyto modely vyjadřující abundance vybraných druhů jako lineární funkci osmi proměnných prostředí byly spočítány s quasi-Poissonovým rozložením korigujícím overdisperzi dat (Schenková *et al.*, accepted, kde jsou uvedeny další detaily analýzy).

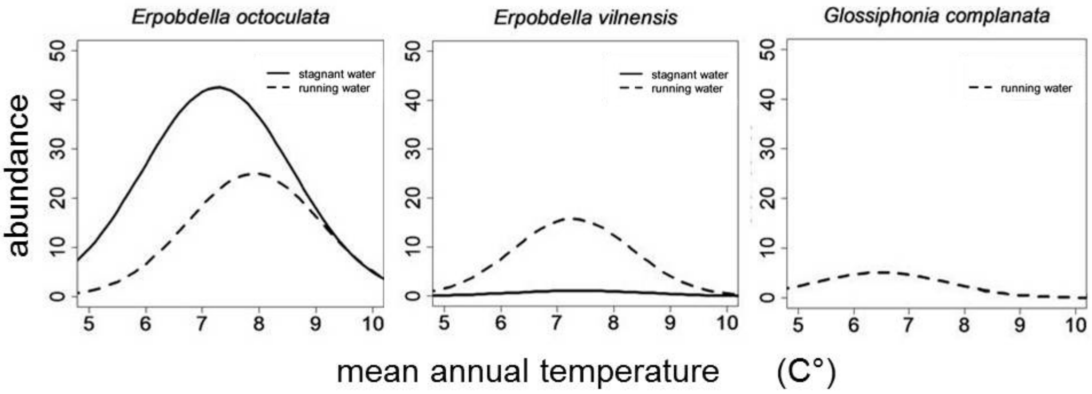
Jako nejzajímavější se jeví vzácný druh *Trichodrilus strandi*, indikátor minerálně bohatých pěnovcových slatinišť, která nejsou antropogenně ovlivněná – v našich analýzách na základě taxocenu opaskovců typ prameništ' A (Obr. 9, Schenková *et al.*, accepted). Jde o druh prameništního specialisty, obývající čisté oligotrofní, chladné, vysoce mineralizované toky (Hrabě 1981, Juget & Dumnicka 1986, Šporka 1998, Bojková *et al.* 2011). Jeho výskyt je pozitivně korelován s koncentrací Ca a Mg natolik, že 50 % variability jeho abundancí lze vysvětlit těmito proměnnými. Ke stejnému typu prameništ' je byť slaběji vázán i druh *Rhyacodrilus falciformis*, který je znám jak vztahem k pramenným a podzemním vodám - krenofilní stygobiot (Schwank 1981, Juget 1987, Dumnicka 2000), tak vlhkým půdám (Schlaghamerský & Kobetičová 2005). My jsme u něj zaznamenali unimodální odpověď ke koncentracím Ca a Mg (vysvětlující 13 % variability) a preferenci habitatů s nízkým množstvím organického substrátu.

Druh *Stylodrilus heringianus*, indikující druhou skupinu prameništ' (B) na základě opaskovců (Obr. 9), je charakteristický pro kyselé slatiniště se 73 % variability vysvětlenými negativním vlivem Ca a Mg (Schenková *et al.*, accepted). Pro tento druh je typická schopnost přežít i občasné vyschnutí toku (Nijboer *et al.* 2004). Pro další druh *Cognettia sphagnetorum* vázající se ke skupině B (i když ne jako indikátor) je všechna zjištěná variabilita vysvětlená negativním vztahem k Ca a Mg koncentracím (47 %) (Schenková *et al.*, accepted) a i když u nás nebyla statisticky významná, je pro jeho prezenci v této skupině důležitá i vazba na nižší teploty vody, kterou se významně liší od zástupce stejného rodu *C. glandulosa* (Timm *et al.* 2001). *Mesenchytraeus armatus*, také se vztahem ke skupině prameništ' B, má veškerou zjištěnou variabilitu danou právě vztahem k chladnějším vodám, mineralizace vody pro něj není důležitá (Schenková *et al.*, accepted), naopak jde o půdní druh s pozitivním vztahem k vlhkosti (Graefe & Schmelz 1999), takže může lépe odolávat dočasnému prosychání prameniště, pravděpodobně charakteristickému pro tuto skupinu pramenišť.

Pijavice *Erpobdella vilnensis*, živící se jako predátor, je nejsilnějším indikátorem třetí skupiny pramenišť označenou C a charakteristickou vyšší trofií; z měřených proměnných pro ni byla významná jen schopnost tolerovat nedostatek kyslíku (vysvětlující 23 % variability) (Schenková *et al.*, accepted). Další indikátorové druhy této skupiny *Tubifex tubifex* a *Limnodrilus hoffmeisteri*, jsou charakteristickými indikátory znečištěných vod (např. Brinkhurst & Jamieson 1971, Uzunov *et al.* 1988), neboť jejich abundance narůstají směrem k vyšším teplotám a množství organického substrátu, respektive k celkovému organickému uhlíku a nitrátům (Schenková *et al.*, accepted). U silně acidofilního druhu *Cognettia sphagnetorum* (van Duinen *et al.* 2006) bylo 47 % variability vysvětleno Ca a Mg (negativní vztah), u acidotolerantního druhu *Lumbriculus variegatus* (Graefe & Schmelz 1999) jen 8 %, silnější vztah byl zaznamenán k vyšším koncentracím celkového uhlíku (40 %) a teplotě vody (15 %). Nerozložená rostlinná potrava v kyselých slatiništích je typickou potravou druhu *Lumbriculus variegatus* (Moore 1978, Brinkhurst & Gelder 1991). Drobné fytofilní druhy z podčeledi Naidinae *Pristina bilobata* a *Nais communis* vykazovaly vztah k nižším koncentracím Ca a Mg, přičemž podhorský druh *P. bilobata* navíc vykazoval pozitivní vztah k teplotě a organickému substrátu a euryvalentní druh *N. communis* negativní vztah k anorganickému substrátu (Schenková *et al.*, accepted).

Pro stanovení preferencí jednotlivých druhů pijavic byl také vyhodnocen široký datový soubor 109 lokalit na území České republiky (Kubová & Schenková 2014). Prediktivní modely bylo možné spočítat pro sedm dominantních a dostatečně frekventovaných druhů pijavic (*A. heteroclita*, *G. complanata*, *H. stagnalis*, *Hemiclepsis marginata*, *T. tessulatum*, *E. octoculata* a *E. vilnensis*) a 12 proměnných prostředí. Vzhledem k šíři variability různých typů vzorkovaných lokalit je na rozdíl od máloštětinatých opaskovců pro pijavice velmi významný typ habitatu, kdy kategoriální proměnná tekoucí vs. stojaté vody byla signifikantní pro všechny studované druhy. Tekoucí vody preferuje pouze *G. complanata*, pro kterou je důležitý i nízký obsah amonných iontů, a *E. vilnensis*, která je naopak k jejich vysokým koncentracím signifikantně tolerantní (Kubová & Schenková 2014), což bylo nové zjištění; pouze Van Haaren (2004) popisuje přítomnost druhu v téměř stojatých vodách holandských kanálů. Také jeho přítomnost v nejvíce znečištěné skupině prameništních slatinišť a tolerance nízkého množství kyslíku podporuje informace o jeho všeobecné odolnosti (Schenková *et al.*, accepted). Ostatní sledované druhy signifikantně preferují vody stojaté (Kubová & Schenková

2014). Téměř všechny sledované druhy mají také významný vztah ke klimatickým podmínkám, kde průměrná roční teplota je pro většinu z nich signifikantní, pro tři druhy *E. octoculata*, *E. vilnensis* a *G. complanata* i v kvadratickém tvaru, jak ukazují odpovědní křivky sestrojené pro tekoucí a stojaté vody zvlášť, u *G. complanata* jen pro tekoucí (Obr. 13). Stejně jako pro máloštětinaté opaskovce, je i pro pijavice významný charakter substrátu. *A. heteroclita*, *G. complanata* a *Helobdella stagnalis* dávají přednost hrubšímu substrátu, který je pro ně optimálním podkladem, pouze parazitický druh *Hemiclepsis marginata* preferuje bahnitý společně s vyšším obsahem nitrátů – oba tyto faktory jsou významné spíše pro jeho rybí hostitele. Také další druhy stojatých vod *A. heteroclita* a *Helobdella stagnalis* obývají spíše vody znečištěné s vysokým obsahem fosforečnanů, respektive s vysokými hodnotami celkového uhlíku (Kubová & Schenková 2014), což je v souladu s pracemi Mann (1955), Sládeček & Košel (1984), van Haaren (2004) a odporuje to pouze výsledkům práce Koperski (2005), kde byl studován velmi omezený datový soubor.



Obrázek 13. (a) Signifikantní odpovědní křivky (P < 0,05) běžných druhů pijavic k průměrné roční teplotě založené na kvadratickém tvaru prediktivních modelů, — stojaté vody, – - – tekoucí vody. Převzato z Kubová & Schenková (2014), kde jsou uvedeny další detaily.

Závěrem kapitoly lze říct, že stanovit preference konkrétních druhů opaskovců je velmi obtížný úkol zejména pro vzácné a málo početné druhy, které se vymykají možnostem statistického zhodnocení dat. Avšak každý příspěvek do této mozaiky byť s limitovanou platností v místních podmínkách přináší možnost vysvětlení chování celého společenstva a tudíž i vysvětlení, jaké podmínky taková druhová skladba indikuje.

3. Závěr

V tomto shrnujícím úvodu bylo mým cílem představit šíři diverzity našich dvou největších skupin opaskovců (Clitellata), máloštětinatých opaskovců a pijavic, často pokládaných za pouhé indikátory organického znečištění. Tito kroužkovci však reagují na celou škálu gradientů prostředí, zejména na chemismus vody a charakter substrátu, takže jejich druhové složení a početnost pomáhají odhalit nejen jednotlivé antropogenní vlivy, od různých typů znečištění, přes oteplení vod až po morfologické změny habitatů, ale indikují nám i vlastnosti habitatů vzácných a čistých, jako jsou prameništní slatiniště. Zatímco výskyt většiny vodních máloštětinatých opaskovců odráží změny v substrátu a v chemismu vodního prostředí, zejména obohacení organickým materiálem, přímo, pijavice nám pomáhají indikovat zvýšení abundance jejich kořisti, tedy početnost ostatních vodních bezobratlých. V dnešní uspěchané době prací silně zaměřených na fylogenezi a „barcoding“ jednotlivých druhů opaskovců se někdy nedostává základních informací o jejich ekologii, a proto jsou předložené práce příspěvkem k doplnění této mezery v poznání zejména zjištěním odpovědí jednotlivých druhů na konkrétní fyzikálně-chemické i biologické gradienty.

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5. Seznam publikovaných vědeckých prací v ISI časopisech k tématu habilitační práce

5.1 Druhová diverzita máloštětinatých opaskovců a její změny podél gradientů prostředí

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Oligochaeta of the Morava and Odra River basins (Czech Republic): species distribution and community composition

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Key words: aquatic Oligochaeta, distribution, ordination, Czech Republic

Abstract

The oligochaete fauna of 57 small streams of the Morava and Odra River basins, Czech Republic, was studied during 1997 and 1998. TWINSPAN and CCA (CANOCO) were used to characterize the relationship between oligochaete species, habitat diversity and environmental variables. Chemical oxygen demand (COD), water hardness, $NO_3{}^-$–N, Ca^{2+}, Mg^{2+}, altitude, and substrate type were found to be the primary factors influencing the distribution of aquatic Oligochaeta.

Introduction

The ecological requirements of aquatic Oligochaeta (Clitellata) and their relationship to abiotic factors in streams of the Czech Republic (central Europe) have received little attention in previous studies. While extensive taxonomic reports on oligochaetes have been published by Hrabě (1979) and Lišková (1976), and on the oligochaete family Enchytraeidae, by Chalupský (1988), few ecological studies on this diverse and often dominant group of lotic benthic organisms have been conducted (Brinkhurst, 1978; Uzunov et al., 1988; Hörner et al., 1995).

The objectives of this study were to determine the distribution of oligochaete species in small, relatively unpolluted streams in the Czech Republic, and to evaluate the impact of environmental factors on the composition of oligochaete taxocenoses using multicriterial analyses.

Description of sites studied

Study sites were located on small streams in the Morava River basin (*sensu lato*, which means the Morava and Dyje River basins together) and in the Odra River basin (Czech Republic) (Fig. 1). The studied area represents an interesting region since all biogeographical subprovinces found in the Czech Republic, are present: Hercynicum, Pannonicum, Carpathicum and Polonicum (Culek, 1996) (Fig. 1). All sites were either unpolluted or moderately influenced by treated and untreated domestic wastewater effluent. Studied localities presented the following characteristics: they were situated from 175 to 750 m a.s.l.; the distance from the source was 0.7 to 16.8 km; the mean width of the streams ranged from 0.93 to 4.25 m; and the mean depth of the streams ranged from 0.06 to 1.1 m.

Materials and methods

Samples of macrozoobenthos were collected from 27 localities during spring, summer, and autumn of 1997, and from 30 localities during the same seasons in 1998. Sampling at each station was completed using

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* The paper is dedicated to the memory of Prof. Dr Sergej Hrabě, on the 100th anniversary of his birth. Dr Hrabě, a major contributor to aquatic oligochaete taxonomy during his extensive career in the Department of Zoology and Anthropology at Masaryk University, actively published from 1927 up to 1984.

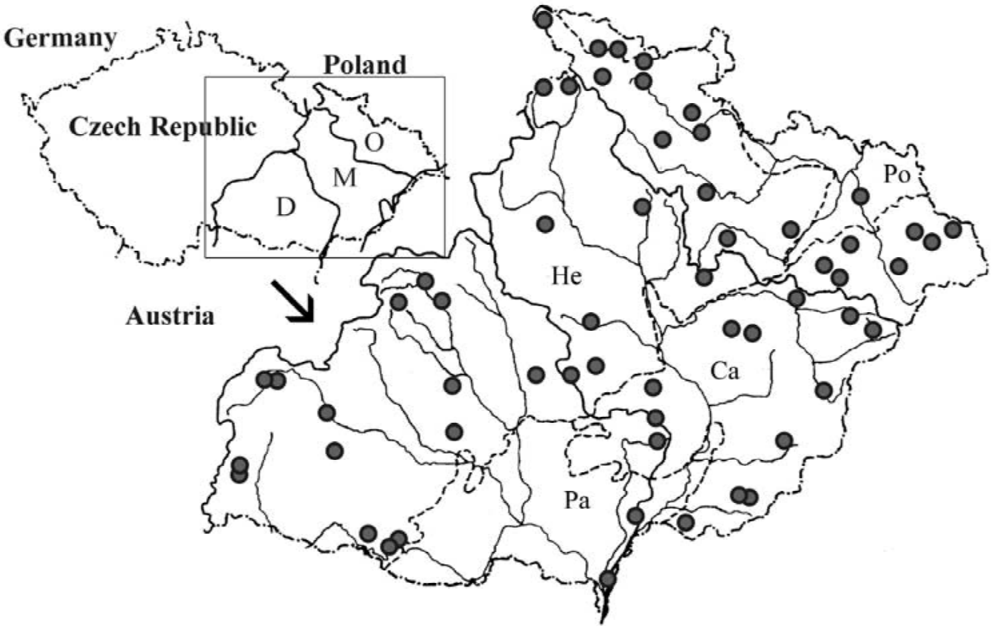

Figure 1. Map of the study area showing site localities and river basins. D – Dyje; M – Morava; O – Odra River basin. Biogeographical subprovinces named according to Culek (1996). He – Hercynicum; Po – Polonicum; Ca – Carpathicum; Pa – Pannonicum.

a standard method of kick sampling for a period of 3 min (Kershaw & Frost, 1968).

During each sampling period, water temperature, width and depth of the stream, hydrogen ion concentration (as pH), conductivity and the substrate composition were measured. The evaluation of the substrate composition at each site followed Wright et al. (1984) by expressing the weighted average of percent presence of each particle size-classes (boulders, cobbles, pebbles, granules, sand, mud and clay). The mean value of *phi*, which is the negative logarithm of the particle size in millimetres, characterised each particle size-class. Laboratory analyses for important ion components present at our sampling sites were performed on samples collected during each sampling period. Alkalinity, water hardness, oxygen content, dissolved oxygen (DO), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total organic carbon (TOC), dissolved organic carbon (DOC), NH_4^+–N, NO_2^-–N, NO_3^-–N, total nitrogen (N TOT), o-PO_4^{3-}–P, total phosphorus (P TOT), Cl^-, SO_4^{2-}, Ca^{2+} and Mg^{2+} were measured. Current velocity was not included in the analyses; single measurements obtained during sampling had no predicable value because of the frequently changing water level at the studied sites.

Samples of macrozoobenthos were fixed on site with 4% formaldehyde. After the samples had been sorted, oligochaetes were prepared for identification by either mounting permanently on slides using Canada balsam, or prepared as temporary mounts using a glycerin-water (1:5) solution.

With the exception of taxa representing five genera in the family Enchytraeidae, which were determined using the keys of Nielsen & Christensen (1959, 1961, 1963), all oligochaetes were identified to the species level using the keys of Hrabě (1954, 1979). Juvenile forms or damaged individuals were determined only to family level.

The resulting data were evaluated and interpreted using TWINSPAN (Hill, 1979). All options were set to default; maximum group size for division was five and maximum level of divisions was six. Relationships between oligochaete species and environmental variables were evaluated by Canonical Correspondence Analysis (CCA) using the CANOCO program for Windows (Ter Braak & Šmilauer, 1998). The scaling type was biplot and species data were not transformed. With the exception of representatives of Enchytraeidae, taxa unidentified to the species level were omitted from the analyses. Statistical significance was then determined by a Monte Carlo permutation test (Ter Braak & Šmilauer, 1998). 27 environmental vari-

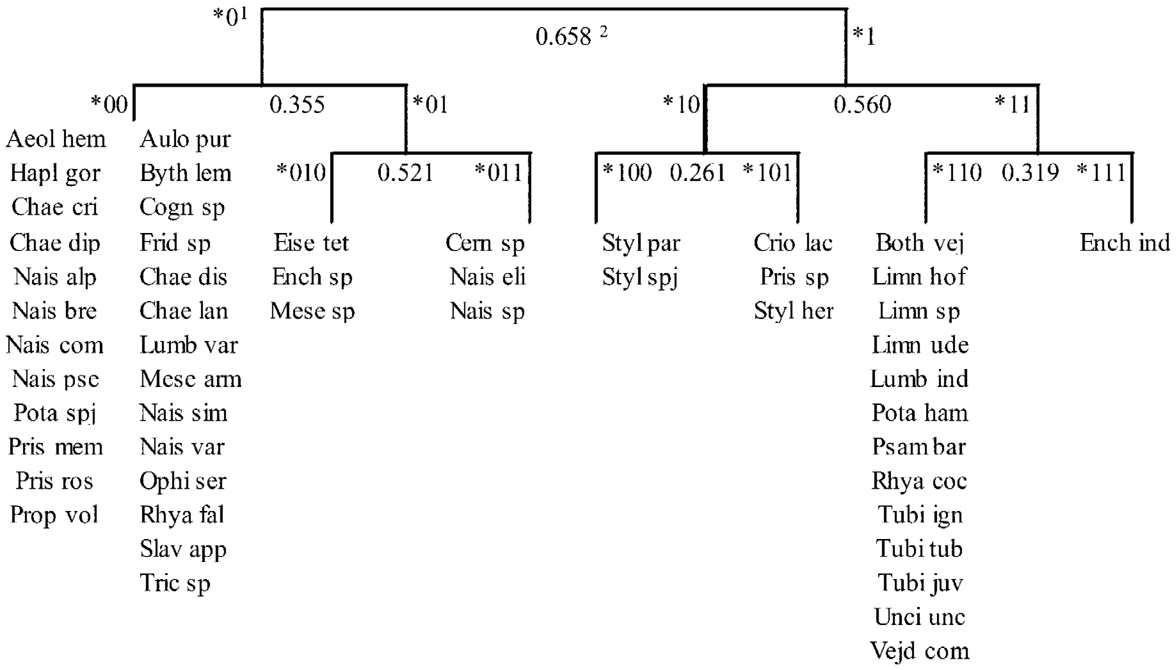

Figure 2. Divisive hierarchic classification (TWINSPAN) of species (for abbreviations see Table 1). [1]TWINSPAN group, [2]eigenvalue.

ables were tested in 999 permutations using automatic forward selection.

Results

During the 1997 and 1998 sampling periods, 22 211 individuals of the class Oligochaeta representing eight families, 27 genera and 36 species were collected (Table 1). One species of Aeolosomatidae (Annelida, Aphanoneura) was also collected. The abbreviation for each identified taxon used in analyses is provided in Table 1.

Hierarchical divisive classification of species

Hierarchical divisive classification of 51 taxa with eigenvalues of divisions is apparent from Figure 2. The eigenvalues express the level of distinction of separated groups at each division. Oligochaeta were separated into seven different groups. Group ∗00, further division of which expressed eigenvalue 0.196 and hence was not significant, consists of representatives of the family Naididae and other species from sites characterized by a sandy bottom with plant detritus. Group *010 includes the taxa *Enchytraeus*, *Mesenchytraeus* and *Eiseniella tetraedra* (Savigny, 1826). Group *011 consists of the cosmopolitan species *Nais elinguis* Müller, 1773 and *Cernosvitoviella* (Enchytraeidae). Group *100 and group *101, with relatively low eigenvalue of division, include species of the genus *Stylodrilus* – inhabiting unpolluted brooks and rivers with sandy bottom, the species *Criodrilus lacuum* Hoffmeister, 1845 – common in pools, and the genus *Pristinella* – which prefers pristine waters. Group *110 includes species in the family Tubificidae, and *Uncinais uncinata* (Ørsted, 1842) (Naididae). Group *111 contains only unidentified Enchytraeidae species.

Canonical correspondence analysis (CCA)

Canonical correspondence analysis (CCA) concerned 27 environmental variables and 46 species or genera, respectively; five genera that might have included more than one species were omitted (Fig. 3). The eigenvalue for the first axis was 0.535, the second 0.335 and the third 0.255. The percentage variance of species environment relation was 35.8 for the first axis, 22.5 for the second and 17.1 for the third axis.

The statistical significance of the data was evaluated using the Monte Carlo permutation test. The following environmental factors were found to be statistically significant: COD, hardness, NO_3^-–N,

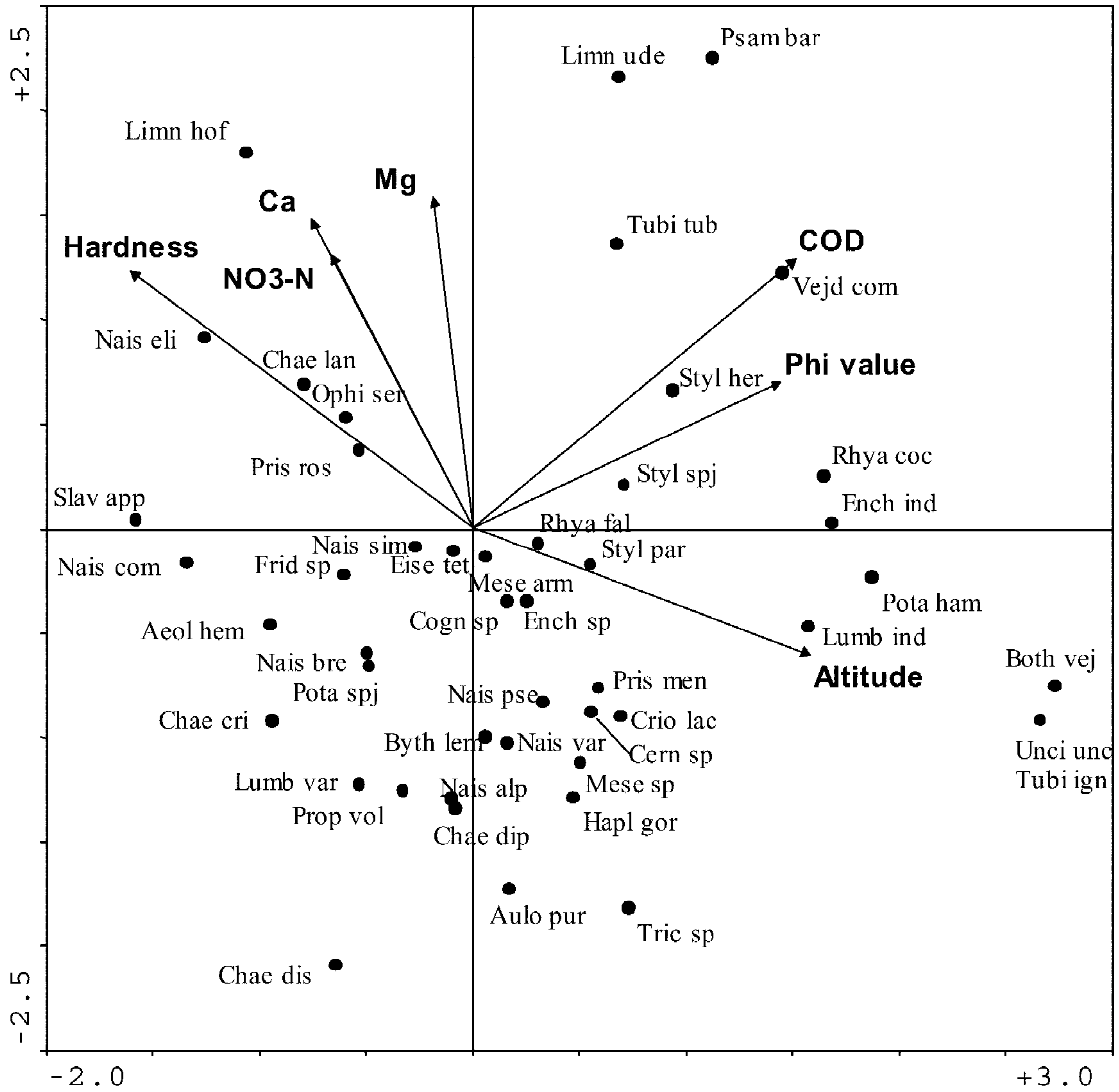

Figure 3. Canonical correspondence analysis (CCA), ordination plot of species (for abbreviations see Table 1) and main environmental variables on the first two CCA axes.

Ca^{2+}, Mg^{2+}, altitude, and substrate type ($P < 0.01$). CCA analysis showed correlations (>0.5): positive for COD, altitude and negative for hardness and the first axis. The distribution of species along the first axis reflects their requirements regarding organic matter supply, altitude and hardness. A positive correlation for Mg^{2+} was shown by the second axis, while positive correlations for Ca^{2+} and Mg^{2+} were shown by the third axis. The distribution of species along the second and third axis can be explained by the water hardness.

Discussion

From the analyses performed, we determined that COD and water hardness together with concentrations of NO_3^-–N, Ca^{2+}, Mg^{2+}, altitude, and substrate type are the primary factors influencing the distribution of Oligochaeta in small streams.

The distribution of the species of the family Tubificidae can be explained by their preference for substrates with higher organic content and fine particle size (Fig. 3). The species are widespread in distribution, from lower to higher altitudes. All species collected are common and abundant, especially in

Table 1. List of oligochaete species from the Morava and Odra River Basins in the Czech Republic and the abbreviation of each species

Species	Abbreviation
Aeolosoma hemprichi Ehrenberg, 1831	Aeol hem
Aulodrilus pluriseta (Piguet, 1906)	Aulo pur
Bothrioneurum vejdovskyanum Štolc, 1886	Both vej
Bythonomus lemani Grube, 1880	Byth lem
Cernosvitoviella sp.	Cern sp
Chaetogaster cristallinus Vejdovský, 1883	Chae cri
Chaetogaster diaphanus (Gruithuisen, 1828)	Chae dip
Chaetogaster diastrophus (Gruithuisen, 1828)	Chae dis
Chaetogaster langi Bretscher, 1896	Chae lan
Cognettia sp.	Cogn sp
Criodrilus lacuum Hoffmeister, 1845	Crio lac
Eiseniella tetraedra (Savigny, 1826)	Eise tet
Enchytraeidae	Ench ind
Enchytraeus sp.	Ench sp
Fridericia sp.	Frid sp
Haplotaxis gordioides (Hartmann, 1821)	Hapl gor
Limnodrilus hoffmeisteri Claparède, 1862	Limn hof
Limnodrilus udekemianus Claparède, 1862	Limn ude
Limnodrilus sp. juv.	Limn spj
Lumbricidae	Lumb ind
Lumbriculus variegatus Grube, 1844	Lumb var
Mesenchytraeus armatus Levinsen, 1884	Mese arm
Mesenchytraeus sp.	Mese sp
Nais alpina Sperber, 1948	Nais alp
Nais bretscheri Michaelsen, 1899	Nais bre
Nais communis Piguet, 1906	Nais com
Nais elinguis O. F. Müller, 1773	Nais eli
Nais pseudoobtusa Piguet, 1906	Nais pse
Nais simplex Piguet, 1906	Nais sim
Nais variabilis Piguet, 1906	Nais var
Nais sp.	Nais sp
Ophidonais serpentina (O. F. Müller, 1773)	Ophi ser
Potamothrix hammoniensis (Michaelsen, 1901)	Pota ham
Potamothrix sp. juv.	Pota spj
Pristinella menoni (Aiyer, 1930)	Pris men
Pristinella rosea (Piguet, 1906)	Pris ros
Pristinella sp.	Pris sp
Propappus volki Michaelsen, 1905	Prop vol
Psammoryctides barbatus (Grube, 1861)	Psam bar
Rhyacodrilus coccineus (Vejdovský, 1875)	Rhya coc
Rhyacodrilus falciformis Bretscher, 1901	Rhya fal
Slavina appendiculata (Udekem, 1855)	Slav app
Stylodrilus heringianus Claparède, 1862	Styl her
Stylodrilus parvus (Hrabě & Černosvitov, 1927)	Styl par
Stylodrilus sp. juv.	Styl spj
Trichodrilus sp.	Tric sp
Tubifex ignotus (Štolc, 1886)	Tubi ign
Tubifex tubifex (O. F. Müller, 1774)	Tubi tub
Tubificidae juv.	Tubi juv
Uncinais uncinata (Ørsted, 1842)	Unci unc
Vejdovskyella comata (Vejdovský, 1883)	Vejd com

polluted waters (Brinkhurst, 1971). COD and the sediment structure, as the most significant factors for oligochaete occurrence, have been mentioned by other authors (Brinkhurst, 1967; Uzunov, 1982).

The distribution of *Limnodrilus hoffmeisteri* Claparède, 1862, *Nais elinguis* Müller, 1773, *Chaetogaster langi* Bretscher, 1896, *Ophidonais serpentina* (Müller, 1773) and *Pristinella rosea* (Piguet, 1906) is positively correlated with water hardness, Ca^{2+}, Mg^{2+} and NO_3^--N and negatively correlated with altitude (Fig. 3). All are known to occur also in stagnant waters. While the values for water hardness, and Ca^{2+} and Mg^{2+} ion concentration are readily influenced by easily eroded bedrock, the presence of these ions also depends also on the concentration of dissolved CO$_2$, which is related to water eutrophication.

Major changes in the structure of oligochaete assemblages related to water mineralization, substrate type, and current velocity were documented by Martínez-Ansemil & Collado (1996), who studied Oligochaeta inhabiting largely unpolluted watercourses in Galicia (Spain) and Northern Portugal. Dumnicka (1978), in her studies of Oligochaeta in the River Nida, Poland, concluded that bottom type and current speed as well as hardness or Ca^{2+} and Mg^{2+} content determine the composition of oligochaete communities.

With the exception of the species noted above, naidids are distributed in streams with the lower content of organic matter and coarse substratum (Fig. 3), which is typical for unpolluted waters. This group is distributed both at low and high altitudes.

While oligochaete distribution in small streams is determined by COD, water hardness (Martínez-Ansemil & Collado, 1996) or current velocity (Dumnicka, 1978), substrate type seems to be the most important factor governing the presence of Oligochaeta in large rivers (Šporka, 1998; Montanholi-Martins & Takeda, 1999; Rempel et al., 2000). This could also be influenced by the presence of a sufficient food supply. The results of this study also suggest that oligochaetes, a group which demonstrate a broad range of ecological tolerance, are nonetheless more restricted at the family level to certain types of environments.

Acknowledgements

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Habitat preferences of aquatic Oligochaeta (Annelida) in the Rokytná River, Czech Republic – a small highland stream

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Key words: Oligochaeta, habitat preferences, distribution pattern, temporal variability, multivariate analysis

Abstract

Research was carried out to determine aquatic oligochaete habitat preferences in the Rokytná River (Thaya River basin), a sixth order highland stream in the Czech Republic during the period of April 1999–April 2001. Quantitative samples were collected and current velocity and basic physico-chemical variables were measured monthly in four typical habitats in the Rokytná River. During this study, 28, 842 individuals representing 44 oligochaete species or higher taxa were collected. Temporal variability of proportional occurrence of trophic groups found on selected habitats (gathering collectors and grazers) was analysed. Habitat preferences of oligochaete species were evaluated by Canonical Correspondence Analysis (CANOCO). Biochemical oxygen demand (BOD) and nitrate (NO_3^-) ion concentration were the most important variables explaining the distribution of Oligochaeta along the first axis. Current velocity (at 40% of the depth) and presence of oligochaetes associated with the habitat where gravel bars never formed were the most important variables along the second axis. Both axes were correlated with the temperature, reflecting the sampling in summer along the first axis and sampling in late spring along the second axis. The amount of organic matter (BOD) and concentrations of NO_3^- ions represented both oligochaete food source and decomposition products contributing to the growth of algae. Current velocity and preferred habitat explained the spatial pattern of oligochaete distributions.

Introduction

A wide range of environmental factors permanently influences aquatic ecosystems. Major physico-chemical factors are mostly defined by geology, geomorphology, and climate, and aquatic ecosystems can also be affected significantly by human activity. The oligochaete community composition reflects the states and changes in these variables. Even under natural conditions, a dynamic process of disturbance and change in the community composition occurs continuously (Hynes, 1970; Williams, 1980). The influence of stream hydrology, and physical and chemical factors on aquatic Oligochaeta (Annelida) have been studied by many authors (e.g., Korn, 1963; Dumnicka & Pasternak, 1978; Prenda & Gallardo, 1992; Martínez-Ansemil & Collado, 1996; Verdonschot, 2001).

A moderately polluted highland stream was selected to study habitat preferences within the aquatic oligochaete community. The first objective was to follow the temporal variability of the community composition over a 2-year study. Oligochaete species were divided according to their feeding habits (Moog, 1995) into two groups – grazers and gathering collectors, and temporal variability in their proportional occurrence was evaluated. Such changes were followed separately on four dominant habitats differing from hydrological point of view.

The second objective was to evaluate, by means of multivariate analysis, environmental variables significant for the oligochaete distribution and thus to establish if selected habitats play an important role in oligochaete community composition.

Study area

The oligochaete fauna was studied in the Rokytná River (Thaya River basin, Czech Republic), a sixth order stream (Strahler, 1957). The Rokytná River is 89.3 km long, with a catchment area of 585.4 km^2; the mean annual discharge at the mouth is 1.27 m^3 s^{-1}. The present study was conducted in a 60 m reach of the river, located at river km 9, at an altitude of 220 m and a slope of 3.2 m km^{-1}.

The hydrology at the study reach is characterised by two (summer and winter) annual maximum discharge levels: 1999 (14.6 and 6.0 m^3 s^{-1} respectively), 2000 (3.8 and 24.3 m^3 s^{-1} respectively), and 2001 (2.2 and 7.5 m^3 s^{-1} respectively). Highly variable discharges are often associated with agricultural landscapes – the catchment area is not able to absorb either summer rainstorms or winter snow-melt. The substrate of the stream within this study area consists of pebbles, gravel, and small amount of sand. For the studied river stretch, an alternating of the straight and meander parts is typical.

On the gravel substratum, periphyton developed in spring, dominantly represented by Bacillariophyceae (Marvan, 1998), but the character of the river bottom did not enable a growth of macrophyta.

Materials and methods

Habitat types

Four major habitat types on two cross-sections were sampled monthly, from April 1999 to April 2001. One sample was taken at cross-section A, situated in the streamline. This part of the river was chosen, as a representative of the natural straight part of the river, where gravel bottom does not form either gravel bars or islands. Three samples were taken in a meander at cross-section B to include all different habitats of this river stretch. One in the littoral, drying up part (depositional zone indicated as B littoral), one on the gravel bar (indicated as B gravel bar) and one in streamline (erosional zone: B streamline), respectively (Fig. 1). Water depth, current velocity at the bottom and at 40% of the depth characterised selected habitats.

"Freeze core" method (Bretschko & Klements, 1986) down to the depth of 0.1 m on each of the selected habitats was used for sediment structure evaluation twice – in October 2000 and June 2001. Detailed results of the sediment analysis including organic matter content down to the 0.7 m depth were published by Helešic et al. (2005). The sediment particles were sorted and percent representation of individual particle sizes was calculated. The roughness of the substrate of each habitat was expressed by the phi value (Cummins, 1962) for all selected habitats as a weighted average of phi values for each particle size. The smallest value (−3.5) and hence the highest roughness was found on B gravel bar, similar to that found on habitat A streamline (−3.2), habitats B littoral and B streamline had higher values (−2.4 and −2.5), all of them in October 2000. The bottom roughness almost did not change on habitats A streamline (−3.1), B gravel bar (−3.4) and B streamline (−2.6) while in B littoral the bottom roughness increased (−3.6) at the end of investigation in June 2001. Due to possible habitat damage it was refrained from more frequent sampling of the substratum.

Although the Rokytná River is quite a dynamic stream, the substratum did not change within a year except in one of the selected habitats. The substrate data were not included in analyses below, but were used to describe the characteristics of studied habitats.

Environmental variables

The following physico-chemical variables were recorded during each sampling period: water temperature, hydrogen ion concentration (as pH), dissolved oxygen, conductivity, current velocity at 40% of depth, current velocity at bottom, water depth, biochemical oxygen demand (BOD), chemical oxygen demand (COD), ammonia and nitrate nitrogen, and phosphate phosphorus (PO_4^{3-}). The values of physico-chemical variables measured were very similar between habitats, however, the current velocity differed considerably. Mean daily

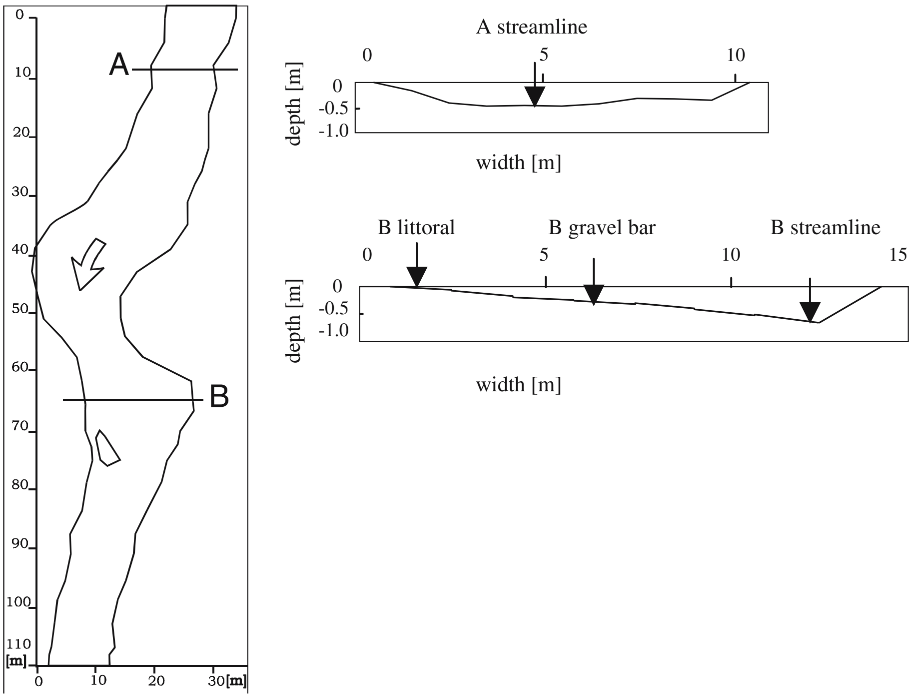

Figure 1. Cross-sections of habitats A and B on the Rokytná River, Czech Republic (5 April 2000), and the position of the sampling points: A streamline, B littoral, gravel bar and streamline (arrows).

discharge values of the sampling dates were obtained from the Czech Hydrometeorological Institute (Table 1). One value per sampling date was obtained, since the values did not differ among studied habitats.

Oligochaeta

The macrozoobenthic populations were sampled using a Kubíček sampler (0.1 m^2, mesh size 0.5 mm) (Helan et al., 1973). Oligochaete specimens were fixed in formalin (4%) in the field, and sorted under the stereomicroscope in the laboratory. All oligochaete species were mounted in Canada Balsam (Cranston, 1982) and determined using keys by Hrabě (1954, 1981), Brinkhurst & Jamieson (1971), Sperber (1950), Timm (1999) and Kathman & Brinkhurst (1999). Identification of *Bythonomus lemani* was confirmed by microscopical examination of mounted specimens that had been embedded in paraffin, sectioned longitudinally, then stained in haematoxylin (Jírovec, 1958).

After the Oligochaeta were divided into trophic groups (Moog, 1995), the temporal variability in proportional occurrence of trophic groups found (gathering collectors and grazers) and their occurrence on selected habitats was evaluated.

Data processing

Habitat preferences of oligochaete trophic groups on selected habitats were compared using the Wilcoxon Matched pair test, because the measured data did not have normal distributions (Kolmogorov–Smirnov test) and were dependent on sampling date. The correlation of two trophic groups and two environmental variables (temperature and daily discharge) were evaluated using the Spearman rank correlation coefficient (Zar, 1984).

Faunistic and environmental data were analysed by Canonical Correspondence Analysis (CCA), CANOCO (Ter Braak & Schmilauer, 2002). This method permits the construction of theoretical variables (ordination axes) that best fit the species data according to a unimodal method

Table 1. Minimum, maximum, mean and median values of environmental variables of the Rokytná River over the period April 1999–April 2001

Variable	Min.	Max.	Mean	Median
Water temperature (°C)	−0.2	19.4	11.0	13.0
pH	4.4	9.0	7.6	7.7
O_2 [mg l^{-1}]	6.59	18.20	10.4	9.43
Conductivity [μS cm^{-1}]	453	725	615	615
Current velocity at bottom [m s^{-1}]	0	0.87	0.34	0.30
Current velocity at 40% of depth [m s^{-1}]	0	1.46	0.67	0.68
Mean daily discharge [m^3 s^{-1}]	0.13	3.95	1.36	1.08
Depth [m]	0.10	0.47	0.22	0.20
BOD [mg O_2 l^{-1}]	1.9	15.8	5.4	4.5
COD [mg O_2 l^{-1}]	26.1	51.1	27.8	32.2
NO_3^- [mg l^{-1}]	12.0	57.5	27.8	20.0
NH_4^+ [mg l^{-1}]	0.25	1.10	0.60	0.60
PO_4^{3-} [mg l^{-1}]	0.05	1.80	0.85	0.90

of ordination. In the canonical ordination these axes are a linear combination of environmental variables. The correlations between environmental variables and the ordination axes are derived and summarised in the eigenvalue of the ordination axis. The result of CCA is a set of scores for both sites and taxa, which can be presented as two-dimensional ordination diagram where points represent sites or taxa and lines outgoing from the origin expresses environmental variables (Ter Braak & Schmilauer, 2002). The length of the lines represents the importance of the respective variable.

The data were not transformed and species were not down-weighted. The statistical significance of the relationship between all species and all variables was tested by Monte Carlo permutation test using 9999 permutations (Ter Braak & Schmilauer, 2002).

Results

Oligochaeta

During this study, 28 842 individuals representing 36 species of the families Lumbriculidae, Naididae, Tubificidae, and Lumbricidae were collected and identified (Table 2). In addition, the family Enchytraeidae was represented by unidentified species in four genera. *Psammoryctides barbatus* and *Bothrioneurum vejdovskyanum* (Tubificidae) and *Nais elinguis* (Naididae) were the dominant species.

Trophic groups

Most of the recorded species belong to two trophic groups, gathering collectors and grazers. Species with more trophic adaptations (Moog, 1995), the dominant feeding habit was used for evaluating. The third group, predators (only within the genus *Chaetogaster*) represented only about 0.1% of the total number of individuals and therefore was omitted in the analyses. Though the proportion of trophic groups (gathering collectors and grazers) varied among habitats, these differences were insignificant ($p > 0.05$) (Wilcoxon Matched pair test).

The occurrence of grazers was positively correlated (Spearman rank correlation coefficient, $p < 0.05$) with the discharge and negatively with temperature on habitat A streamline (Fig. 2a). The grazers were also negatively correlated with temperature habitat B streamline (Fig. 2b) and gathering collectors were positively correlated with temperature on habitat A streamline (Fig. 2c), (Spearman rank correlation coefficient, $p < 0.05$).

Table 2. List of oligochaete taxa recorded, taxon code, frequency of occurrence, percentage of occurrence and trophic group (gat – gathering collector, gra – grazer and pre – predator)

Taxon	Abbreviation	No. of individuals	%	Trophic group
Lumbricidae				
Allolobophora chlorotica Savigny, 1826)	Allo chl	28	0.10	gat
Eiseniella tetraedra (Savigny, 1826)	Eise tet	26	0.09	gat
Enchytraeidae				
Enchytraeus g. sp. div.	Ench sp	64	0.22	gat
Fridericia g. sp. Div.	Frid sp	54	0.19	gat
Cernosvitoviella g. sp. div.	Cern sp	3	0.01	gat
Cognettia g. sp. div.	Cogn sp	32	0.11	gat
Tubificidae				
Aulodrilus pluriseta (Piguet, 1906)	Aulo plu	2	0.01	gat
Bothrioneurum vejdovskyanum Štolc, 1886	Both vej	2604	9.03	gat
Limnodrilus claparedeanus Ratzel, 1868	Limn cla	3	0.01	gat
Limnodrilus hoffmeisteri Claparede, 1862	Limn hof	167	0.60	gat
Limnodrilus udekemianus Claparede, 1862	Limn ude	6	0.02	gat
Limnodrilus g. sp. div. juv.	Limn sp	2292	7.95	gat
Moraviodrilus pygmaeus Hrabě, 1935	Mora pyg	386	1.34	gat
Potamothrix hammoniensis (Michaelsen, 1901)	Pota ham	7	0.02	gat
Psammoryctides barbatus (Grube, 1861)	Psam bar	5482	19.01	gat
Psammoryctides moravicus (Hrabě, 1934)	Psam mor	5	0.02	gat
Rhyacodrilus coccineus (Vejdovský, 1875)	Rhya coc	41	0.14	gat
Rhyacodrilus falciformis Bretscher, 1901	Rhya fal	1395	4.84	gat
Rhyacodrilinae juv. with hair chaetae	Rhya juv	1634	5.67	gat
Spirosperma ferox Eisen, 1879	Spir fer	1	0.003	gat
Tubifex tubifex (O. F. Műller, 1774)	Tubi tub	50	0.17	gat
Tubificidae juv. with hair chaetae	Tubi juv	957	3.32	gat
Naididae				
Chaetogaster diaphanus (Gruithuisen, 1828)	Chae dip	21	0.07	pre
Chaetogaster diastrophus (Gruithuisen, 1828)	Chae dis	7	0.02	pre, gat, gat
Nais alpina Sperber, 1948	Nais alp	461	1.60	gra, gat
Nais barbata O. F. Műller, 1773	Nais bar	108	0.37	gra, gat
Nais bretscheri Michaelsen, 1899	Nais bre	857	2.97	gra, gat
Nais communis Piguet, 1906	Nais com	144	0.50	gra, gat
Nais elinguis O. F. Műller, 1773	Nais eli	5580	19.34	gra, gat
Nais pardalis Piguet, 1906	Nais par	6	0.02	gra, gat
Nais pseudobtusa Piguet, 1906	Nais pse	48	0.17	gra, gat
Nais simplex Piguet, 1906	Nais sim	20	0.07	gra, gat
Nais variabilis Piguet 1906	Nais var	1	0.003	gra, gat
Ophidonais serpentina O. F. Műller, 1773	Ophi ser	4	0.01	gra, gat
Pristina aequiseta Bourne, 1981	Pris aeq	56	0.19	gra, gat
Pristina bilobata (Bretscher, 1903)	Pris bil	19	0.07	gra, gat
Pristina rosea (Piguet, 1906)	Pris ros	323	1.12	gra, gat
Stylaria lacustris (Linnaeus, 1767)	Styl lac	2	0.01	gra, gat
Vejdovskyella intermedia (Bretscher, 1896)	Vejd int	22	0.08	gra, gat

Continued on p. 122

Table 2. (Continued)

Taxon	Abbreviation	No. of individuals	%	Trophic group
Lumbriculidae				
Bythonomus lemani (Grube, 1879)	Byth lem	1401	4.86	gat
Lumbriculus variegatus O. F. Műller, 1774	Lumb var	14	0.05	gat
Stylodrilus parvus (Hrabě & Černosvitov, 1927)	Styl par	813	2.82	gat
Stylodrilus heringianus Claparede, 1862	Styl her	49	0.17	gat
Stylodrilus g. sp. div. juv.	Styl juv	3647	12.64	gat
Total		28 842	100.00	

Habitat preferences

Canonical correspondence analysis (Fig. 3) was used to extract species–environment relationships; 44 taxa and 13 environmental variables and 8 nominal variables in 84 samples were evaluated. Four nominal variables represented selected habitats – A streamline, B littoral, B gravel bar, and B streamline, and four nominal variables represented seasons – spring (March–April), spring/summer (May–June), summer (July–August), and autumn/winter (September–February). These sampling periods correspond more closely with temporal changes in oligochaete community composition than the four 'standard' seasons (e.g., winter = 21 December through 20 March). The statistical significance of environmental variables was assessed by Monte Carlo permutation test. Ranked from the most to the least: BOD, temperature, habitat A streamline, NO_3^-, current velocity at 40%, habitat B streamline, summer sampling and conductivity were significant.

The eigenvalues for the four axes are 0.325, 0.171, 0.084, and 0.075, respectively. According to the weighted correlation matrix, the first axis (40.8% variance of the species–environment relation) is related to BOD (0.592) and NO_3^- (0.350) and thus represents the food source (NO_3^- as indicator of a nutrient source for algae and diatoms). The second axis (21.6% of variance) is correlated with current velocity at 40% of the depth (0.400) and presence on habitat A streamline (−0.408), explained spatial variability. Both axes are negatively correlated with temperature (−0.334, −0.396 respectively), which reflects the summer sampling along the first axis (−0.518) and the spring/summer sampling (−0.421) along the second axis respectively (Fig. 3). The third ordination axis (10.6% of variance) is positively correlated with the presence of oligochaetes on habitat B streamline (0.391), BOD (0.376), temperature (0.360) and negatively with NO_3^- (−0.401).

In the ordination diagram representatives of the family Naididae were in right upper corner showing development at lower temperatures, in spring. Most of Tubificidae representatives were negatively correlated with current velocity in 40% of the depth and occurred on habitat A streamline.

Discussion

Most oligochaete species found in the Rokytná River occurred within a wide range of environmental conditions, demonstrating a large temporal and spatial variability. Oligochaete composition on four selected habitats did not differ significantly when comparing proportion of two feeding groups. We suggest that density changes of grazers, which were negatively correlated with the temperature and positively with discharge, were directly caused by periphyton growth. The grazers represented by members of the family Naididae have maximal development in winter and spring (Pfannkuche, 1981) when sufficient light enables the development of periphyton, also Šporka (1996) have found that the abundance of the family Naididae is dependent on the quantity of periphyton. The positive correlation of gathering collectors and the temperature again correspond with availability of food sources. They reach maximal densities in summer (Lazim & Learner, 1986), when decaying organic matter is available. Most significant relationships were associated with a very stable habitat A; the fact that gravel bars never form there enhance oligochaete growth.

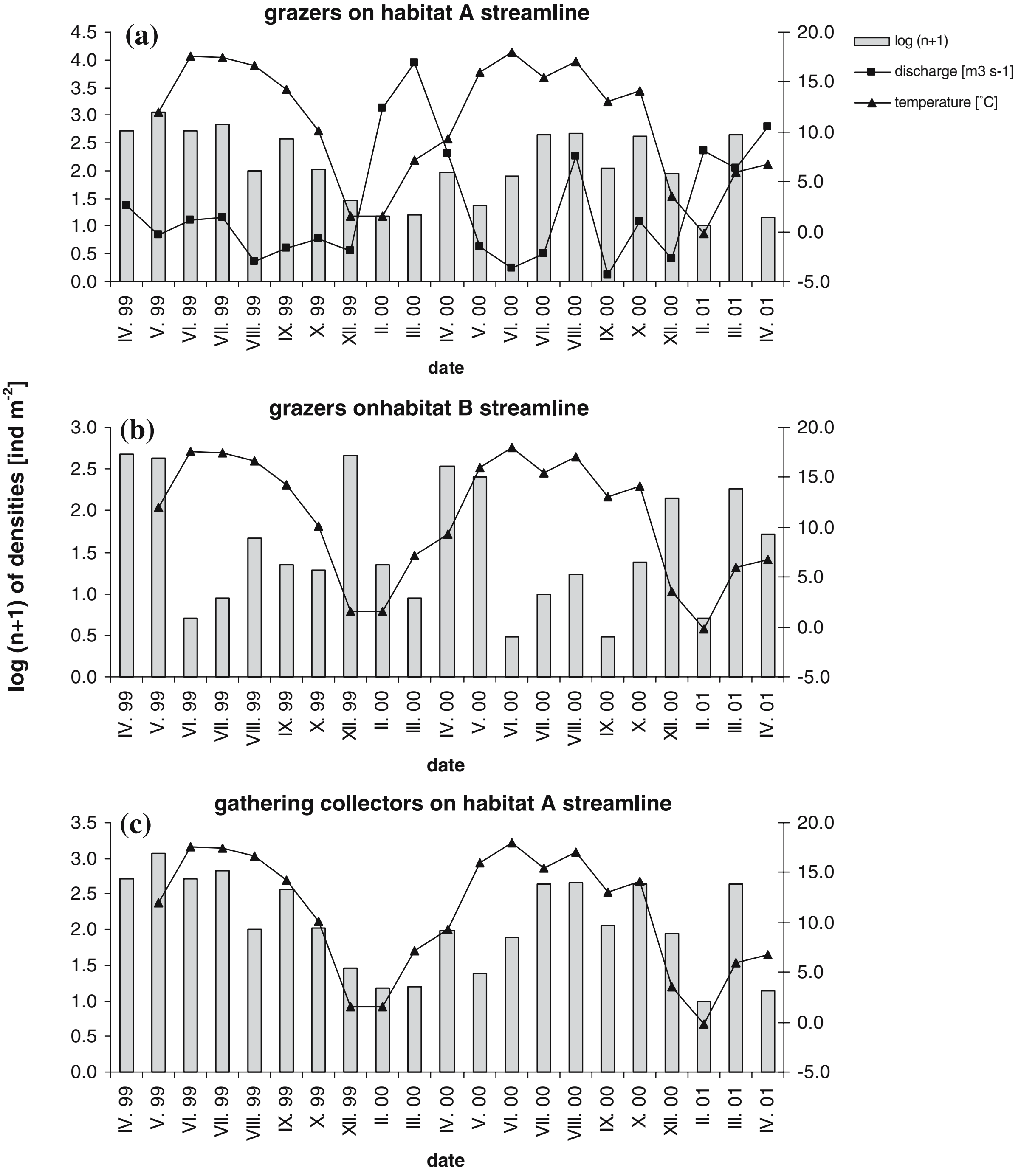

Figure 2. The temporal variability of grazers on habitat A streamline, positively correlated with the discharge and negatively with temperature (a) The temporal variability of grazers on habitat B streamline, negatively correlated with temperature (b) The temporal variability of gathering collectors on habitat A streamline, positively correlated with temperature (c) (Spearman rank correlation coefficient, $p < 0.05$).

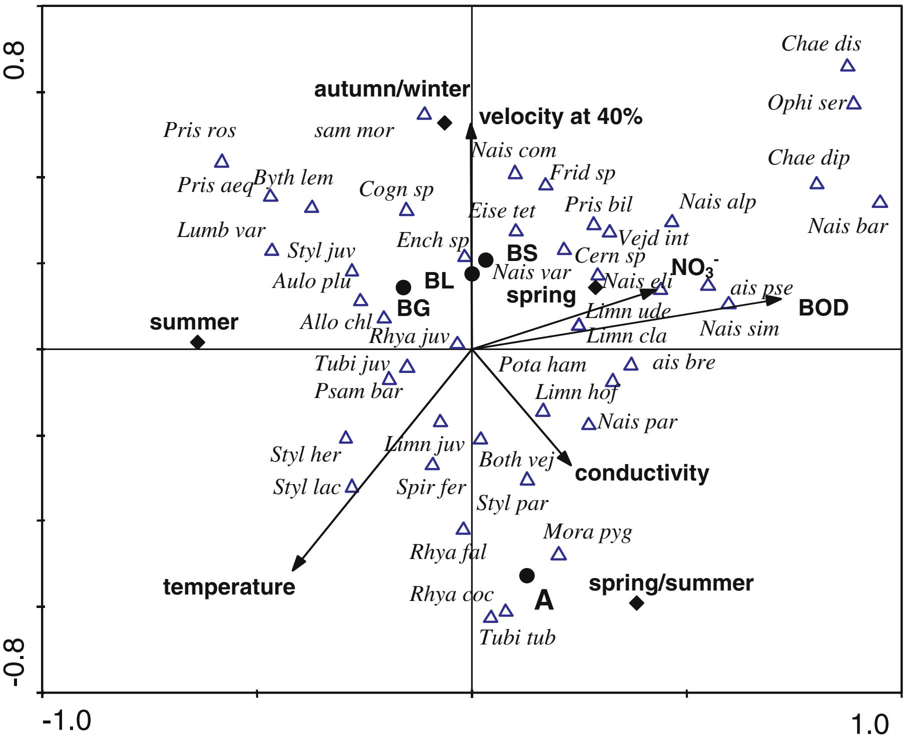

Figure 3. CCA diagram of the axis 1 and 2 of oligochaete taxa (for abbreviations see Table 2) in relation to environmental variables (arrows), seasons (diamonds) and sampled habitats (points): A = A streamline, BL = B littoral, BG = B gravel bar, BS = B streamline – recorded in the Rokytná River.

Variability of current velocities in different habitats of cross-section B resulted in either the movement of worms between habitats or their downward burrowing into hyporheic layers.

Habitat preferences of various species were evaluated using CANOCO. Oligochaete community composition was dependent upon organic matter input (BOD) and sources of inorganic ions NO_3^- (aerobic degradation product and secondary periphyton nutrient source) along the first axis. The second axis was correlated with current velocity and presence on habitat A streamline. The most important variable determining oligochaete community composition in habitats assessed during this study was habitat A streamline in which little if any shifting of substrate occurred – it offered stable conditions for oligochaete occurrence, especially permanent availability of organic matter in substratum. Both axes, however, were also correlated with temperature and seasons of sampling, thus representing temporal variability. The values for chemical variables monitored during this study indicate that the Rokytná River varies from slightly to strongly polluted stream within a year. Detecting the habitat preferences of species in polluted waters is problematic. Some authors (Dumnicka & Pasternak, 1978; Culp et al., 1983; Prenda & Gallardo, 1992) showed that certain oligochaete species were not habitat selective. In CCA analysis, two of the four selected habitats (B littoral and B gravel bar) showed only small reaction with the oligochaete distribution. Although both were differing in environmental conditions with respect to velocity and depth, the oligochaete composition was quite similar there. The position of these environmental variables is similar in the diagram (Fig. 3), although they are not correlated. CCA analysis showed that certain habitats are vital for oligochaete occurrence (habitat A streamline was the third most important environmental variable); other variables were less important. Results were significantly influenced by temporal variability – oligochaete community composition differed both in time and in space.

We conclude that, at least in the lower reaches of a highland stream, habitats do not play as an important role as has been demonstrated in high order streams (Korn, 1963; Montanholi-Martins & Takeda, 1999, 2001). Although some authors (Montanholi-Martins & Takeda, 1999) applied a very similar sampling scheme, they found spatial distribution to prevail over the temporal one. Our conclusions are similar to those of Verdonschot (2001), who studied the influence of different substrates in lowland rivers. There were no significant relationships with the habitats if the most of the study streams included them. According to Verdonshot (2000) the disturbance caused by agricultural activities in the catchment area can cause that only ubiquists without habitat preferences settle the bottom. Most of the oligochaete species show wide ecological valences and habitat preferences are not significant when comparing relatively close sampling points in streams particular in agricultural areas. Relatively natural character of the stream is why we found two (stable) habitats important for oligochaete occurrence. On the other hand, the impact of agriculture causes extreme discharges and this promotes ubiquist species whose community composition and abundance, therefore, varies with time rather than with habitat.

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Seasonal dynamics of *Bythonomus lemani* and *Bothrioneurum vejdovskyanum* (Oligochaeta, Annelida) in relation to environmental variables

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Abstract: The seasonal dynamics of *Bythonomus lemani* (Lumbriculidae) were studied for the first time and the knowledge of *Bothrioneurum vejdovskyanum* (Tubificidae) was extended based on four quantitative samples of oligochaetes taken monthly in the Rokytná River (Czech Republic) during a two-year study (April 1999 – April 2001). The influence of water temperature, velocity, depth, discharge, pH, conductivity, dissolved oxygen amount and biochemical oxygen demand (BOD) on their life cycles was evaluated. Habitat preferences of the juvenile and adult stages were recorded. Time series analysis was used to determine the worm densities, seasonality and trends. For *Byth. lemani* one distinct reproduction cycle per year was found and this was regulated by temperature, while *Both. vejdovskyanum* showed a one-year cycle not significantly dependent on measured environmental variables. The overall trend was an increase in density for *Byth. lemani* and a decrease in density for *Both. vejdovskyanum*. *Byth. lemani* showed a significant negative correlation between the trend of its density and BOD concentration.

Key words: *Bythonomus lemani*, *Bothrioneurum vejdovskaynum*, aquatic Oligochaeta, seasonal dynamics, time series analysis.

Introduction

The knowledge on the seasonal population dynamics of certain aquatic oligochaetes is limited. As early as in the first half of the last century, Hrabě (1935a, b) studied the reproduction of *Bothrioneurum vejdovskyanum* Štolc, 1888 (Tubificidae) and described its asexual reproduction, at that time not known in any representative of this family. Brinkhurst & Kennedy studied other tubificid species in the second half of the last century (Brinkhurst & Kennedy, 1965; Brinkhurst, 1966; Kennedy, 1966). The best known are the life cycles of *Tubifex tubifex* (Müller, 1774), *Limnodrilus hoffmeisteri* Claparède, 1862 and *L. udekemianus* Claparède, 1862 (Ladle, 1971; Bonomi, 1979, 1980; Pfannkuche, 1981; Lazim & Learner, 1986; Lazim et al., 1989) because of their high abundance in polluted waters. Other species of this family which have been studied include *Potamothrix hammoniensis* (Michaelsen, 1901) (Thorhauge, 1976; Risnoveanu & Vadineanu, 2002), *Psammoryctides barbatus* (Grube, 1861) and *Spirosperma ferox* Eisen, 1879 (Adreani et al., 1984).

Freshwater species of the family Lumbriculidae and Propappidae represented by *Stylodrilus heringianus* Claparède, 1862, *S. parvus* (Hrabě-Černosvitov, 1927) and *Propappus volki* Michaelsen, 1916 were studied by Dumnicka & Kukuła (1990) and Petto & Humpesch (1992). Some species from the family Naididae were also studied (e.g., Learner et al., 1978; Dumnicka, 1982).

Recently there has been a shift from studies following only changes in the numbers of worms to more sophisticated techniques such as modelling of seasonal dynamics. In this study we aim to evaluate the seasonal dynamics of *Bythonomus lemani* (Grube, 1879) (Lumbriculidae) and *Bothrioneurum vejdovskyanum* (Tubificidae) because they are very rare and rare species, respectively, in the Czech Republic and information about their natural reproduction strategy is scarce (especially for *Byth. lemani*). Distribution of the Palaearctic species *Byth. lemani* covers S and C Europe and the study area lies on its northern margin. The conservation of this rare species requires more knowledge about its ecological demands. Whereas some authors (Brinkhurst, 1978; Hrabě, 1981) considered *Byth. lemani* to be a stagnant water (lakes) species, others (Rodriguez & Armas, 1983) considered it an inhabitant of running waters; certain authors have considered it as a species of oligosaprobic waters (Uzunov et al., 1988). The second species, *Both. vejdovskyanum*, with a cosmopolitan distribution, is remarkable for its reproduction by architomy (fission followed by development of the rest of the body), which is quite rare in the Tubificidae family.

The species studied represent two different forms of life strategies, for *Byth. lemani* sexual reproduction is typical, while *Both. vejdovskyanum* reproduces by the above mentioned architomy. The aim of our study was to describe and compare their life cycles, to evaluate which environmental variable(s) regulate them and to find habitat preferences of juvenile and adult stages of *Byth. lemani.*

Material and methods

Study area and sampling

The sixth order Rokytná River (the Thaya River Basin, Czech Republic), which is influenced relatively little by human impact, was chosen for this study. The sampling site was situated on the ninth river km near Moravský Krumlov (49°03′ N, 16°19′ E), with 3.2% slope at 220 m a.s.l. (Fig. 1). The river bottom mainly consisted of small pebbles, gravel, sand and, locally, silt. During late winter and in spring periphytic algae covered the substratum; from the beginning of May the river channel was shaded and water showed high turbidity.

Oligochaetes were sampled along two cross-sections (A and B) across a 60 m long river reach. Cross-section A was a straight part of the channel, while B was a meandering one; they differed from one another in the variability of their width during the year (Tab. 1). Four typical habitats were sampled at the same time, one on cross-section A and three on B (Fig. 1). The habitat on cross-section A was located in the middle of the channel, where small pebbles (20–40 mm) comprised 40% of bottom substratum and gravel bars did not form there. This is hereafter referred to as 'streamline A'. The habitats on cross-section B were: 'littoral', located close to the right bank of an inner river bend, 'gravel bar', located in the middle of the channel and 'streamline B', on the left side with the strongest water current up to 1.46 [m s^{-1}] and the most coarse substratum. Small pebbles (20–40 mm) formed on cross-section B: 13%, 12% and 17% of the bottom substrata of littoral, gravel bar and streamline habitats, respectively. The river channel in cross-section A remained stable during the two-years sampling period, but on cross-section B the substratum was continually shifting, although the general shape of the river channel and character of the three studied habitats remained approximately the same.

Oligochaeta

Oligochaeta were sampled monthly from April 1999 to April 2001 within each habitat except in winter, when two monthly sampling was used. Quantitative samples were taken using a Kubíček's sampler (HELAN et al., 1973) (mesh size 0.5 mm) from a triangular area of 0.1 m^2. Sampled animals were stored in 4% formalin in the field and sorted in the laboratory. Oligochaeta were identified to species level using the keys of HRABĚ (1954, 1981). The specific identification of *Byth. lemani* was tested using cross sectioning. Abundance of juvenile and adult individuals of *Byth. lemani* and *Both. vejdovskyanum* were counted. Identification of juvenile *Byth. lemani* was possible because they were the only lumbriculids with simple pointed setae present in the area.

Fig. 1. Map showing the study site and selected river stretch with the sampling points. AS – streamline; BL – littoral; BG – gravel bar; BS – streamline.

Environmental characteristics

On each sampling date one sample of water for chemical analysis was taken on cross-section A and biochemical oxygen demand (BOD) was evaluated in the laboratory. Considering the dimensions of the river, values of BOD on cross-section B were considered the same as from A. The water temperature, velocity at 40% of the depth and velocity close to the bottom, depth, pH, conductivity and dissolved oxygen were measured at each of the four habitats. Mean daily discharges were obtained from the hydrological station situated 6 km downstream from the sampling site.

Statistical analysis

The presence of species in different parts of the stream and differences in their abundance were evaluated using χ^2 test with a null hypothesis of equal distribution of worms among habitats. The proportion of adults and juveniles of *Byth. lemani* was determined by contingency table analysis. Proportions of their densities were weighted by the total number of worms.

Time series analysis was used to evaluate trends in worm abundance, based on the mean of worm densities and the means of abiotic parameters. Twenty-five dates were used in the time series of species density analysis; 21 sampling events and four missing values (taking into account two monthly samplings in winter) estimated from adjacent points in the time period. Data were log transformed. Moving average smoothing (3 numbers) was applied for visualization of the time series.

The time series analysis enables division of the total trend in oligochaete densities into two components: 'smooth' – representing a particular trend (increasing/decreasing) during the whole sampling period and 'cyclic' – following

Table 1. Physico-chemical characteristic of the selected sites, cross-sections A and B and habitats AS, BS, BG and BL.

Cross-section	A				B			
	Mean		Min.–Max.		Mean		Min.–Max.	
Width (m)	10.35		9.56–14.42		13.02		9.06–18.14	
Habitat	AS		BS		BG		BL	
	Mean	Min.–Max.	Mean	Min.–Max.	Mean	Min.–Max.	Mean	Min.–Max.
Temperature (℃)	11.0	−0.1–19.4	11.0	−0.2–19.4	11.0	−0.2–19.4	11.0	−0.2–17.6
pH	7.5	4.4–9.0	7.7	5.9–9.0	7.6	5.9–8.8	7.5	5.9–8.3
Dissolved O_2 (mg L^{-1})	10.2	6.9–12.4	10.6	7.3–17.5	10.3	7.3–14.2	10.4	7.3–14.2
Conductivity (μS cm^{-1})	620	468–700	617	479–725	612	536–725	612	536–725
Velocity close to the bottom (m s^{-1})	0.36	0.16–0.64	0.37	0.13–0.92	0.29	0–0.98	0.35	0.0–0.76
40% velocity (m s^{-1})	0.70	0.29–1.10	0.84	0.53–1.46	0.57	0.11–0.94	0.55	0.03–1.35
Depth (m)	0.26	0.11–0.47	0.27	0.11–0.42	0.16	0.02–0.36	0.20	0.06–0.38
	Mean				Min.–Max.			
BOD (mg L^{-1})	5.4				1.9–15.8			
COD (mg L^{-1})	27.8				26.1–51.1			
NO_3^- (mg L^{-1})	27.9				12.0–57.5			
NH_4^+ (mg L^{-1})	0.60				0.25–1.10			
PO_4^3 (mg L^{-1})	0.85				0.05–1.80			
Discharge (m^3 s^{-1})	1.36				0.13–3.95			

regularly repeating changes in densities over the course of time. The smooth component of time series was evaluated by Daniel's test (Daniels, 1950) which in fact is the Spearman rank correlation between oligochaete densities and their rank in the time series. For the subsequent analysis of cyclic component of the time series, the time series was detrended (i.e., the smooth component was removed) by linear regression. The cyclic component was computed by two methods; (i) autocorrelation of abundances and (ii) spectral analysis of seasonality.

Relationships between abundances and abiotic parameters were computed by cross-correlation (i.e., correlation of densities with values of environmental variables shifted in time) and the lag between them was evaluated (Box & Jenkins, 1970). The Spearman rank correlation coefficient was used also to compare trends between species densities and environmental parameters.

The analyses were performed using Statistica 7 for Windows (StatSoft, 2004).

Results

Environmental variables

Seasonal changes of physico-chemical variables in the studied river stretch over two years were recorded (Tab. 1). All values were calculated from the same number of measurements ($n = 21$). In the highland river water quality became near oligosaprobity during the winter, while in the summer sufficient autochthonous and allochthonous organic matter sources created a status close to β-mesosaprobity (ČSN 75 7358, 1997).

Oligochaeta

Of the total number of 28,842 oligochaetes (36 species), 900 juvenile and 506 adult individuals of *Byth. lemani* and 2,590 juvenile and 14 adult individuals of *Both. vejdovskyanum* were found. In *Byth. lemani*, the following identification characters were revealed: the clitellum began in the half of segment IX and extended into segment XIII, the paired mushroom-like spermatecae were situated in segment IX, two pairs of testes were in the IX and X segment, atria entered into small penes in segment X (Fig. 2). Adults of *Byth. lemani* were observed throughout the year. Adults of *Both. vejdovskyanum* occurred only rarely because of asexual reproduction, with seven individuals found in May 1999, four in May 2001 and three in June 2001. Mean density of *Byth. lemani* was 220 ind. m^{-2} while that of *Both. vejdovskyanum* was 370 ind. m^{-2}.

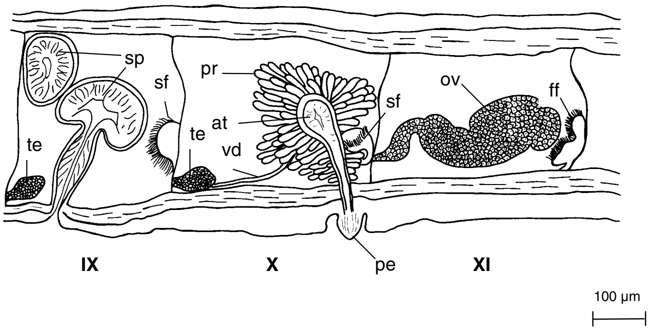

Fig. 2. *Bythonomus lemani.* Schematic drawing of reproductive organs, IX, X, XI segment, lateral view: at – atrium; ff – female funnel; ov – ovarium; pe – penis; pr – prostate gland; sf – sperm funnel; sp – spermatheca; te – testes; vd – vas deferens.

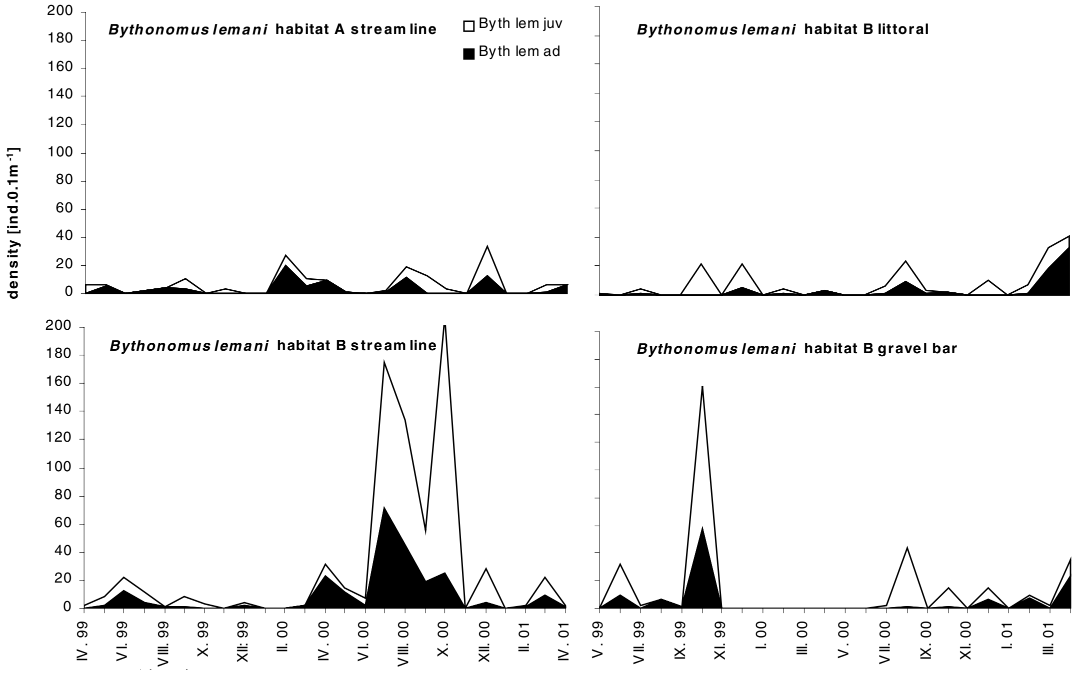

Fig. 3. *Bythonomus lemani.* Seasonal changes in densities of juvenile and adult individuals in four selected habitats.

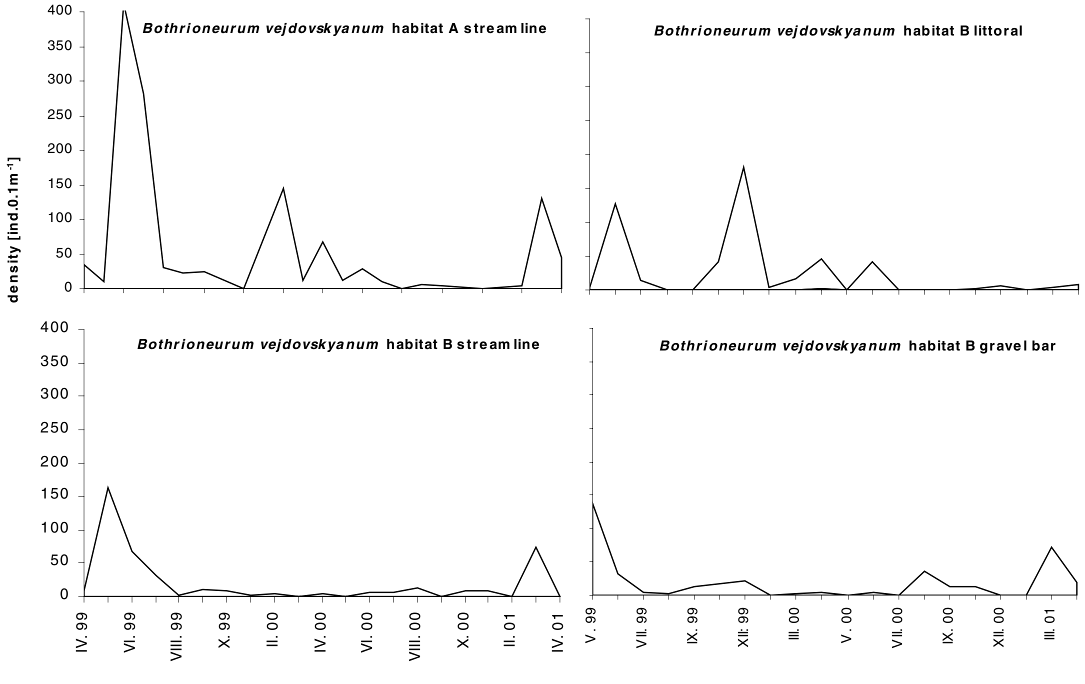

Fig. 4. *Bothrioneurum vejdovskyanum.* Seasonal changes in densities in four selected habitats.

Habitat preferences

Numbers of *Byth. lemani* differed between the four habitats (Fig. 3) whereas habitat preference was less marked for *Both. vejdovskyanum* (Fig. 4). Significant inter-habitat differences were found for both *Byth. lemani*, ($P < 0.01$; $\chi^2 = 598$; df = 3) and *Both. vejdovskyanum*, ($P < 0.01$; $\chi^2 = 454$; df = 3). *Byth. lemani* preferred stream line B, a dynamic stretch of the river with the fastest current (mean water velocity at of depth 40% was 0.84 m s^{-1}). *Both. vejdovskyanum* preferred stream line A, a relatively straight and shallow river stretch with less fluctuating water level, with no shifting of substratum (mean water velocity at of depth 40% was 0.70 m s^{-1}).

Significant differences in densities of adult and juvenile *Byth. lemani* were found among habitats ($\chi^2 = 24.5$, $P < 0.01$). The adults predominated in the habitat stream line A while juveniles were mostly in littoral

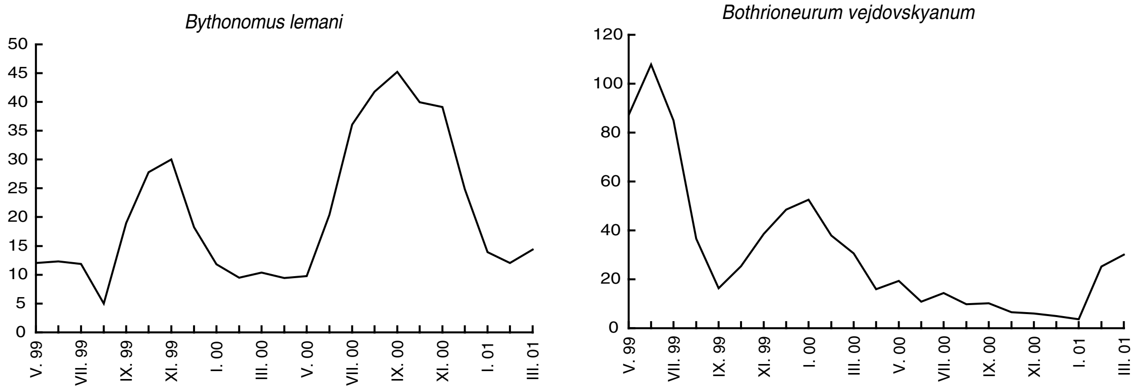

Fig. 5. Time series of *Bythonomus lemani* and *Bothrioneurum vejdovskyanum* based on mean densities, moving average smoothing was applied.

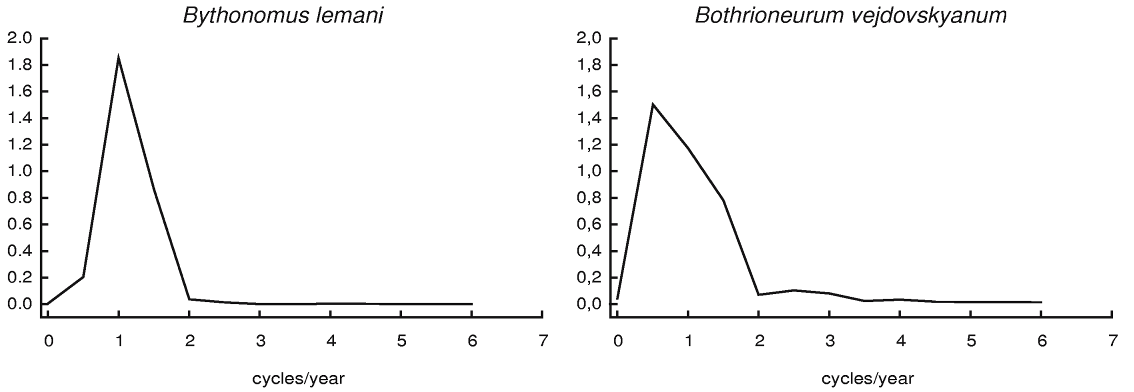

Fig. 6. Peridiograms of *Bythonomus lemani* and *Bothrioneurum vejdovskyanum* after extraction of smooth component.

of B. In habitat B stream line, the one generally preferred by this species, the ratio of adults to juveniles was balanced.

Time series analysis

The evaluation of smooth components showed significant trends for both species (Fig. 5). The density of *Byth. lemani* was increasing with Daniel's test value 0.51 ($P = 0.017$), while that of *Both. vejdovskyanum* was decreasing with Daniel's test value 0.54 ($P = 0.011$). None of the selected environmental variables showed a significant trend (using Daniel's test).

The evaluation of cyclic components using autocorrelation showed certain cyclic trends, but they were insignificant. Peridiograms showed one distinct cycle per year in both species (Fig. 6).

The cross-correlations for the density of *Byth. lemani* and *Both. vejdovskyanum* and environmental variables showed significant positive cross-correlation of *Byth. lemani* with water temperature (two-month lag). No other significant relationships between either species or environmental parameters were found. Comparison of trends of species densities and environmental parameters revealed some significant trends; a negative correlation between abundance of *Byth. lemani* and BOD ($r_\mathrm{s} = 0.59$; $P < 0.05$).

Discussion

Although Oligochaeta are generally considered as ubiquists that live in various habitats, significant habitat preferences were found in the two selected species. *Byth. lemani* preferred sites with the highest water velocity (0.53–1.46 m s^{-1}), hence we can confirm it as a running water species (Rodriguez & Armas, 1983) requiring a sufficient level of dissolved oxygen. Other habitats were colonised with significantly lower densities – the straight part of the stream was preferred by adults, the littoral by juveniles. The arrangement of sexual organs in this species corresponds to that found by Rodriguez (1988), but we did not record prostate cells in discrete masses as stated by Brinkhurst & Jamieson (1971).

Both. vejdovskyanum as a typical tubificid inhabited the straight part of the stream, the substratum of which did not shift for most of the year. This distribution decreased the competition between *Byth. lemani* and *Both. vejdovskyanum*, which were the dominant species at this site.

Based on worm densities in the Rokytná River during a two-year study, a model of the reproductive cycle of *Byth. lemani* was created. The spectral analysis was computed and its peridiogram showed one distinct reproduction cycle per year. Although some adults were present throughout the year, main breeding activity started in April. The correlation with water temperature indicates that increasing temperature in March may have triggered the development of sexual organs and the subsequent copulation of worms. Increasing abundances of juveniles between August and November indicates that worms were hatching from cocoons at that time. The juvenile oligochaetes had probably grown during the winter months and matured during the spring or summer of the next year. Such a life cycle is comparable with that of another lumbriculid, *Stylodrilus heringianus* (Petto & Humpesch, 1992). These authors also found a one-year cycle and significant correlation with water temperature. A similar one-year life cycle was recorded for *S. parvus*, with numerous adults in May and the highest percentage of juveniles in October (Dumnicka & Kukuła, 1990).

The significant negative correlation of *Byth. lemani* densities and BOD values can be explained by ecological adaptation of this species to changing conditions within the stream. According to Uzunov et al. (1988), it is an oligo- (80%) or β-mesosaprobic (20%) water species preferring a small amount of organic pollution in water. During the part of the year when concentrations of the dissolved organic matter are highest, most adults die after deposition of cocoons and the development of juveniles inside the cocoons takes place. Juveniles' hatching and growth start in the autumn after BOD values have decreased. The total trend showing an increase in the abundance of the oligosaprobic water species *Byth. lemani* and a decrease in the β-mesosaprobic species *Both. vejdovskyanum* (Uzunov et al., 1988) probably reflected overall changes in water quality at the site. Our data, however, are insufficient to specify the impact of particular environmental variables.

The reproductive strategy of *Both. vejdovskyanum* (Tubificidae) is different. The species reproduces by architomy, and adults are found only sporadically. Our peridiogram showed a one-year developmental cycle. These results can be compared with a one year study by Hrabě (1935a, b) who found worms, with up to 140 segments in winter, whose fission started in the second half of April, continued during summer, declined in autumn and completely stopped in winter. Similarly, in laboratory experiments, *Both. vejdovskyanum* reproduce by fragmentation without any marks of degeneration for several years (Timm, 1984).

When looking at the asexual reproduction of the Naididae family, we can find the numbers increasing with temperature in the genus *Nais* (e.g., Poddubnaya, 1968; Pfannkuche, 1981; Dumnicka, 1982), except for *Nais elinguis* Müller, 1773 (Learner et al., 1978). No periodic cycle as a result of asexual reproduction was found in the genus *Chaetogaster* (Petto & Humpesch, 1992).

We can conclude that the life history of the sexually reproducing lumbriculid *Byth. lemani* consists of a one-year reproduction cycle regulated by temperature; further knowledge of its seasonal dynamics could be obtained by sampling the smallest juveniles and cocoons. Under the same conditions asexual reproduction of the tubificid *Both. vejdovskyanum* was not affected by temperature, although it also showed a one-year reproduction cycle. For this species, a longer study period in the field will be necessary to find out which environmental parameter(s) regulate reproduction.

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The distribution of chironomid larvae and oligochaetes within a stony-bottomed river stretch: the role of substrate and hydraulic characteristics

Vít Syrovátka*, Jana Schenková and **Karel Brabec**[1]

With 9 figures, 4 tables and 1 appendix

Abstract: The taxonomic and functional structure of oligochaete and chironomid communities in a stony-bottomed river reach was investigated with respect to substrate and hydraulic conditions. Combined oligochaete and chironomid data were analyzed to search for main gradients and the separate data sets were analyzed to identify the most important variables determining the structure of the communities. Preferences for particular hydraulic conditions, expressed as Froude number, were calculated for 29 most abundant taxa. The results of Correspondence Analysis (CA) indicated that the main gradient in the data could be explained by hydraulic conditions and, inversely, by the amount of sedimented particulate organic matter (POM). The second CA axis probably reflected the quantity of the available food and space resources. Canonical Correspondence Analysis (CCA) showed the amount of sedimented POM as the most important variable explaining 21 and 24 % of the variance in the oligochaete and chironomid data, respectively. Among the other variables, the occurrence of aquatic vegetation, variability of near-bottom current velocity and substrate roughness were the most important. In the case of chironomids, the proportions of functional feeding strategies changed along the gradient of hydraulic conditions: the proportions of predators, active filter-feeders and collectors/gatherers decreased with the increasing hydraulic stress, while the proportion of grazers/scrapers increased. Nevertheless, in the case of oligochaetes, the proportions of functional feeding strategies did not follow the gradient of hydraulic conditions. Although the total abundance of both oligochaetes and chironomids was independent of hydraulic conditions, only few oligochaete taxa were able to succeed in hydraulically rough conditions and most oligochaete taxa were found only in pools. On the other hand, chironomids showed high taxa richness, which seemed to be limited by the quantity of the available food and space resources rather than hydraulic stress.

Key words: chironomidae, oligochaeta, distribution, substrate, hydraulic conditions, stream.

Introduction

Hydromorphological features of rivers reflecting long-term climatic and geomorphological processes are being influenced by anthropogenic impacts and global changes. The knowledge of distributional patterns of river biota and its habitat preferences is fundamental to effective assessment of possible effects of climate fluctuations and/or anthropogenic impacts on fluvial ecosystems. In this respect, the following paper aims to contribute to the knowledge of aquatic macroinvertebrate distributional patterns.

Physical factors, food availability and biological interactions are generally proposed as determinants of spatial variation in the stream community structure (e.g. Hynes 1970, Cummins et al. 1984, Statzner et al. 1988). At a larger scale (e.g. catchment) the main changes in the benthic macroinvertebrate community structure are usually found along the longitudinal gradient from source to mouth of a stream and the influ-

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ence of particular physical and chemical factors is difficult to separate because all the factors usually change along with the distance from the source (Lindegaard & Brodersen 1995).

At a finer scale (mesohabitat – sensu Armitage et al. 1995) hydraulic and substrate conditions are the major factors influencing the benthic macroinvertebrate community structure. These factors are highly interrelated and each in part depends on the other. The substrate grain size is determined by past and present flows (Leopold 1994, Allan 1995) and the grain size determines surface roughness which, in turn, creates fine-scale patterns of near-bed flow that influence organic matter retention and the distribution of benthic organisms (e.g. Culp et al. 1983, Quinn et al. 1996, Bouckaert & Davis 1998, Hoover et al. 2006). Some studies emphasize the importance of substrate – especially its organic components – in determining the density, taxa richness, and overall structure of benthic invertebrate community (Ward 1975, Death & Winterbourn 1994, Way et al. 1995, Beisel et al. 1998, Habdija et al. 2004, Ortiz et al. 2006) but most of the recent studies are in agreement that the hydraulic conditions play the major role (Quinn & Hickey 1994, Rempel et al. 2000, Doisy & Rabeni 2001, Mérigoux & Dolédec 2004, Brooks et al. 2005).

The mean flow velocity is not an adequate descriptor of the flow conditions. Therefore, some complex hydraulic parameters incorporating the velocity, depth and eventually substrate characteristics were proposed (Davis & Barmuta 1989). These hydraulic parameters (e.g. Froude number, boundary Reynolds number, roughness shear velocity) have proved to be better predictors of the benthic invertebrate community structure than the measured current velocity not only in cases when the substrate roughness and depth varied within the studied locality (e.g. Qiunn & Hickey 1994, Doisy & Rabeni 2001, Fesl 2002, Mérigoux & Dolédec 2004, Brooks et al. 2005). Moreover, easily accessible Froude number (incorporates only the velocity, depth and acceleration due to gravity) was found to be strongly related to invertebrate abundance in many other studies (e.g. Orth & Maughan 1983, Wetmore et al. 1990, Jowett et al. 1991, Benbow et al. 1997). Froude number was also identified as a good hydraulic parameter for objective identification of pool, run, and riffle habitats (Jowet 1993).

Oligochaeta (sensu oligochaetous Clitellata; Erséus 2005) and chironomid larvae often comprise a large proportion of benthic macroinvertebrate communities but due to their small body size and difficulties associated with their identification, their habitat preferences and distributional patterns at the mesohabitat scale are not well known. For chironomid larvae only the works of Ruse (1994) and Fesl (2002) deal with the spatial distribution with regard to hydraulic conditions, and some other studies focus on the preferences of particular species to particular hydraulic conditions (Collier 1993, Benbow et al. 1997). Only few studies bring any information about meso- or microhabitat preferences of oligochaete taxa (Verdonschot 1999, 2001).

Oligochaetes and chironomid larvae differ in many ways (their physique, life cycle, reproduction), but they are similar in their relatively small, prolonged and flexible body. Their body proportions together with the locomotion type – sprawling/walking, and the absence of legs enable them to inhabit bed sediments into notable depths (Schmid 1993, Rulík 1995). Some taxa of both groups are able to survive in low-oxygen to anoxic conditions. Collecting/gathering and grazing/scraping predominate within these groups, though they differ in feeding mechanisms: contrary to the oligochaetes, chironomids use chitinous mouthparts such as mandibles and mentum to graze. Moreover, some chironomid larvae can utilize particulate organic matter (POM) using active or passive filtration which is very rare within the oligochaetes (Learner et al. 1978).

In this study, spatial distribution of oligochaetes and chironomid larvae at the mesohabitat scale was investigated. The two groups were chosen because of their high abundance in benthic stream communities and their ecological similarity. Sampling was carried out simultaneously at a single river reach which enabled elimination of temporal variability and of factors associated with the longitudinal stream zonation.

The objectives were to i) determine the main gradients in the structure of oligochaete and chironomid communities, ii) identify the most important factors influencing the taxonomic structure of these communities, iii) identify oligochaete and chironomid community types and relate them to particular environmental conditions, and iv) determine habitat preferences of frequent taxa.

Study site

The Svratka River (Czech Republic) is a rather stable simple channelled stream, which drains the Czech-Moravian Highlands with siliceous bedrock. The catchment area of about 354 km^2 is dominated by coniferous forests (42 %) and arable land (32 %), followed by agricultural areas with significant areas of natural vegetation (14 %), pastures (6 %), urban fabric (3 %) and transitional woodlands-shrubs (2 %). Mean annual rainfall in the catchment is about 746 mm year^{-1}.

The samples were taken from an approx. 300 m long reach located in the Czech-Moravian Highlands, 480 m above sea level (49° 36′ 51″ N, 16° 13′ 59″ E). The Svratka is a mid-sized river of the 5th Strahler's order at this site. The mean slope of the sampled reach is 0.28 % and the mean channel width is 12.5 m. Both river banks are bordered by willows and shrubs that pass further to a coniferous forest on the left and arable land on the right side. The median annual discharge in 2002 was 1.9 $m^3 s^{-1}$ (10 % and 90 % quantiles were 1.1 $m^3 s^{-1}$ and 8.2 $m^3 s^{-1}$, respectively). The mean annual water temperature in 2002 was 6.9 °C with the summer maximum of 20.2 °C.

On the sampling date, water conductivity 164 µS cm^{-1} and pH 7.36 were recorded. The stream-bed substrate was dominated by cobbles (mesolithal) but ranged from sand (psammal) to boulders (macrolithal). The mineral substrate was covered with fine particulate organic matter (FPOM) in the deposition areas with low current, where also patches of leaf accumulation occurred. Living parts of terrestrial plants (grass leaves, roots) were observed at the margins. Patches of aquatic mosses (*Fontinalis antipyretica* Hedw. and *Hygrohypnum ochraceum* (Turn. ex Wils.) Loeske) and *Ranunculus* sp. were recorded in the streamline and the "bare" mineral substrate was usually overgrown with diatoms.

Methods

Sampling and physical measurements

The sampling was carried out on one occasion (3–4 October 2002) during stable and low flow conditions (1.25 $m^3 s^{-1}$). The closest high flow event was recorded 50 days before sampling, which ought to be sufficient time for stream recovery and recolonization of habitats.

Mesohabitats (visually distinct areas) were initially visually assessed according to surface flow characteristics, current velocity, depth and substrate, and their proportions within the studied river reach were estimated (± 5 %). In total, 27 samples were taken. Twenty macroinvertebrate sampling points were distributed among the mesohabitats proportionally to their share (one sampling point was assigned for each 5 % of the river reach). Within each mesohabitat the sampling points were randomly positioned. Additional 7 macroinvertebrate sampling points were randomly positioned at mesohabitats represented by less than 5 % in order to collect samples from the majority of hydraulic conditions available throughout the study site.

A short underwater video sequence was taken prior to sampling at each sampling point. It was used to estimate the amount of organic matter at the sampling points. The dominant and subdominant substrate was visually classified into categories according to Hering et al. (2004) and the presence of aquatic vegetation at the sampling point was recorded. The depth was measured using a simple mechanic device (Fig. 1a,b) at 25 points arranged in a matrix of 5 by 5 points with 5 cm distance intervals. Although this procedure was very fast (a few seconds) it might have disturbed the benthic organisms. A sampling net was therefore placed downstream the investigated area before the depth measurements, and the sample was taken immediately afterwards.

Samples were taken using a hand net (mesh size 250 µm) from an area of 0.0625 m^2 (25 × 25 cm) where the depth was measured. During sampling the substrate was disturbed by hands into the depth of approx. 5 cm. Samples were preserved in 4 % formaldehyde solution.

Current velocity was measured after the sampling (portable water flow meter, The Marsh-McBirney Model 2000 Flow-Mate) in 4 depths (2 cm from the bottom and in 0.2, 0.4 and 0.8 depth from the bottom) at 5 points distributed along the right and upper edge of the disturbed sampled area.

Water temperature and discharge data were obtained from the nearest gauge station (Dalečín, approx. 3 km downstream). There are no major tributaries between the gauge station and the study site.

Laboratory procedure

The samples were sorted under dissecting microscope in the laboratory. All the macroinvertebrates were picked up and stored in 75 % ethanol. Only oligochaetes and chironomid larvae were identified under microscope and counted for this study. Small oligochaete specimens (genera *Nais* and *Pristina*) were slide-mounted in the glycerine-water solution (1:5), larger oligochaetes and all chironomid head capsules in the Canada balsam. Oligochaetes were identified according to Hrabě (1954, 1981), Brinkhurst & Jamieson (1971), Timm (1999) and Timm & Veldhijzen van Zanten (2002) and chironomid larvae according to Cranston (1982), Fittkau & Roback (1983), Nocentini (1985) and Schmid (1992).

Calculated and estimated environmental parameters

For the data analyses averages of the five measured values of the current velocity for each depth were calculated and their standard deviations were used as a measure of the current velocity variability in particular depths. Depth was calculated as the median value of the 25 measured depths.

A smooth inclined plane may provide high substrate roughness if calculated as an ordinary standard deviation of the measured depths. Therefore, a plane was fitted linearly through the 25 measured depths (arranged in the matrix of 5 × 5 points) and the residues of the measured depths from the plane were calculated. The standard deviation of these residues was used as the measure of substrate roughness within each sampling area.

The amount of particulate organic matter (POM) was estimated using the underwater video sequences. Values 0–3 were assigned to each sampling area according to the quantity of visible POM (0 – no visible sedimented organic particles (usually riffles), 1 – some sedimented organic particles present, 2 – fine organic particles covering most of the mineral substrate, and 3 – the substrate composed mainly of organic parts).

Substrate types were coded according to the estimated particle size of the dominant mineral substrates. Values 0–6 were assigned for each substrate type: organic substrate (0), psammal (1), akal (2), microlithal (3), mesolithal (4), macrolithal (5) and megalithal (6). For the description and the particle size of the

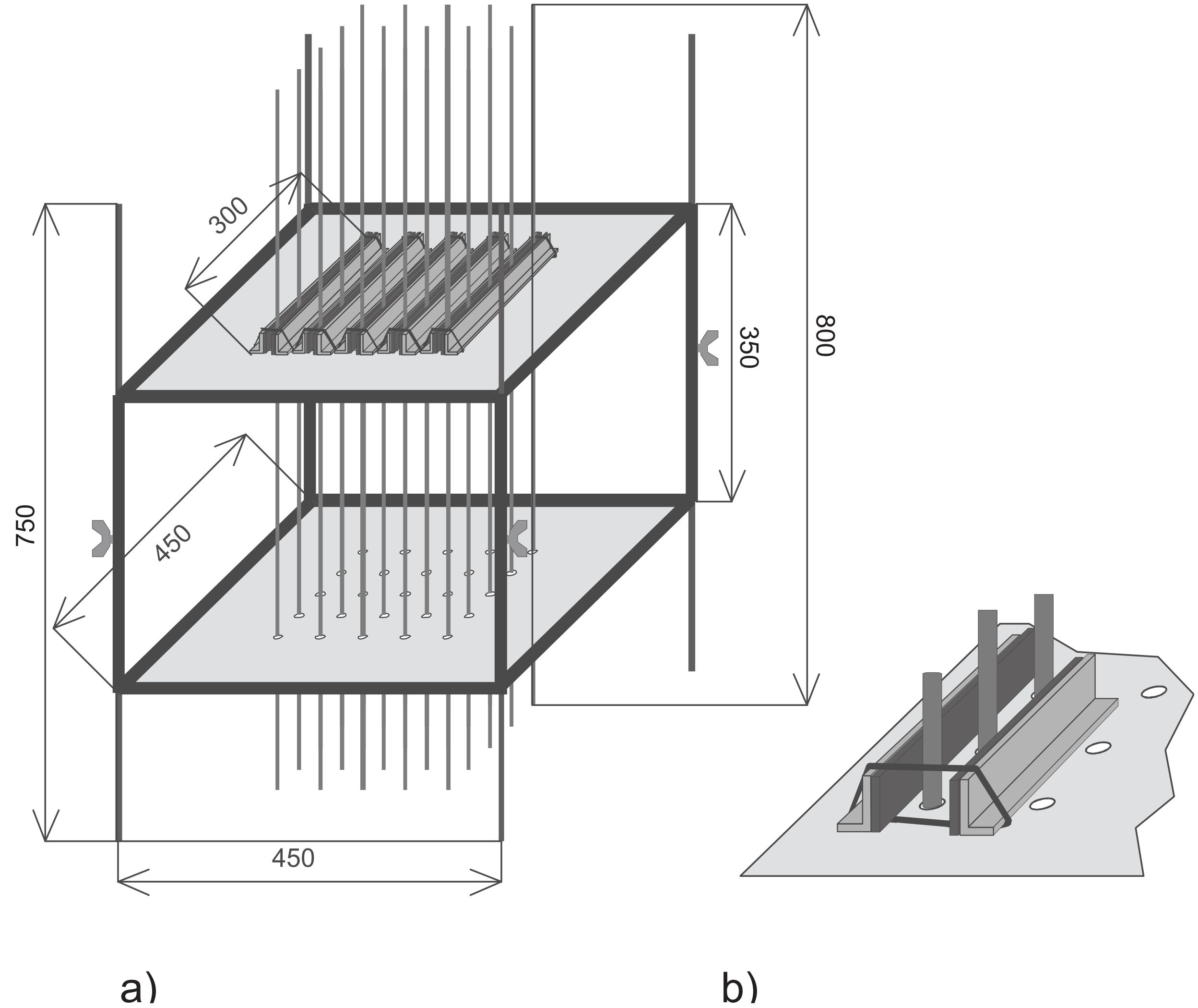


Fig. 1. The device used for substrate roughness measurements. Two plastic boards are perforated in the middle (25 holes, Ø 11 mm) and fitted in the metal frame equipped with four adjustable legs. 25 aluminium tubes (Ø 10 mm) go through the holes in both boards and are blocked on the upper board's surface by two 300 mm long L-shaped aluminium sticks tight together with two rubber bands. A 5 mm thick cellular plastic strip is glued to the inner surface of each aluminium stick to adhere better to the surface of the tubes. Each pair of the aluminium sticks operates 5 tubes. After the positioning of the device at the sampled area, the boards were set horizontally using the adjustable legs. Then the pairs of the aluminium sticks were pulled apart, so that the tubes could slide down to the stream-bed. The aluminium sticks were then released again and locked the tubes in their position. As soon as all the tubes were locked, the whole device was removed from the stream and the tubes were measured on the bank. **a)** the whole device; **b)** a detail of the aluminium sticks in the open position.

substrate types see Hering et al. (2004). Hydraulic parameters that were calculated included:

Froude number (Fr) = $U/(gD)^{1/2}$
Reynolds number (Re) = $(UD)/v$
roughness shear velocity (U_*) = $U/\ (5.75 \log_{10}(12\ D/k_v))$
inferred boundary Reynolds number (Re_*) = $(U_*\ k_v)/v$

where D = depth, g = acceleration due to gravity, k_v = substrate roughness, U = current velocity at 0.4 depth and v = kinematic viscosity of water (1.394×10^{-6} m^2 s^{-1} at 7.8 °C). All the measured and calculated environmental variables and their ranges are listed in Table 1.

Community parameters

As the measure of the oligochaete and chironomid diversity, number of taxa standardized according to Heck et al. (1975) to 50 individuals of each taxonomic group per sample was used. If there were fewer individuals in a sample, the taxa richness was not calculated and the sample was omitted.

The proportions of particular feeding strategies within samples (further in text as proportions of collectors/gatherers, predators etc.) were calculated using species traits characteristics listed in the autecological database used in software ASTERICS (AQEM consortium 2002; see also www.aqem.de and

Table 1. Ranges of measured and calculated environmental variables.

Variable	Abbreviation	Range of values
Water depth (cm)	depth	12.4–50.1
Substrate roughness (SD of 25 water depths)	roughness	0.66–5.77
Current velocity in 2 cm from the bottom (cm s^{-1})	cur_2	0.0–54.8
Current velocity in 0.2 depth from the bottom (cm s^{-1})	cur_0.2	0.2–65.2
Current velocity in 0.4 depth from the bottom (cm s^{-1})	cur_0.4	0.2–95.8
Current velocity in 0.8 depth from the bottom (cm s^{-1})	cur_0.8	3.2–132.0
Variability of current velocity in 2 cm (SD)	SDvel2 cm	2.55–37.44
Variability of current velocity in 0.2 depth (SD)	SDvel0.2	2.05–32.28
Variability of current velocity in 0.4 depth (SD)	SDvel0.4	1.41–47.25
Variability of current velocity in 0.8 depth (SD)	SDvel0.8	1.79–26.27
Froude number	Fr	0–0.56
Reynolds number	Re	0–2061
Roughness shear velocity	U_*	0–40.35
Inferred boundary Reynolds number	Re_*	0–52.56
Presence of aquatic vegetation	VEG	0/1
Category of the amount of particulated organic matter	POM	0–3
Category of mineral substrate particle size	mineral	0–6

www.freshwaterecology.info/). Species traits characteristics of related taxa were used when the characteristics of the respective taxon were not available (6 chironomid taxa).

To compare the success of two most frequent feeding strategies (collecting/gathering and grazing/scraping) all taxa were classified into the following feeding groups: pure collectors/gatherers (col), partial collectors/gatherers and partial grazers/scrapers (col/gr), pure grazers/scrapers (gr), and others (other). Taxa richness and abundance of particular feeding categories were computed for each sample from 100 random selections of 100 individuals. Random selections were performed using MATLAB Version 6.1.0.450. The classification of taxa is given in Appendix 1.

Data analyses

For multivariate analyses the species data were log (x+1) transformed. Detrended Correspondence Analysis (DCA) was used to identify the length of the main gradient in the faunal data. In all cases the gradient lengths were between 1.5 and 3.0 standard deviation units (2.3, 2.0, and 1.9 SDU in the oligochaete, chironomid, and combined data respectively) indicating that both the linear and unimodal models were suitable (Ter Braak & Prentice 1988).

Correspondence Analysis (CA) was used to search for the main gradients in the combined faunal data and Spearman rank correlation coefficients of the sample scores with the environmental and the community parameters were computed to explain those gradients. Canonical Correspondence Analysis (CCA) (Ter Braak 1986) using stepwise forward selection was performed to identify environmental parameters which could account for the maximum variation in the oligochaete and chironomid data separately. All environmental variables were used in this analysis and the significance of each variable was tested by 999 unrestricted Monte Carlo permutations.

CANOCO for Windows version 4.5 was used for all ordinations. Correlations were performed using STATISTICA for Windows version 7.

In the next step, two cluster analyses and subsequent two Indicator Species Analyses (Dufrene & Legendre 1997) were performed to group communities according to the oligochaete and chironomid taxonomic structure separately, and to identify the taxa characteristic of the community types obtained by the cluster analysis. Bray/Curtis (Sørensen) dissimilarity index was used as the distance measure and the grouping was done using the Flexible Beta linkage method with beta value set at -0.4. The significance of indicator taxa was tested by 999 Monte Carlo permutations. Only the taxa with the Indicator Value higher than 50 were considered good indicators. Clustering and Indicator Species Analysis were performed using PC-ORD for Windows Version 4.17.

Hydraulic conditions were identified as the major factor influencing the structure of the oligochaete and chironomid communities, and therefore the preferences of particular taxa for particular hydraulic conditions expressed as the Froude number were assessed. Medians and 10, 25, 75, and 90 % quantiles of the Froude number weighted by the abundance of a given taxon were calculated for each taxon and the values were used to demonstrate different hydraulic preferences of the taxa. Only the taxa represented by at least 50 individuals were analyzed. The result of this analysis might be affected by the irregularity in the distribution of Froude numbers within the data set. As there were more samples from habitats with low Froude number than from habitats with high Froude number, eight samples were removed to obtain as even distribution of Froude numbers as possible.

Results

Faunal data

In total 6,275 specimens of 35 oligochaete taxa and 10,272 specimens of 56 chironomid taxa were collected. The abundance of oligochaetes and chironomid

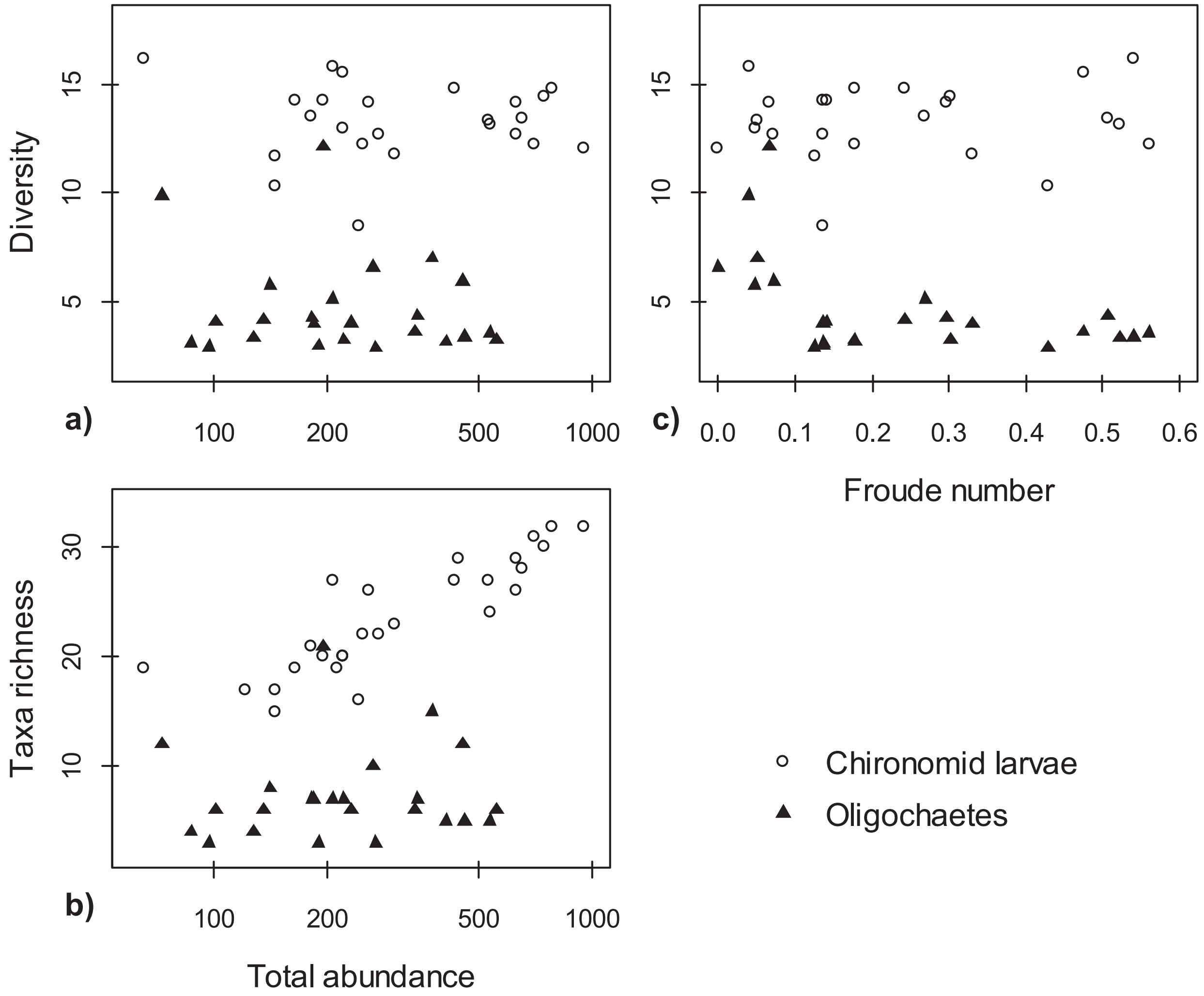


Fig. 2. Scatter plots showing the relationships between **a)** the diversity and total abundance of the oligochaete and chironomid communities, **b)** their taxa richness and total abundance, **c)** their diversity and Froude number. Only the communities with more than 50 individuals are displayed.

larvae in samples ranged from 21 to 558 (median = 195) and from 65 to 957 (median = 258) individuals respectively. The number of oligochaete taxa ranged between 3 and 21 (median = 6), the number of chironomid taxa ranged between 15 and 32 (median = 23) and the taxa richness of both taxonomic groups combined ranged between 18 and 50 (median = 28). Total abundances and frequencies of all taxa are listed in Appendix 1.

Abundance and taxa richness patterns

The total abundance and diversity of chironomid communities were independent of hydraulic conditions (Fig. 2c). Nevertheless, the taxa richness of chironomids was logarithmically dependent on their total abundance (Linear regression model, Adjusted R-squared = 0.9836, $p < 0.001$) (Fig. 2b), suggesting their high diversity (Fig. 2a) and their ability to find favourable conditions in different environments within the sampled river section. Both chironomid total abundance and taxa richness were limited by the availability of food and space resources rather than hydraulic conditions.

The total abundance of oligochaetes was also independent of hydraulic conditions but their diversity was negatively related to the Froude number (Spearman Rank Correlation, R = 0.47, $p < 0.05$) (Fig 2c). Many oligochaete taxa occurred in pools of the studied river section but only few were able to succeed and reach high densities in habitats with Froude number higher than 0.1.

Table 2. Eigenvalues of the CA axes and Spearman rank correlations between the CA axes and environmental and community parameters. Only parameters that significantly correlated with at least one CA axis are included. Stars indicate the significance of the correlations (*** – $p < 0.001$, ** – $p < 0.01$, * – $p < 0.05$). S – total taxa richness, S_chir – chironomid taxa richness, chir diversity – chironomid diversity, N_chir – chironomid abundance, S_olig – oligochaete taxa richness, olig diversity – oligochaete diversity, COL_S – the taxa richness of collectors/gatherers, GR_S – taxa richness of grazers/scrapers, GR_N – abundance of grazers/scrapers, %GA – proportion of collectors/gatherers, %AF – proportion of active filter-feeders, %PF – proportion of passive filter-feeders, %PR – proportion of predators, %GR_chir – proportion of grazers/scrapers within chironomids, %GA_chir – proportion of collectors/gatherers within chironomids, %AF_chir – proportion of active filter-feeders within chironomids, %PF_chir – proportion of passive filter-feeders within chironomids, %PR_chir – proportion of predators within chironomids. For abbreviations of environmental variables see Table 1.

	Variable	AX1	AX2	AX3	AX4
Environmental variables	depth	0.12	-0.21	0.45 *	0.08
	cur_2	-0.82 ***	0.00	-0.34	0.18
	cur_0.2	-0.85 ***	0.01	-0.34	0.27
	cur_0.4	-0.84 ***	0.12	-0.37	0.20
	cur_0.8	-0.87 ***	0.12	-0.40 *	0.22
	SDvel2cm	-0.73 ***	0.43 *	-0.57 **	0.12
	SDvel0.2	-0.67 ***	0.43 *	-0.62 ***	0.06
	SDvel0.4	-0.63 ***	0.39 *	-0.78 ***	0.18
	SDvel0.8	-0.39 *	0.44 *	-0.68 ***	0.20
	Fr	-0.84 ***	0.15	-0.41 *	0.20
	Re	-0.67 ***	0.02	-0.09	0.22
	U*	-0.77 ***	0.05	-0.28	0.22
	Re*	-0.86 ***	0.19	-0.34	0.22
	VEG	-0.63 ***	0.51 **	-0.60 **	0.04
	POM	0.81 ***	-0.01	0.31	-0.06
	mineral	-0.46 *	0.09	-0.45 *	-0.51 **
Community parameters	S	0.37	0.56 **	-0.33	-0.22
	S_chir	0.22	0.73 ***	-0.25	-0.16
	chir diversity	0.04	0.52 **	-0.23	-0.43 *
	N_chir	0.16	0.58 **	-0.20	-0.10
	S_olig	0.48 *	0.24	-0.23	-0.16
	olig diversity	0.47 *	0.27	-0.30	-0.19
	COL_S	0.83 ***	0.19	0.11	-0.19
	GR_S	-0.86 ***	0.18	-0.56 **	0.16
	GR_N	-0.64 ***	0.55 **	-0.54 **	0.14
	%GA	0.00	-0.39 *	0.18	0.34
	%AF	0.70 ***	-0.09	0.52 **	0.05
	%PF	-0.12	0.57 **	-0.44 *	-0.22
	%PR	0.91 ***	0.13	0.31	-0.25
	%GR_chir	-0.88 ***	-0.21	-0.35	0.25
	%GA_chir	0.46 *	-0.24	0.39 *	0.39 *
	%AF_chir	0.71 ***	-0.16	0.67 ***	0.04
	%PF_chir	-0.16	0.54 **	-0.42 *	-0.28
	%PR_chir	0.89 ***	0.12	0.33	-0.30
	Eigenvalues	0.289	0.12	0.093	0.083
	Cumul. % var.	23.6	33.5	41.1	47.9

Main gradients in the combined data

The first four CA axes explained almost 48 % of the variance in the faunal data and the sum of all eigenvalues was 1.223 (Table 2, Fig. 3).

The sample scores on the first CA axis were correlated most with the amount of POM (positively) and with hydraulic parameters (negatively) such as the inferred boundary Reynolds number, Froude number, roughness shear velocity, current velocity and the current velocity variability in different depths (Table 2). Considering the community parameters, the first CA axis correlated positively with the proportion of predators and active filter-feeders and with the taxa richness

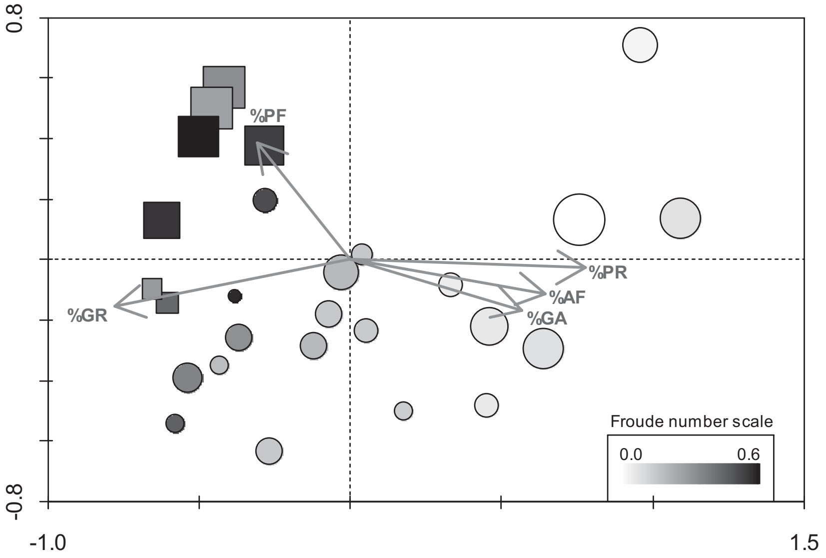


Fig. 3. CA ordination diagram showing the position of samples on the first and second CA axes. The area of samples reflects the total chironomid abundance and the symbols' grey tones indicate Froude number. Proportions of chironomid feeding strategies are displayed as passive variables. (%AF proportion of active filter-feeding, %GA collecting/gathering, %GR grazing/scraping, %PF passive filter-feeding, %PR predation, ☐ samples from habitats with aquatic vegetation, ○ the other samples)

of collectors/gatherers, and negatively with the taxa richness and abundance of grazers/scrapers (Table 2). This axis correlated also with the proportions of collectors/gatherers (positively) and grazers/scrapers (negatively), but only if calculated from chironomid data alone (Table 2, Fig. 3).

The sample scores on the second CA axis were positively correlated with the presence of aquatic vegetation and weakly with the current velocity variability in different depths. Of the community parameters, the second CA axis correlated most with the taxa richness and total abundance of chironomids (positively) (Table 2). These parameters separated habitats rich in chironomids from poorer ones so that the vegetated riffles and runs together with pools rich in POM appeared in the upper part of the CA ordination diagram, while the habitats with bare mineral substrate in the lower part (Fig. 3). The second CA axis correlated also with the proportion of the passive filter-feeders, and with the abundance of grazers and weakly with the total taxa richness (positively) and the proportion of the collectors/gatherers (negatively) (Table 2, Fig. 3).

The sample scores on the third CA axis were negatively correlated with the current velocity variability in different depths and with the presence of aquatic vegetation. These correlations were stronger than those between the second CA axis and the same parameters (Table 2). The third CA axis also weakly correlated with the Froude number, current velocity in 0.8 depth, water depth and the category of the mineral substrate particle size (Table 2). Of the community parameters, the third CA axis correlated with the abundance and taxa richness of grazers and the proportion of the passive filter-feeders (negatively) and the active filter-feeders (positively) (Table 2).

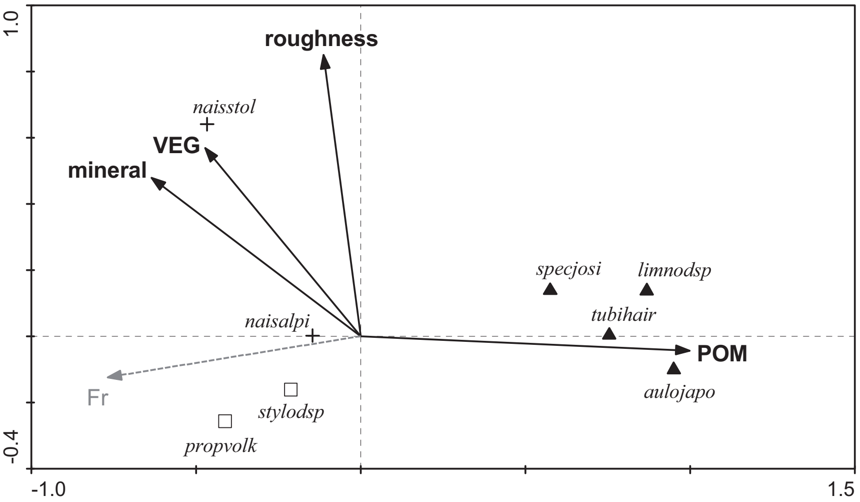
It is difficult to interpret the directions of the second and the third largest variability in the data using the available parameters. Both the second and the third CA axes were correlated with similar parameters and most of these correlations were rather weak. Nevertheless, it seems that the direction of the second largest variability might be explained by the taxa richness and abundance of chironomids, while the direction of the third one by the presence of aquatic vegetation and the associated variability in current velocity, though the presence of aquatic vegetation influences also the taxa richness and abundance of chironomids.

The fourth ordination axis weakly correlated only with the particle size of the mineral substrate (Table 2).

Oligochaete community structure in relation to environmental parameters

The CCA suggested that the amount of POM followed by hydraulic parameters were the most significant parameters explaining the highest portion of variability within the oligochaete data. Using stepwise forward selection the amount of POM, category of the mineral substrate particle size, the substrate roughness and the presence/absence of aquatic vegetation, explaining 21.36, 7.51, 6.35, and 5.2 % of the total variance in the oligochaete data respectively, were selected as statistically significant parameters influencing the oligochaete community structure (Fig. 4).

Fig. 4. CCA ordination diagram (the first and second axes) showing the positions of oligochaete taxa in relation to environmental variables significantly influencing the oligochaete community structure. Only the taxa with species fit and species weight > 10 % are displayed. Froude number is displayed as a passive variable. (mineral – the category of mineral substrate grain size, POM – the category of the amount of particulate organic matter, roughness – substrate roughness, VEG – the occurrence of aquatic vegetation, Fr – Froude number, ☐ indicator taxa of the second community type, ▲ indicator taxa of the third community type, + other taxa).



Oligochaete community types

Cluster analysis identified three types of oligochaete communities (Fig. 5), though *Stylodrilus* sp. and *N. alpina* comprised the majority (from 60 % to 98 %) of the oligochaete community at almost all sampled patches:

The first type included communities from pool habitats with mineral substrate and from riffles with aquatic mosses (10 patches). The communities of these quite different habitats were grouped probably because of their low diversity and total abundance (Fig. 6). Only the most common taxa occurred at these habitats. The communities were dominated by *N. alpina* followed by *Stylodrilus* sp. or *N. stolci* that was found in runs and riffles with vegetation only (Fig. 4). Of the other taxa, *Propappus volki* was the only one occurring within these communities in considerable proportions. As all these taxa were common in other samples as well, no indicator taxa were identified for this community type.

The second type included communities from run and riffle habitats with mineral substrate and usually with aquatic vegetation (10 patches). The diversity was similar to the previous type. The communities were dominated by *Stylodrilus* sp. followed by *N. alpina* and *P. volki*. The proportions of *Stylodrilus* sp. and *P. volki* were higher than within the previous community type and *P. volki* was identified as a good indicator species (Table 3) (Fig. 4).

The third community type was characteristic of pools (Fig. 6) with high amount of POM (7 patches) and also living parts of terrestrial plants (grass leaves) occurred at three patches. It had almost twice as high taxa richness as both the previous types (Fig. 6). *N. alpina* dominated followed by *Stylodrilus* sp., *Aulodrilus japonicus*, Tubificidae with hairs, *Specaria josinae* and *Limnodrilus* sp. occurred in considerably high abundances and were identified as indicators of this community type (Table 3) (Fig. 4).

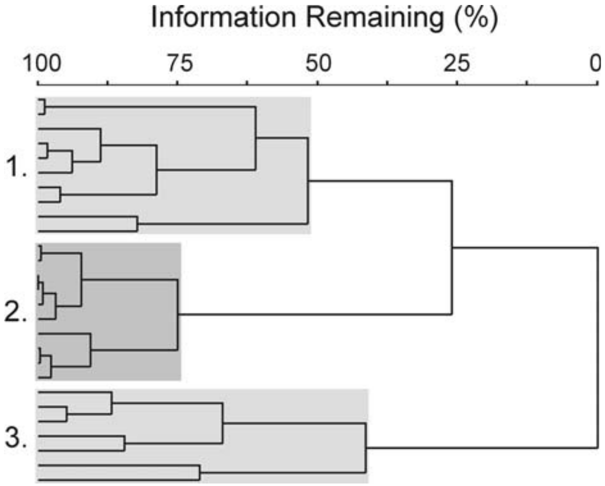


Fig. 5. Dendrogram showing the clustering of oligochaete communities into the three oligochaete community types.

Chironomid community structure in relation to environmental parameters

Similarly to the oligochaetes, the CCA suggested that the variability in the chironomid data was best explained by the amount of POM followed by hydraulic parameters. Using stepwise forward selection, the amount of POM, the current velocity variability near the bottom and the particle size of the mineral substrate, explaining 23.72, 8.63 and 4.31 % of the total variance respectively, were selected as statistically

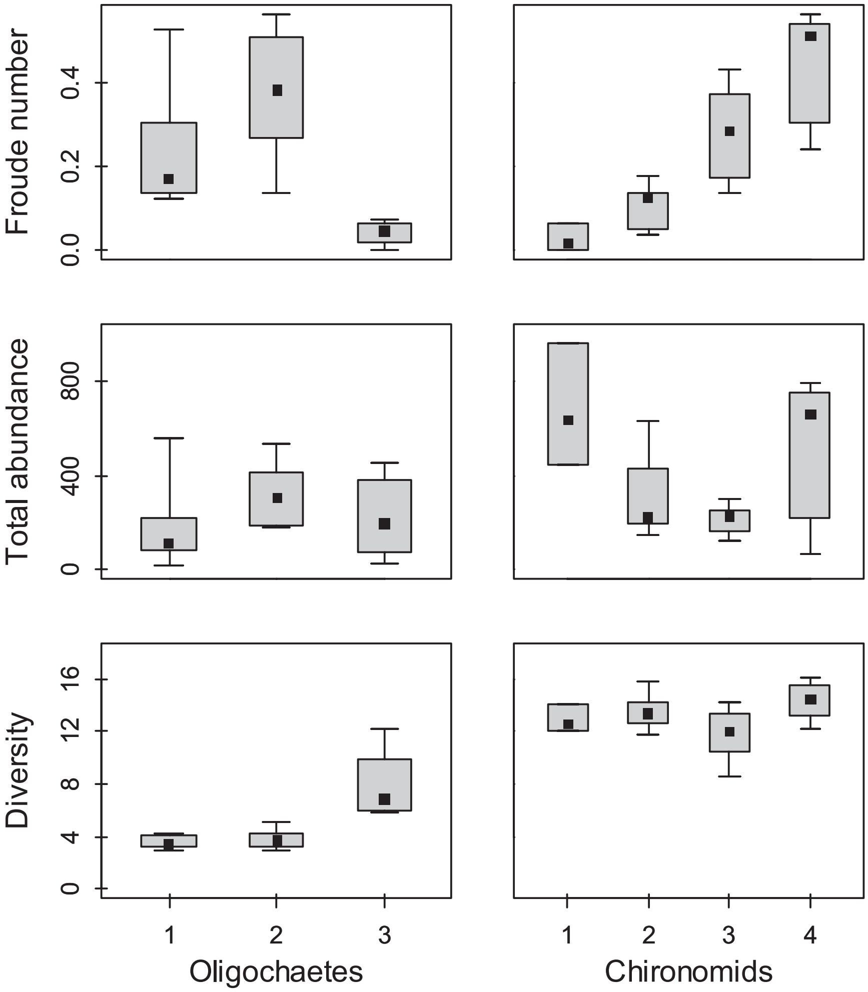


Fig. 6. Box-plots of the diversity, total abundance, and Froude number of the three oligochaete and four chironomid community types (square – median, box – quartiles, whiskers – ranges).

Table 3. Indicator taxa of the oligochaete community types. Indicator value (Ind. value), p-value (p) and median abundance within each community type are shown for each taxon. Values in grey refer to the habitat type of which the taxa are indicative.

			Community types		
Taxon name	Ind. value	p	1. Median abundances	2.	3.
Propappus volki	64.4	0.001	2	39.5	1
Stylodrilus spp. juv.	46.3	0.001	11	136	29
Aulodrilus japonicus	93.4	0.001	-	-	3
Tubificidae juv. with hair chaete	83.1	0.001	-	-	11
Limnodrilus spp. juv.	81.1	0.001	-	-	12
Specaria josinae	60.8	0.001	-	-	7
Rhyacodrilinae juv. with hair chaete	42.9	0.012	-	-	-
Pristina aequiseta	47.2	0.018	-	-	1
Stylaria lacustris	38.8	0.036	-	-	-

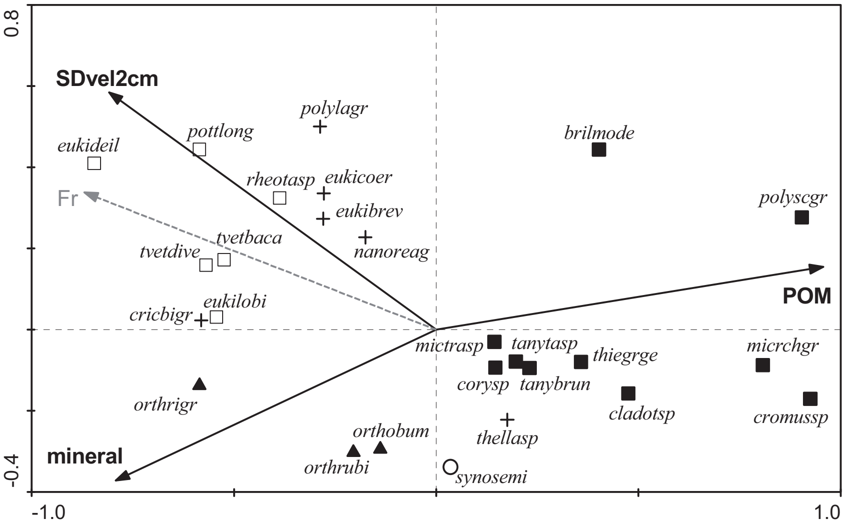


Fig. 7. CCA ordination diagram (the first and the second axes) showing the positions of chironomid taxa in relation to environmental variables significantly influencing the chironomid community structure. Only the taxa with species fit and species weight > 10 % are displayed. Froude number is displayed as a passive variable. (mineral – the category of mineral substrate grain size, POM – the category of the amount of particulate organic matter, SDvel2 cm – the current velocity variability 2 cm above the bottom. Fr – Froude number, ■ indicator taxa of the first community type, ○ indicator taxa of the second community type, ▲ indicator taxa of the third community type, □ indicator taxa of the fourth community type, + other taxa)

significant parameters influencing the structure of the chironomid community (Fig. 7).

Chironomid community types

In general, the taxonomic composition of chironomid communities was similar across all sampled patches. Most taxa occurred in the majority of habitat types but their proportions differed. Four different types of chironomid communities were identified using cluster analysis (Fig. 8). These community types may be interpreted in terms of different hydraulic conditions (Fig. 6).

The first community type occurred at three sampled patches with minor current (Fig. 6) and high amount of POM. The total chironomid abundance was high (Fig. 6) and *Microtendipes pedellus*-Gr. dominated. Further characteristics of this community type were high relative abundances of *Prodiamesa olivacea*, *Thienemannimyia*/*Conchapelopia*-Gr., *Corynoneura* sp. and taxa from the Tanytarsini tribe (*Micropsectra* sp., *Tanytarsus* sp., *Tanytarsus brundini*/*curticornis* and *Cladotanytarsus* sp.), and the presence of different taxa from the subfamily Tanypodinae (*Ablabesmyia* sp., *Apsectrotanypus trifascipennis*, *Natarsia* sp. and *Zavrelimyia* sp.). In contrast to the other community types, relative abundances of *Synorthocladius semivirens* and *Orthocladius* species were low. Ten good indicators of this group were identified (Table 4) (Fig. 7).

The second community type (9 patches) occurred at habitats with low current (Fig. 6) and mineral substrate sometimes covered with fine particulate organic matter (FPOM). It was characterized by the dominance of *S. semivirens* and high relative abundances of *Orthocladius* cf. *obumbratus* and *Orthocladius* cf. *rubicundus*. *M. pedellus*-Gr., *Thienemannimyia*/*Conchapelopia*-Gr., *Corynoneura* sp. and taxa from the Tanytarsini tribe (*Tanytarsus* sp., *T. brundini*/*curticornis*, *Micropsectra* sp. and *Cladotanytarsus* sp.) were again the most abundant taxa. If compared to the first community type, the same taxa from the Tanypodinae subfamily were present although in lower densities. Most of the taxa were common in other community types. *S. semivirens* was identified as a single indicator with low indicator value (Table 4) (Fig. 7).

The third community type occurred in runs and riffles (Fig. 6) with mineral substrate sometimes with aquatic mosses (8 patches). The lowest total chironomid abundances were observed (Fig. 6). *O.* cf. *obumbratus*, *S. semivirens* and *O.* cf. *rubicundus* were the most dominant taxa, together accounting for 50 – 80 % of the entire community. No Tanypodinae taxa were present except for *Thienemannimyia*/*Conchapelopia*-Gr., and practically no individuals of *M. pedellus*-Gr. Similarly, the relative abundances of *Corynoneura* sp. and the Tanytarsini taxa were lower. On the contrary, the proportions of *Orthocladius rivicola*-Gr., *Eukiefferiella* and *Tvetenia* species and *Rheotanytarsus* sp. were higher than in the previous community types. One good and two weak indicators were identified (Table 4) (Fig. 7).

The last community type was characteristic of runs and riffles (Fig. 6), often with aquatic vegetation. Similarly to the first community type, high total chironomid abundances were observed (Fig. 6). *Rheotanytarsus* sp. and both *Tvetenia* species dominated at the expense of *O.* cf. *obumbratus*, *O.* cf. *rubicundus* and

Table 4. Indicator taxa of the chironomid community types. Indicator value (Ind. value), p-value (p) and median abundance within each community type are shown for each taxon. Values in gray refer to the habitat type of which the taxa are indicative.

Taxon name	Ind. value	p	Community types 1.	 2.	 3.	 4.
			Median abundances			
Prodiamesa olivacea	92.9	0.001	5	-	-	-
Natarsia sp.	90	0.001	1	-	-	-
Apsectrotanypus trifascipennis	79.8	0.001	6	1	-	-
Corynoneura sp.	35	0.001	42	15	5	10
Microtendipes pedellus -Gr.	60.7	0.002	213	25	-	-
Thienemannimyia/Conchapelopia -Gr.	43.5	0.003	72	14	1	6
Phaenopsectra sp.	66.7	0.007	2	-	-	-
Micropsectra sp.	38.6	0.008	20	7	5	11
Paratendipes albimanus -Gr.	66.7	0.009	1	-	-	-
Polypedilum scalaenum -Gr.	65.4	0.009	7	-	-	-
Cryptochironomus sp.	65.2	0.009	5	1	-	-
Ablabesmyia sp.	54.5	0.009	1	-	-	-
Zavrelimyia sp.	44.4	0.014	1	-	-	-
Brillia bifida	59.5	0.018	4	-	-	1
Polypedilum convictum	48.8	0.021	1	-	-	-
Tanytarsus brundini/curticornis	37.5	0.039	19	10	2	5
Tanytarsus sp.	37.7	0.04	36	10	2	6
Cladotanytarsus sp.	44.7	0.049	9	5	-	-
Synorthocladius semivirens	32.7	0.003	17	70	51	9
Orthocladius rivicola -Gr.	58.2	0.005	-	-	9	1
Orthocladius cf. *rubicundus*	37.5	0.009	-	21	36	10
Orthocladius cf. *obumbratus*	34.3	0.014	4	28	49	12
Potthastia longimana	63	0.001	1	-	-	7
Tvetenia bavarica/calvescens	55	0.001	1	2	4	83
Tvetenia discoloripes/verralli	54.5	0.001	1	2	5	53
Eukiefferiella devonica/ilkleyensis	65.1	0.002	-	-	-	14
Eukiefferiella lobifera	54.2	0.002	-	-	2	6
Rheotanytarsus sp.	50.7	0.003	3	2	2	98
Orthocladius thienemanni	60.6	0.007	-	-	-	1

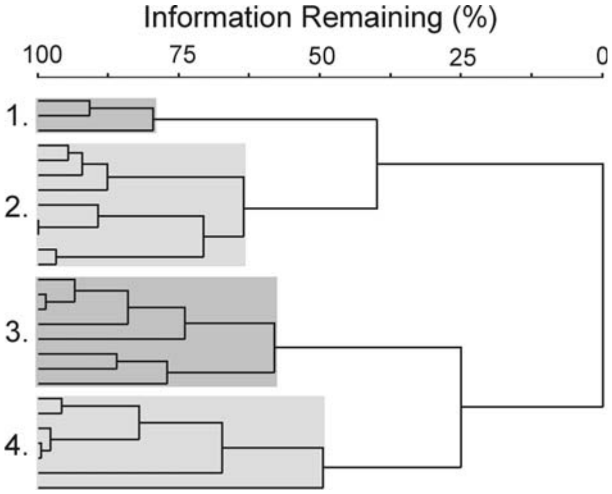


Fig. 8. Dendrogram showing the clustering of chironomid communities into the four chironomid community types.

S. semivirens, whose proportions decreased. Conversely, the proportions of *Eukiefferiella* species and *Potthastia longimana* increased to their observed maximum. The proportions of *Corynoneura* sp. and the Tanytarsini taxa were similar to those observed in the previous community type. Seven good indicators were identified (Table 4) (Fig. 7).

Hydraulic preferences of selected taxa

Hydraulic preferences of 6 oligochaete and 23 chironomid taxa were assessed (Fig. 9). The taxa showed different preferences for hydraulic conditions expressed as the Froude number. Generally, it was possible to identify i) taxa occurring only in pools with very low Froude number, ii) taxa occurring only in riffles and runs, and iii) taxa occupying most of (or all) the sam-

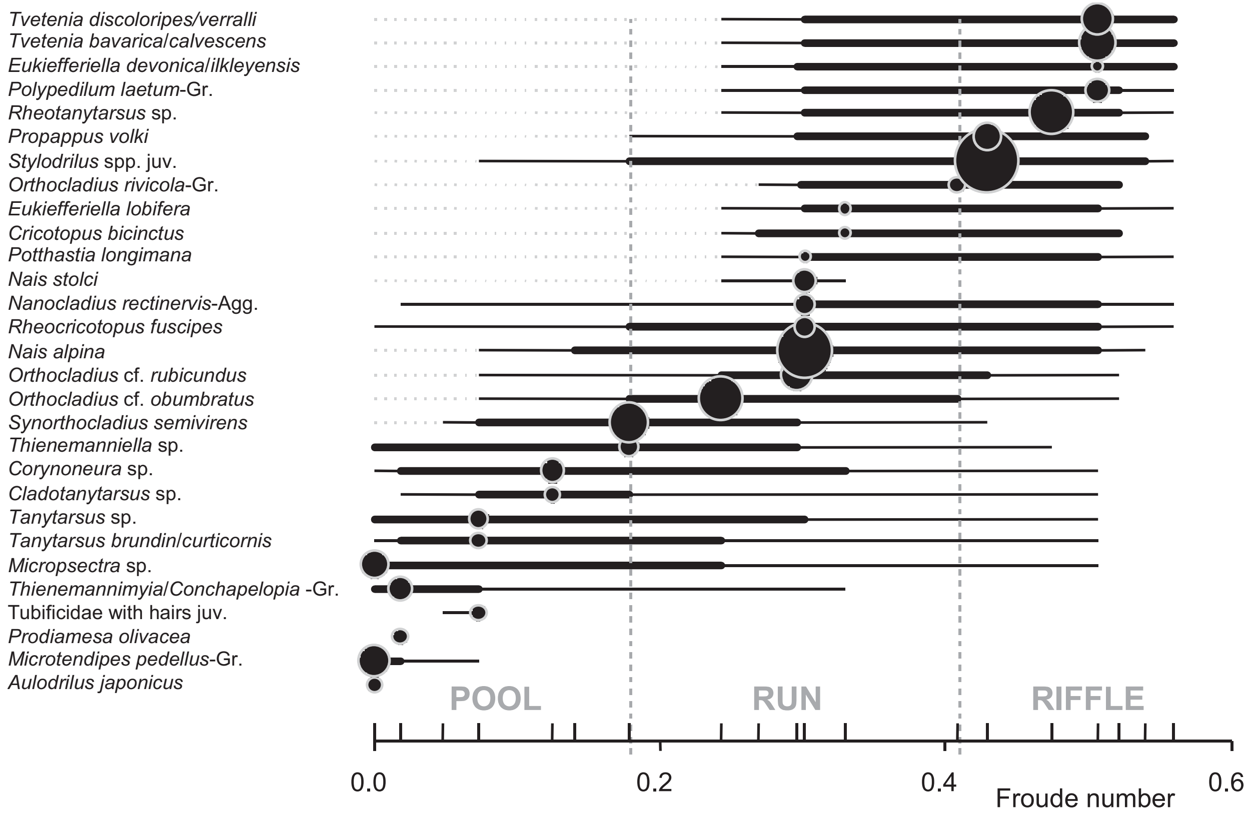


Fig. 9. Hydraulic preferences of frequent taxa. Medians (circles), lower and upper quartiles (thick lines) and 10 % and 90 % quantiles (thin lines) of Froude number are weighted by the abundances of taxa. The area of the circles reflects the total abundance of particular taxa. Tick marks pointing upwards indicate the position of the samples on the gradient of the Froude number. Pool, run and riffle habitats are identified according to Froude number (Jowett 1993).

pled habitats and having low or no preferences for hydraulic conditions.

Among the oligochaetes, three taxa preferred narrow ranges of Froude number: *A. japonicus* and Tubificidae with hairs occurred only in pools with very low Froude number, while *N. stolci* preferred runs (Fig. 9). The other three oligochaete taxa did not show such strong preferences: *N. alpina* was almost evenly distributed across all the sampled patches, *Stylodrilus* sp. slightly preferred runs and riffles and only *P. volki* occurred almost exclusively in runs and riffles (Fig. 9).

Among the chironomids, only *M. pedellus* Gr. and *P. olivacea* showed strong preferences for particular hydraulic conditions. They occurred only in pools with low Froude number (Fig. 9). Some taxa showed rather strong preferences for riffles (*Tvetenia bavarica/calvescens*, *T. discoloripes/verralli*, *Eukiefferiella devonica/ilkleyensis*, *Polypedilum laetum*-Gr. and *Rheotanytarsus* sp.) or runs and riffles (*O. rivicola*-Gr., *E. lobifera*, *Cricotopus bicinctus*-Gr. and *P. longimana*) (Fig. 9). The other chironomid taxa occurred more or less across the whole range of the available hydraulic conditions and usually showed weak preferences for pools or runs: e.g. taxa from the Tanytarsini tribe except for *Rheotanytarsus* sp. (*Cladotanytarsus* sp., *Micropsectra* sp. and both the *Tanytarsus* sp. and *T. brundini/curticornis*) preferred pools, but occurred also in runs and riffles (Fig. 9).

Discussion

The importance of hydraulic conditions

The major influence of hydraulic conditions on the structure of benthic macroinvertebrate communities has already been reported (e.g. Orth & Maughan 1983, Jowett et al. 1991, Lancaster & Hildrew 1993, Quinn & Hickey 1994, Benbow et al. 1997, Mérigoux & Dolédec 2004). Also in this study, hydraulic conditions, inversely related to the amount of sedimented POM, best explained the main gradient in the combined data, along which both the taxonomic and functional structure of the communities changed. Surprisingly, of the measured and calculated hydraulic variables, current velocity in 0.8 depth (from bottom) was the best explanatory variable of this gradient, although it was closely followed by the inferred boundary Reynolds number, current velocity in 0.2 and 0.4 depth, Froude number and roughness shear velocity. The substitutability of the simple current velocity for complex hydraulic variables might be caused by a relatively low heterogeneity in water depth and substrate roughness (Quinn & Hickey 1994, Brooks et al. 2005) in the Svratka River.

It is also surprising that the amount of sedimented POM, estimated from underwater video sequences and classified into only four categories, was identified as a more important variable, explaining the structure of both separate oligochaete and chironomid communities better than the measured and calculated hydraulic variables. Nevertheless, already Rabeni & Minshall (1977) demonstrated the influence of POM on the structure of macroinvertebrate community, and the spatial distribution of chironomid larvae in gravels of the River Pank was also most correlated with fine detritus (Ruse 1994). In this study, the amount of POM explained about 21 and 24 % while the hydraulic variables about 14 and 20 % of the variance in the oligochaete and chironomid data respectively. In the case of oligochaetes, the samples seemed to be divided into organically rich with many oligochaete taxa and poor containing only few common taxa. Within the organically poor samples the substrate roughness and the occurrence of aquatic vegetation played the major role and hydraulic conditions were less important. In the case of chironomids the amount of POM seemed to be replaceable by some hydraulic variables and the community structure changed more gradually along the gradient of the amount of sedimented POM (hydraulic conditions).

Potential influence of substrate characteristics

A part of the variability within the data could be explained by some substrate characteristics. In the case of oligochaetes, the substrate characteristics might have represented the thickness of the colonisable substrate layer (Stanford & Ward 1988). It is quite curious that both the substrate particle size and substrate roughness were chosen by the CCA, which indicates that these two variables probably expressed different substrate qualities.

In the case of combined data, the second largest variability could be best explained by chironomid taxa richness and abundance that seemed to reflect the quantity of available food and space resources across the whole range of hydraulic conditions. The quantity of available food and space resources could be probably interpreted in terms of different substrate characteristics. In this respect, aquatic vegetation in runs and riffles and large quantities of sedimented POM in pools provided the most favourable conditions. Aquatic vegetation highly enlarges the substrate surface and may also alter near-bed hydraulic conditions and promote sedimentation of fine particles (Glime & Clemons 1972, Sand-Jensen & Mebus 1996). Higher abundance and taxa richness of chironomids at vegetated habitats was reported earlier e.g. by Wright et al. (1983), Nolte (1989) and Schmid (1993). On the other hand, pools rich in POM offer plenty of food to collecting/gathering and active filter-feeding taxa with low oxygen demands and are likely to satisfy food demands of large numbers of individuals (e.g. Culp et al. 1983).

Functional feeding strategies

As well as the taxonomic structure, also the functional structure of macroinvertebrate communities changes and reflects the spatial variability within a stream (Poff & Ward 1990, Scarsbrook & Townsend 1993, Townsend & Hildrew 1994, Resh et al. 1994, Wohl et al. 1995, Lamouroux et al. 2004). The taxa richness of collectors/gatherers and grazers/scrapers changed along the main CA axis. Negative association of collectors/gatherers with hydraulic variables was recently reported e.g. by Quinn & Hickey (1994), Rempel et al. (2000), Doisy & Rabeni (2001) or Mérigoux & Dolédec (2004). Lower current velocities enable sedimentation of fine organic particles and therefore support collectors/gatherers. On the other hand, higher velocities benefit periphyton in nutrient-poor streams (Horner et al. 1990) and therefore support grazers/scrapers – the consumers of periphytic algae.

The taxa richness of collectors/gatherers and grazers/scrapers changed along the gradient of hydraulic conditions, and therefore similar changes in the proportions of collectors/gatherers and grazers/scrapers were expected. Nevertheless, the proportions of these feeding strategies significantly correlated with the first CA axis only if calculated from chironomid data alone. This was the case because the two most abundant oligochaete collectors/gatherers (*Stylodrilus* spp. juv. and *P. volki*) preferred riffles and runs, while the most abundant oligochaete species, a partial grazer/scraper *N. alpina* did not show any preference for particular hydraulic conditions. In this respect it seemed that the proportions of functional feeding groups within the oligochaete community did not follow the gradient of hydraulic conditions as was observed in the chironomid community (with two exceptions of uncommon predators, all the oligochaete taxa were classified either as pure collectors/gatherers or partial collectors/gatherers and partial grazers/scrapers). This can probably be explained by different behaviour of oligochaetes versus chironomids. Oligochaetes usually exploit interstitial spaces hidden from the rough hydraulic conditions (Usseglio-Polatera et al. 2000), while tube-building chironomids can stand quite rough hydraulic conditions in the upper layer of the substrate, where the food resources are influenced most by hydraulic conditions.

Similarly to the course of collecting/gathering strategy within chironomid community, also the proportions of active filter-feeders and predators decreased along with the increasing hydraulic stress. Active filter-feeders feed on sedimented POM and therefore are favoured at habitats with high sedimentation rates. Predators in pools can probably move about on the substrate without the danger of being swept away by the current. In contrast, Doisy & Rabeni (2001) found no relationship between hydraulic variables and the proportion of predators, while in the study of Rempel et al. (2000) predators were even positively associated with Reynolds number. This disagreement was probably caused by different fractions of the macroinvertebrate communities that were analyzed (only a part of the macroinvertebrate community, e.g. without predatory stoneflies, was analyzed in this study) and by different identification levels (chironomids were identified at the family and subfamily level in Doisy & Rabeni (2001) and Rempel et al. (2000) respectively). It is also possible that the proportion of predators differ in different rivers.

The proportion of passive filter-feeders, attributable only to a chironomid *Rheotanytarsus* sp., was possibly influenced by the occurrence of aquatic vegetation. It can be explained by the benefit of passive filter-feeders from the unimpeded velocities and turbulence transmitted through the open plant stands (Wallace 1980, Iversen et al. 1985, Sand-Jensen & Pedersen 1999). This hypothesis is supported by Schmid (1993), who found *Rheotanytarsus nigricauda* Fittkau, 1960 in distinctly higher densities in the epilithic moss *Fontinalis antipyretica* than in the neighbouring mineral substrate.

Notes on the ecology of frequent taxa

The oligochaete taxa richness patterns confirmed the general preference of most oligochaete taxa for hydraulically smooth conditions promoting creation of patches rich in sedimented POM (Schmedtje & Colling 1996). Nevertheless, the most abundant oligochaete *Stylodrilus* sp., accounting for 40 % of the whole oligochaete community, preferred runs and riffles where it dominated. *P. volki* even indicated runs and riffles. Both these taxa were able to succeed in hydraulically rough conditions because of their adaptation to living in floating sands and gravel bars: *P. volki* is able to exploit interstitial spaces in the substrate and it can attach itself to the substrate surface by adhesive substance (Bird 1982, Petto & Humpesch 1992). *Stylodrilus* sp. can form relatively firm mucous tubes into which it hides during disturbances (Mrázek 1926).

N. stolci preferred runs too and was strongly associated with aquatic vegetation though it is referred to be a typical species of stony substrates (Learner et al. 1978, Verdonschot 1999). In contrast, *N. alpina* could be considered a very adaptive species since it occurred in all samples and dominated in most of them. Chironomids *S. semivirens*, *O.* cf. *rubicundus* and *O.* cf. *obumbratus* showed similar plasticity. All these taxa are classified as partial collectors/gatherers and partial grazers/scrapers (AQEM consortium 2002) and feeding on both the FPOM and algae probably enables them to succeed in wide range of hydraulic conditions.

Unlike the oligochaetes, habitat preferences of most chironomids reflected their feeding behaviour. Collectors/gatherers and active filter-feeders (primarily taxa from the Chironomini tribe) preferred pools, while grazers/scrapers (*Tvetenia* and *Eukiefferiella* species) and the passive filter-feeder *Rheotanytarsus* sp. preferred riffles and runs.

Conclusions

Even though a limited number of samples were analyzed, large spatial variability in the taxonomic and the functional structure of the investigated communities was observed and it was further possible to relate this variability to environmental variables. The sampling design was set up to reduce the temporal and large-scale spatial variation. The results of this study are therefore associated with the studied river section and the sampling season, but nevertheless contribute to the knowledge of lotic macroinvertebrates spatial distribution patterns. Although further work is needed to understand the spatial variability in the macroinvertebrate communities and the hydraulic preferences of particular taxa at more general level, it was shown that the investigated communities clearly reflected environmental conditions. This indicates that any alteration of physical habitat induced for example by human activities or global changes (see Pedersen 2009 in this issue) might influence both the taxonomic and the functional structure of the macroinvertebrate communities in whole river segments.

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Appendix 1. Abundances and frequencies of taxa (in all samples / in the subset of 19 samples used for the analysis of hydraulic preferences of selected taxa) and their classification to feeding groups (col – collectors/gatherers, col/gr – partial collectors/gatherers and partial grazers/scrapers, gr – grazers/scrapers, other – others.

Abbrev.	Taxon	Abundances	Frequenices	Trophic group
	OLIGOCHAETA			
	NAIDIDAE			
chaediap	*Chaetogaster diaphanus* (Gruithuisen, 1828)	3	3	other
chaedias	*Chaetogaster diastrophus* (Gruithuisen, 1828)	1	1	other
naisalpi	*Nais alpina* Sperber, 1948	2,305/1,621	27/19	col/gr
naisbret	*Nais bretscheri* Michaelsen, 1899	4	3	col/gr
naiscomm	*Nais communis* Piguet, 1906	27	9	col/gr
naiselin	*Nais elinguis* O. F. Műller, 1773	3	2	col/gr
naispard	*Nais pardalis* Piguet, 1906	27	2	col/gr
naispseu	*Nais pseudoobtusa* Piguet, 1906	2	2	col/gr
naisstol	*Nais stolci* Hrabě, 1981	308/302	12/10	col/gr
ophiserp	*Ophidonais serpentina* (O. F. Műller, 1773)	1	1	col
prisrose	*Pristina rosea* (Piguet, 1906)	10	3	col
prisaequ	*Pristina aequiseta* Bourne, 1891	12	6	col
prisbilo	*Pristina bilobata* (Bretscher, 1903)	5	3	col
slavappe	*Slavina appendiculata* (D'udekem, 1855)	10	5	col
specjosi	*Specaria josinae* (Vejdovský, 1883)	72	9	col
styllacu	*Stylaria lacustris* (Linnaeus, 1767)	13	4	col/gr
	TUBIFICIDAE			
aulojapo	*Aulodrilus japonicus* Yamaguchi, 1953	177/156	9/5	col
auloplur	*Aulodrilus pluriseta* (Piguet, 1906)	1	1	col
limnodsp	*Limnodrilus* spp. juv.	78	7	col
rhyachair	Rhyacodrilinae juv. with hair chaete	6	3	col
spirferx	*Spirosperma ferox* (Eisen, 1879)	1	1	col
tubiigno	*Tubifex ignotus* (Štolc, 1886)	2	2	col
tubihair	Tubificidae with hairs juv.	163/131	7/4	col
	PROPAPPIDAE			
propvolk	*Propappus volki* Michaelsen, 1916	484/408	23/17	col
	HAPLOTAXIDAE			
haplgord	*Haplotaxis gordioides* (Hartmann, 1821)	5	5	col
	ENCHYTRAEIDAE			
achasp.	*Achaeta* sp.	1	1	col
cognglan	*Cognettia glandulosa* (Michaelsen, 1888)	1	1	col
cognsp.	*Cognettia* sp.	3	1	col
enchsp.	*Enchytraeus* sp.	1	1	col
fridsp.	*Fridericia* sp.	1	1	col
	LUMBRICIDAE			
eisetetr	*Eiseniella tetraedra* (Savigny, 1826)	5	4	col
luicussp	*Lumbricus* sp.	1	1	col
	LUMBRICULIDAE			
lumbvari	*Lumbriculus variegatus* O. F. Műller, 1774	1	1	col
stylbrac	*Stylodrilus brachystylus* Hrabě, 1929	2	2	col
stylodsp	*Stylodrilus* spp. juv.	2,539/2,117	27/19	col
	CHIRONOMIDAE			
	TANYPODINAE			
ablabesp	*Ablabesmyia* sp.	6	4	other
apsetrif	*Apsectrotanypus trifascipennis* (Zetterstedt, 1838)	25	8	other
natasp.	*Natarsia* sp.	4	4	other
nilodubi	*Nilotanypus dubius* (Meigen, 1804)	24	12	other
thiegrge	*Thienemannimyia/Conchapelopia* - Gr.	495/312	25/17	other
zavyiasp	*Zavrelimyia* sp.	5	5	other

Appendix 1. Continued.

Abbrev.	Taxon	Abundances	Frequencies	Trophic group
	DIAMESINAE			
pottgaed	*Potthastia gaedii* (Meigen, 1838)	1	1	col/gr
pottlong	*Potthastia longimana* (Kieffer, 1922)	57/55	14/12	col/gr
	PRODIAMESINAE			
prodoliv	*Prodiamesa olivacea* (Meigen, 1818)	147/142	4/3	col
	ORTHOCLADIINAE			
brilmode	*Brillia bifida* (Kieffer, 1909)	31	11	col
brilflav	*Brillia flavifrons* Johannsen, 1905	12	3	col
corysp.	*Corynoneura* sp.	408/271	26/19	gr
cricannu	*Cricotopus annulator* Goetghebuer, 1927	7	5	gr
cricbici	*Cricotopus bicinctus* (Meigen, 1818)	8	7	col/gr
cricbigr	*Cricotopus bicinctus*-Gr.	68/57	16/13	col/gr
crictrgr	*Cricotopus tremulus*-Gr.	32	12	gr
critriia	*Cricotopus trifascia* Edwards, 1929	5	3	col/gr
diplcult	*Diplocladius cultriger* Kieffer in Kieffer & Thienemann, 1908	1	1	col
eukibrev	*Eukiefferiella brevicalcar* (Kieffer, 1911)	30	11	gr
eukicoer	*Eukiefferiella coerulescens* (Kieffer in Zavrel, 1926)	22	13	gr
eukideil	*Eukiefferiella devonica/ilkleyensis*	94/91	13/10	gr
eukigrgr	*Eukiefferiella gracei*-Gr.	2	1	gr
eukilobi	*Eukiefferiella lobifera* Goetghebuer, 1934	69/55	19/14	gr
eukimino	*Eukiefferiella minor* (Edwards, 1929)	3	2	gr
eukisimi	*Eukiefferiella similis* Goetghebuer, 1939	1	1	gr
hetemarc	*Heterotrissocladius marcidus* (Walker, 1856)	3	2	col
nanoreag	*Nanocladius rectinervis*-Agg.	202/168	21/15	col
orthrigr	*Orthocladius rivicola*-Gr.	124/116	15/11	gr
orthrubi	*Orthocladius* cf. *rubicundus* (Meigen, 1818)	695/530	24/16	col/gr
orthfrig	*Orthocladius frigidus* (Zetterstedt, 1838)	4	3	gr
orthobum	*Orthocladius* cf. *obumbratus* Johannsen, 1905	1,167/903	27/19	col/gr
orththie	*Orthocladius thienemanni* Kieffer in Kieffer & Thienemann, 1906	13	9	gr
paracrsp	*Paracricotopus* sp.	2	2	col
parastyl	*Parametriocnemus stylatus* (Kieffer, 1924)	9	6	gr
pararufi	*Paratrichocladius rufiventris* (Meigen, 1830)	16	9	col/gr
rheofusc	*Rheocricotopus fuscipes* (Kieffer, 1909)	199/162	23/16	col/gr
synosemi	*Synorthocladius semivirens* (Kieffer, 1909)	1,575/809	27/19	col/gr
thellasp	*Thienemanniella* sp.	266/178	24/16	col/gr
tvetbaca	*Tvetenia bavarica/calvescens*	632/606	22/15	gr
tvetdive	*Tvetenia discoloripes/verralli*	480/461	22/17	gr
	CHIRONOMINAE-Tribus Chironomini			
cromussp	*Cryptochironomus* sp.	56	9	other
demisp.	*Demicryptochironomus* sp.	1	1	col
micrchgr	*Microtendipes pedellus*-Gr.	836/503	14/7	col
paraalgr	*Paratendipes albimanus*-Gr.	3	2	col
phaepssp	*Phaenopsectra* sp.	6	2	col/gr
polyconv	*Polypedilum convictum* (Walker, 1856)	17	5	col
polylagr	*Polypedilum laetum*-Gr.	299/283	18/13	col
polyscgr	*Polypedilum scalaenum*-Gr.	75	10	col
xenoxeno	*Xenochironomus xenolabis* (Kieffer in Thienemann & Kieffer, 1916)	1	1	other
	CHIRONOMINAE-Tribus Tanytarsini			
cladotsp	*Cladotanytarsus* sp.	214/105	17/11	col
mictrasp	*Micropsectra* sp.	455/383	26/18	col
paratasp	*Paratanytarsus* sp.	4	2	col/gr
rheotasp	*Rheotanytarsus* sp.	897/874	23/15	other
stembrgr	*Stempellinella brevis*-Gr.	3	3	col
tanybrun	*Tanytarsus brundini/curticornis*	201/130	24/16	col/gr
tanytasp	*Tanytarsus* sp.	260/162	24/17	col

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Aquatic oligochaetes (Annelida: Clitellata) of the Czech Republic: check-list, new records, and ecological remarks

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Abstract

This study contributes to the knowledge of central European clitellates by creating a check-list of Oligochaeta (sensu oligochaetous Clitellata; Erséus 2005) of the Czech Republic, exclusive of taxa in the family Enchytraeidae. In total, 95 aquatic oligochaete species representing 43 genera are reported for the Czech Republic. Rare species are highlighted and associated with the categories for threatened species as outlined by the International Union for the Conservation of Nature. The first records of *Trichodrilus strandi* Hrabě, 1936, *Pristina jenkinae* (Stephenson, 1931), *Pristina osborni* (Walton, 1906), *Rhyacodrilus subterraneus* Hrabě, 1963, *Aulodrilus limnobius* Bretscher, 1899, and *Aulodrilus pigueti* Kowalewski, 1914 in the Czech Republic are presented. Their ecology, morphology, and distribution are discussed.

Key words: Oligochaeta, *Trichodrilus strandi*, *Pristina jenkinae*, *Pristina osborni*, *Rhyacodrilus subterraneus*, *Aulodrilus limnobius*, *Aulodrilus pigueti*, habitat preferences, first records, red list, alien species

Introduction

Aquatic Oligochaeta (sensu oligochaetous Clitellata; Erséus 2005), are typical inhabitants of bottom sediments, forming communities whose species composition is a relevant information source for water quality assessment (Brinkhurst & Cook 1974; Chapman 2001; Verdonschot 2006). Hence, reliable species identification and a complete knowledge of their ecology are essential. Since the turn of the 20th century many phylogenetic changes and taxonomic shifts in Oligochaeta have been made. Therefore, the monographs published by renowned Czech oligochaetologists in the 20th century have become insufficient for valid identification. The list of oligochaete species of the Czech Republic is scattered among old monographs, mostly written in Czech, and recent papers that have documented distributional records for particular species. Furthermore, the territory of the present Czech Republic was previously a part of several different countries, so its records of oligochaete fauna formed a subset of data published on larger territories.

Taxonomic surveys on aquatic oligochaetes have a long tradition in the Czech Republic. Many world-famous taxonomists, whose names have been assigned by patronymy to oligochaete species and genera, came from the Czech territory. At the end of the 19th century, when the Czech Republic was a part of Austria-Hungary, František Vejdovský (1849–1939) and Antonín Štolc (1863–1917) published numerous works dealing with the classifications and morphology of Oligochaeta (e.g. Vejdovský 1876, 1883, 1884, 1892; Štolc 1886, 1888). At the beginning of the 20th century, Alois Mrázek (1868–1923) and Lev Černosvitov (1902–1946, born in Russia) continued their works with studies on anatomy and reproduction (e.g. Mrázek 1913a, 1913b), and morphology and faunistics (e.g. Černosvitov 1928, 1930, 1935). Fundamental research on this group was made by Sergěj Hrabě (1899–1984), who was born in Russia where he lived with his Czech parents until the October Revolution. After the revolution, during the civil war and under difficult circumstances, he moved back to what was then Czechoslovakia. He published an admirable volume of almost 100 scientific papers and monographs, in which he (and other authors), among others, described 11 new species to science

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from Czechoslovakia and 15 genera new for science and 77 new species of oligochaeta from other countries. Both fundamental monographs by Hrabě (1954, 1981) still serve as the list of Czech oligochaetes and remain the major source of information on the morphology of many species. These works were published prior to 1992, when the former Czechoslovakia was split into the Czech Republic and Slovakia, and therefore include species from both countries irrespective of the new political boundaries. This list was supplemented by species documented by Eva Lišková (1964, 1976), and more recently by species documented in faunistic studies (e.g. Wohlgemuth & Schenková 1999) and ecological studies (e.g. Uzunov *et al.* 1988; Schenková *et al.* 2001a, 2001b; Schenková 2005; Schenková & Kroča 2007). Aquatic and semiaquatic species of the family Lumbricidae were treated in the monograph by Pižl (2002), which dealt with predominantly terrestrial species. Up to now, aquatic species of the family Enchytraeidae have been investigated insufficiently (cf. Hrabě 1954) or even omitted (cf. Hrabě 1981). Extensive studies focusing on the terrestrial and semiaquatic Enchytraeidae occurring in terrestrial habitats were published by Chalupský (1988, 1991, 1994). New records for the Czech Republic were recently reported by Jiří Schlaghamerský (e.g. Schlaghamerský 2007; Schlaghamerský & Kobetičová 2005, 2006; Šídová & Schlaghamerský 2007; Schlaghamerský & Pižl 2009). With respect to taxonomic development, the distribution of the family Enchytraeidae in the Czech Republic needs detailed investigation of both freshwater and terrestrial habitats, and a revision of the published historical data.

The objectives of this study are (i) to summarise and update the check-list of aquatic oligochaete species for the Czech Republic, (ii) to evaluate the frequency of all species occurrences in the Czech Republic and update the Red list and (iii) to discuss new records with extended information on their distribution, preferred habitats, ecological requirements, and morphological characteristics. Six oligochaete species reported herein as new for the Czech Republic—*Trichodrilus strandi* (Lumbriculidae), *Pristina jenkinae* and *Pristina osborni* (Naididae: Pristininae), *Rhyacodrilus subterraneus* (Naididae: Rhyacodrilinae), and *Aulodrilus limnobius* and *Aulodrilus pigueti* (Naididae: Tubificinae)—were studied in detail.

Material and methods

The check-list of aquatic oligochaetes (exclusive of taxa in the family Enchytraeidae) was compiled from published records (Hrabě 1954, 1981; Schenková 2005; Schenková & Kroča 2007; Pižl 2002) and from unpublished material determined and/or revised by the authors. Semiaquatic species of the family Lumbricidae were selected based on the data published in the monograph by Pižl (2002), after personal communication with the author. Nomenclature for species discussed in this paper follows Erséus *et al.* (2008) and a synonymy of species (according to Timm 2009) is presented for the six species reported as new records for the Czech Republic.

Each species discussed herein was given a category for its frequency of occurrence in the Czech Republic based upon published data and unpublished records. The unpublished records were compiled from those discussed in running water quality assessments and other projects in which the authors have participated since 1996. This dataset includes approximately 1300 sites across the whole of the Czech Republic. Species were sorted into four categories: F1—scarce (up to 10 sites), F2—medium frequent (10–100 sites), F3—very frequent (more than 100 sites) and A—absent (i.e., not recorded from the Czech Republic since 1996). For scarce or rare species, a category of threat in the Czech Republic according to the International Union for Conservation of Nature 1994 was recommended. As we applied the IUCN criteria at national level, we used guidelines prepared by the IUCN/SSC Regional Applications Working Group (Gärdenfors *et al.* 2001, IUCN 2003, Miller *et al.* 2007). A national and regional category for a particular species may not be the same as its global category, because at global level the whole range of a species is evaluated.

Because many oligochaete species dwell in different freshwater habitats, they were sampled by various methods. The conservation and processing of samples and species identification were the same.

Trichodrilus strandi was collected quantitatively using a metal frame of 25 × 25 cm^2 in spring fen habitats. Vegetation and upper bottom layer were gathered to the depth of 5 cm and this substrate was elutriated through a 250 μm mesh net.

Pristina jenkinae, *Rhyacodrilus subterraneus*, and *Aulodrilus limnobius* were collected in reaches of the river (20–100 m in length) using a three-minute kick-sampling of all visually distinguishable habitats, which were sampled proportionally to their occurrences in the reach of the river using a hand net (25 × 25 cm^2, mesh size 500 µm) (Kokeš *et al.* 2006).

Aulodrilus pigueti was collected using the same type of hand net (25 × 25 cm^2, mesh size 500 µm) in the bottom substrate of a fishpond and in submerged littoral vegetation.

Pristina osborni was collected in a large river from a depth up to 3 m using an "air lift" vacuum sampler designed for sampling in large, fast-flowing rivers (Pehofer 1998) and the grab "van Veen" (Lie & Pamatmat 1965). The samples were elutriated through the 250 µm mesh net.

Samples obtained by all these methods were fixed by 4% formaldehyde in the field and then sorted under a stereomicroscope in the laboratory. Oligochaetes were picked up, permanently mounted in Canada balsam and identified under a light microscope using the keys by Hrabě (1954, 1981), Timm & Veldhijzen van Zanten (2002) and Timm (2009).

New records of selected species included the following information associated with specimens: name of the water body, name of the settlement (town, village, city), closest site from which the specimen(s) was/were collected, latitude and longitude coordinates for the collecting site, name(s) of the person(s) who collected and/or identified the species and date of collection. Abbreviations for the names of collectors and persons who identified the species are presented below: IS—Ivan Skála, JB—Jindřiška Bojková, JK—Jiří Kokeš, JSc—Jana Schenková, JSy—Jan Sychra, JZ—Jiří Zahrádka, KB—Karel Brabec, KP—Karla Petřivalská, LO—Libuše Opatřilová, PH—Pavel Horák, PP—Petr Pařil. Data on habitat characteristics - abiotic and chemical parameters, water saprobity (Kolkwitz & Marsson 1909), ecology, morphology (if new or untypical features were recorded) and distribution of species are noted.

Results and discussion

The check-list of 95 aquatic/semiaquatic oligochaete species for the Czech Republic comprises the families Lumbriculidae (12 species), Naididae sensu Erséus *et al.* (2008) (a total of 72 species)—which includes the Naidinae (36 species), Pristininae (eight species), Tubificinae (22 species), and Rhyacodrilinae (six species), Propappidae (one species), Criodrilidae (one species), Haplotaxidae (one species) and Lumbricidae (8 species) (Table 1).

The majority of the evaluated species (44) were moderately or widely distributed (F2 and F3) in the Czech Republic. They are mostly ubiquitous and euryecious species (e.g., several species of Tubificinae inhabiting running and stagnant waters) and many species can tolerate high organic matter content and pollution. There were also two species, *Potamothrix moldaviensis* Vejdovský et Mrázek, 1903 and *Potamothrix bavaricus* (Oschman, 1913) (both in the subfamily Tubificinae), that are considered alien species in Europe (Drake 2009). However, their non-indigenous status in central Europe is questionable because they were recorded historically from areas within the border of today's Czech Republic. *P. moldaviensis* has previously been described in the Vltava (Moldau) River (the Elbe basin) in the beginning of 20th century and *P. bavaricus* was recorded in the Elbe basin in the 1930s (Hrabě 1939). Both species recently occur in moderately polluted rivers—*P. moldaviensis* prefers larger rivers, while *P. bavaricus* prefers smaller streams.

Thirty-eight species were considered rare (F1). Some of these species are not necessarily endangered, since they can be locally abundant in either restricted areas or particular habitats. For instance, *Stylodrilus lemani* (Grube, 1879) is abundant in a few eutrophic streams in southern Moravia (located in the south-eastern part of the Czech Republic) and *Haemonais waldovogeli* Bretscher, 1900 is dominant in the bottom fauna of small fishponds in the Czech-Moravian Highland Mountains. By contrast, one species, *Branchiura sowerbyi* Beddard, 1892, is a non-indigenous thermophilic species which has been spreading throughout Europe, most likely via water transport (Lišková 1964; Gruszka 1999). However, its invasion is very slow because of a lack of suitable habitats (warmer waters). We have documented four records of *B. sowerbyi* since 1964, when it was found (Lišková 1964) for the first time in Czechoslovakia. Two other species, *Psammoryctides moravicus*

(Hrabě, 1934) and *Paranais frici* Hrabě, 1941—both considered aliens of Ponto-Caspian origin in Europe (Drake 2009)—were recently found sporadically in the Labe and Morava Rivers that serve as immigration gateways into Czech waters.

TABLE 1. Aquatic Oligochaeta of the Czech Republic. Lumbriculidae, Naididae (Naidinae, Pristininae, Rhyacodrilinae, Tubificinae), Propappidae, Haplotaxidae, Criodrilidae, and Lumbricidae. Frequency of occurrence since 1996: F1—scarce (up to 10 sites), F2—medium frequent (10–100 sites), F3—very frequent (more than 100 sites) and A—absent. Lumbricidae, affinity to wet habitats and frequency of occurrence refers to Pižl (2002, and personal communication).

Taxon	Author	Reference	Frequency
Lumbriculidae			
Lamprodrilus mrazeki	Hrabě, 1929	Hrabě 1954, 1981	A
Lumbriculus variegatus	(Müller, 1774)	Hrabě 1954, 1981	F3
Rhynchelmis limosella	Hoffmeister, 1843	Hrabě 1954, 1981	F2
Stylodrilus absoloni	(Hrabě, 1970)	Hrabě 1981	A
Stylodrilus brachystylus	Hrabě, 1929	Hrabě 1954, 1981	F3
Stylodrilus heringianus	Claparède, 1862	Hrabě 1954, 1981	F3
Stylodrilus lemani	(Grube, 1879)	Hrabě 1954, 1981	F1
Stylodrilus parvus	(Hrabě & Černosvitov, 1927)	Hrabě 1954, 1981	F1
Trichodrilus allobrogum	Claparède, 1862	Hrabě 1954, 1981	F1
Trichodrilus moravicus	Hrabě, 1937	Hrabě 1954, 1981	F1
Trichodrilus pragensis	Vejdovský, 1876	Hrabě 1954, 1981	A
Trichodrilus strandi	Hrabě, 1936	**new record**	F1
Naididae: Naidinae			
Amphichaeta leydigi	Tauber, 1879	Hrabě 1954, 1981	F1
Arcteonais lomondi	(Martin, 1907)	Hrabě 1954, 1981	F1
Aulophorus furcatus	(Müller, 1773)	Hrabě 1954, 1981	F1
Chaetogaster cristallinus	Vejdovský, 1884	Hrabě 1954, 1981	F1
Chaetogaster diaphanus	(Gruithuisen, 1828)	Hrabě 1954, 1981	F2
Chaetogaster diastrophus	(Gruithuisen, 1828)	Hrabě 1954, 1981	F2
Chaetogaster langi	Bretscher, 1896	Hrabě 1954, 1981	F2
Chaetogaster limnaei	Baer, 1827	Hrabě 1954, 1981	A
Chaetogaster setosus	Světlov, 1925	Hrabě 1954	A
Dero digitata	(Müller, 1774)	Hrabě 1954, 1981	F2
Dero dorsalis	Ferronière, 1899	Hrabě 1954, 1981	A
Dero nivea	Aiyer, 1929	Hrabě 1954	A
Dero obtusa	Udekem, 1855	Hrabě 1954, 1981	A
Haemonais waldvogeli	Bretscher, 1900	Hrabě 1954, 1981	F1
Nais alpina	Sperber, 1948	Hrabě 1954, 1981	F3
Nais barbata	Müller, 1774	Hrabě 1954, 1981	F2
Nais behningi	Michaelsen, 1923	Hrabě 1954, 1981	F1
Nais bretscheri	Michaelsen, 1899	Hrabě 1954, 1981	F2
Nais christinae	Kasprzak, 1973	Hrabě 1981	F1
Nais communis	Piguet, 1906	Hrabě 1954, 1981	F2

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Taxon	Author	Reference	Frequency
Nais elinguis	Müller, 1774	Hrabě 1954, 1981	F3
Nais pardalis	Piguet, 1906	Hrabě 1954, 1981	F2
Nais pseudobtusa	Piguet, 1906	Hrabě 1954, 1981	F1
Nais simplex	Piguet, 1906	Hrabě 1954, 1981	F2
Nais stolci	Hrabě, 1981	Hrabě 1954	F1
Nais variabilis	Piguet, 1906	Hrabě 1954, 1981	F2
Ophidonais serpentina	(Müller, 1774)	Hrabě 1954, 1981	F2
Paranais frici	Hrabě, 1941	Hrabě 1954, 1981	F1
Piguetiella blanci	(Piguet, 1906)	Schenková 2005	F1
Ripistes parasita	(Schmidt, 1847)	Hrabě 1954, 1981	F1
Slavina appendiculata	(Udekem, 1855)	Hrabě 1954, 1981	F2
Specaria josinae	(Vejdovský, 1884)	Hrabě 1954, 1981	F1
Stylaria lacustris	(Linnaeus, 1767)	Hrabě 1954, 1981	F3
Uncinais uncinata	(Ørsted, 1842)	Hrabě 1954, 1981	F1
Vejdovskyella comata	(Vejdovský, 1884)	Hrabě 1954, 1981	F2
Vejdovskyella intermedia	(Bretscher, 1896)	Hrabě 1954, 1981	F1
Naididae: Pristininae			
Pristina aequiseta	Bourne, 1891	Hrabě 1954, 1981	F1
Pristina amphibiotica	Lastočkin, 1927	Hrabě 1954	A
Pristina bilobata	(Bretscher, 1903)	Schenková & Kroča 2007	F1
Pristina jenkinae	(Stephenson, 1931)	**new record**	F1
Pristina longiseta	Ehrenberg, 1828	Hrabě 1954, 1981	F1
Pristina menoni	(Aiyer, 1930)	Hrabě 1954	F1
Pristina osborni	(Walton, 1906)	**new record**	F1
Pristina rosea	(Piguet, 1906)	Hrabě 1954, 1981	F2
Naididae: Rhyacodrilinae			
Bothrioneurum vejdovskyanum	Štolc, 1886	Hrabě 1954, 1981	F3
Branchiura sowerbyi	Beddard, 1892	Hrabě 1981	F1
Epirodrilus pygmaeus	(Hrabě, 1935)	Hrabě 1954, 1981	F2
Rhyacodrilus coccineus	(Vejdovský, 1875)	Hrabě 1954, 1981	F3
Rhyacodrilus falciformis	Bretscher, 1901	Hrabě 1954, 1981	F2
Rhyacodrilus subterraneus	Hrabě, 1963	**new record**	F1
Naididae: Tubificinae			
Aulodrilus japonicus	Yamaguchi, 1953	Hrabě 1954, 1981	F2
Aulodrilus limnobius	Bretscher, 1899	**new record**	F1
Aulodrilus pigueti	Kowalewski, 1914	**new record**	F1
Aulodrilus pluriseta	(Piguet, 1906)	Hrabě 1954, 1981	F2
Haber speciosus	(Hrabě, 1931)	Hrabě 1954, 1981	A
Ilyodrilus templetoni	(Southern, 1909)	Hrabě 1954, 1981	F2
Limnodrilus claparedeanus	Ratzel, 1868	Hrabě 1954, 1981	F3
Limnodrilus hoffmeisteri	Claparède, 1862	Hrabě 1954, 1981	F3

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Taxon	Author	Reference	Frequency
Limnodrilus profundicola	(Verrill, 1871)	Hrabě 1981	F1
Limnodrilus udekemianus	Claparède, 1862	Hrabě 1954, 1981	F2
Peipsidrilus pusillus	Timm, 1977	Hrabě 1981	A
Potamothrix bavaricus	(Oschmann, 1913)	Hrabě 1954, 1981	F2
Potamothrix bedoti	(Piguet, 1913)	Hrabě 1954, 1981	A
Potamothrix hammoniensis	(Michaelsen, 1901)	Hrabě 1954, 1981	F2
Potamothrix moldaviensis	Vejdovský & Mrázek, 1903	Hrabě 1954, 1981	F2
Psammoryctides albicola	(Michaelsen, 1901)	Hrabě 1954, 1981	F2
Psammoryctides barbatus	(Grube, 1861)	Hrabě 1954, 1981	F3
Psammoryctides moravicus	(Hrabě, 1934)	Hrabě 1954, 1981	F1
Spirosperma ferox	Eisen, 1879	Hrabě 1954, 1981	F2
Tubifex ignotus	(Štolc, 1886)	Hrabě 1954, 1981	F2
Tubifex nerthus	Michaelsen, 1908	Hrabě 1981	A
Tubifex tubifex	(Müller, 1774)	Hrabě 1954, 1981	F3
Propappidae			
Propappus volki	Michaelsen, 1916	Hrabě 1954	F2
Haplotaxidae			
Haplotaxis gordioides	(Hartmann, 1821)	Hrabě 1954, 1981	F2
Criodrilidae			
Criodrilus lacuum	Hoffmeister, 1845	Hrabě 1954	F1
Lumbricidae			
Aporrectodea georgii	(Michaelsen, 1890)	Pižl 2002	F1
Aporrectodea handlirschi	(Rosa, 1879)	Pižl 2002	F1
Aporrectodea limicola	(Michaelsen, 1890)	Pižl 2002	F1
Eisenia fetida	(Savigny, 1826)	Pižl 2002	F2
Eisenia spelaea	(Rosa, 1909)	Pižl 2002	F1
Eiseniella tetraedra	(Savigny, 1826)	Pižl 2002	F3
Helodrilus oculatus	Hoffmeister, 1845	Pižl 2002	F1
Octoclasion tyrtaeum	(Savigny, 1826)	Pižl 2002	F2

Thirteen species have not been recorded in studies conducted in the Czech Republic since 1996. Their absence from more recent collections could be caused by various reasons. Firstly, particularly unique habitats were not studied, thus species limited in distribution to these areas where not recorded—e.g., a cave species *Stylodrilus absoloni* (Hrabě, 1970), a groundwater species *Trichodrilus pragensis* Vejdovský, 1876, and a snail parasite *Chaetogaster limnaei* Baer, 1827. Secondly, most of the material discussed in this paper was collected in running water habitats; stagnant waters were studied to a lesser extent. This could be the reason for the absence of the temporal pool specialist *Lamprodilus mrazeki* Hrabě, 1929 and stagnant water specialists such as *Dero dorsalis* Ferronière, 1899, *Dero nivea* Aiyer, 1929, *Dero obtusa* Udekem, 1855, and *Chaetogaster setosus* Světlov, 1925. Other species that have not been reported since 1996 include *Pristina amphibiotica* Lastočkin, 1927, *Haber speciosus* (Hrabě, 1931), *Peipsidrilus pusillus* Timm, 1977, *Potamothrix bedoti* (Piguet, 1913), and *Tubifex nerthus* Michaelsen, 1908—each of these had previously been reported only once in the country, and we consider them to be either very rare or regionally extinct in the Czech Republic.

TABLE 2. Proposal of the Red list of aquatic Oligochaeta of the Czech Republic. Two species from the family Lumbricidae according to Pižl (2005) are included.

endangered (EN)		**vulnerable (VU)**	
Arcteonais lomondi	(Martin, 1907)	*Amphichaeta leydigi*	Tauber, 1879
Criodrilus lacuum	Hoffmeister, 1845	*Aporrectodea limicola*	(Michaelsen, 1890)
Haber speciosus	(Hrabě, 1931)	*Pristina aequiseta*	Bourne, 1891
Lamprodrilus mrazeki	Hrabě, 1929	*Pristina bilobata*	(Bretscher, 1903)
Peipsidrilus pusillus	Timm, 1977	*Pristina jenkinae*	(Stephenson, 1931)
Piguetiella blanci	(Piguet, 1906)	*Pristina longiseta*	Ehrenberg, 1828
Stylodrilus absoloni	(Hrabě, 1970)	*Pristina osborni*	(Walton, 1906)
Trichodrilus pragensis	Vejdovský, 1876	*Nais behningi*	Michaelsen, 1923
Trichodrilus strandi	Hrabě, 1936	*Nais christinae*	Kasprzak, 1973
		Nais stolci	Hrabě, 1981
		Rhyacodrilus subterraneus	Hrabě, 1963
		Specaria josinae	(Vejdovský, 1884)
near threatened (NT)		*Stylodrilus lemani*	(Grube, 1879)
Apporrectodea georgii	(Michaelsen, 1890)	*Uncinais uncinata*	(Ørsted, 1842)

The Red List of aquatic oligochaetes of the Czech Republic (Schenková 2005, Pižl 2005) was modified on the basis of the new extensive data by excluding *Aulodrilus japonicus* Yamaguchi, 1953, *Ilyodrilus templetoni* (Southern, 1909), *Epirodrilus pygmaeus* (Hrabě, 1935), *Psammoryctides albicola* (Michaelsen, 1901), and *Slavina appendiculata* (Udekem, 1855), which had been formerly considered endangered. The number of recent records and distribution of these species within the Czech Republic indicate the stability of their populations. On the basis of those new data, we changed the protection status of another three species—*Pristina bilobata* (Bretscher, 1903), *Pristina longiseta* Ehrenberg, 1828, and *Uncinais uncinata* (Ørsted, 1842)—from endangered (EN) to vulnerable (VU). However, we now suggest that the status of endangered species (EN) to be allocated to *Trichodrilus strandi*, *Haber speciosus*, *Lamprodrilus mrazeki*, *Peipsidrilus pusillus*, and *Stylodrilus absoloni* with respect to their very restricted occurrence—not only in the Czech Republic, but also in neighbouring countries. Vulnerable protection status (VU) was proposed for *Amphichaeta leydigi* Tauber, 1879, *Nais behningi* Michaelsen, 1923, *Nais christinae* Kasparzak, 1973, *Nais stolci* Hrabě, 1981, *Pristina aequiseta* Bourne, 1891, *P. osborni*, *P. jenkinae*, *R. subterraneus*, *Specaria josinae* (Vejdovský, 1884), *S. lemani* and *Vejdovskyella intermedia* (Bretscher, 1896), based on their rare occurrence and scattered distributions. The new Red list of Czech aquatic oligochaete species with suggested changes is given in Table 2.

Six species collected during this present study are reported here as new records for the Czech Republic: *Trichodrilus strandi*, *Pristina jenkinae*, *P. osborni*, *Rhyacodrilus subterraneus*, *Aulodrilus limnobius* and *A. pigueti*. The extent of their physical and chemical characteristics of finding sites is summarised in Table 3.

Species accounts

Family: Lumbriculidae

Trichodrilus strandi Hrabě, 1936

Records. Bílé Potoky Spring Fen, Valašské Klobouky, *49°06'52"N / 18°01'40"E*, lgt. JB, det. JSc (2006); Hrubý Mechnáč Spring Fen, Lopeník, *48°56'27"N / 17°47'51"E*, lgt. JB, det. JSc (2006); Kalábová Spring

Fen, Březová, *48°56'22"N / 17°44'39"E*, lgt. JB, det. JSc (2006); Chmelinec Spring Fen, Vyškovec, *48°56'22"N / 17°51'20"E*, lgt. JB, det JSc (2006); Hutě Spring Fen, Žítková, *48°59'27"N / 17°54'33"E*, lgt. JB, det. JSc (2006).

Characteristics of sites. *T. strandi* was recorded in 2006 in the Western Carpathian spring fens, which are situated in the easternmost part of the Czech Republic, on the boundary with Slovakia (Fig. 1). We have collected this species from altitudes 350–642 m a.s.l. in extremely mineral-rich spring fens with precipitation of cold water travertine (tufa). The bottom substrate of spring fens was dominated by inorganic material (gravel and sand) and coarse particulate organic matter (leaves and wood). Tufa precipitated on all submerged surfaces forms either small incrustations or strong layers.

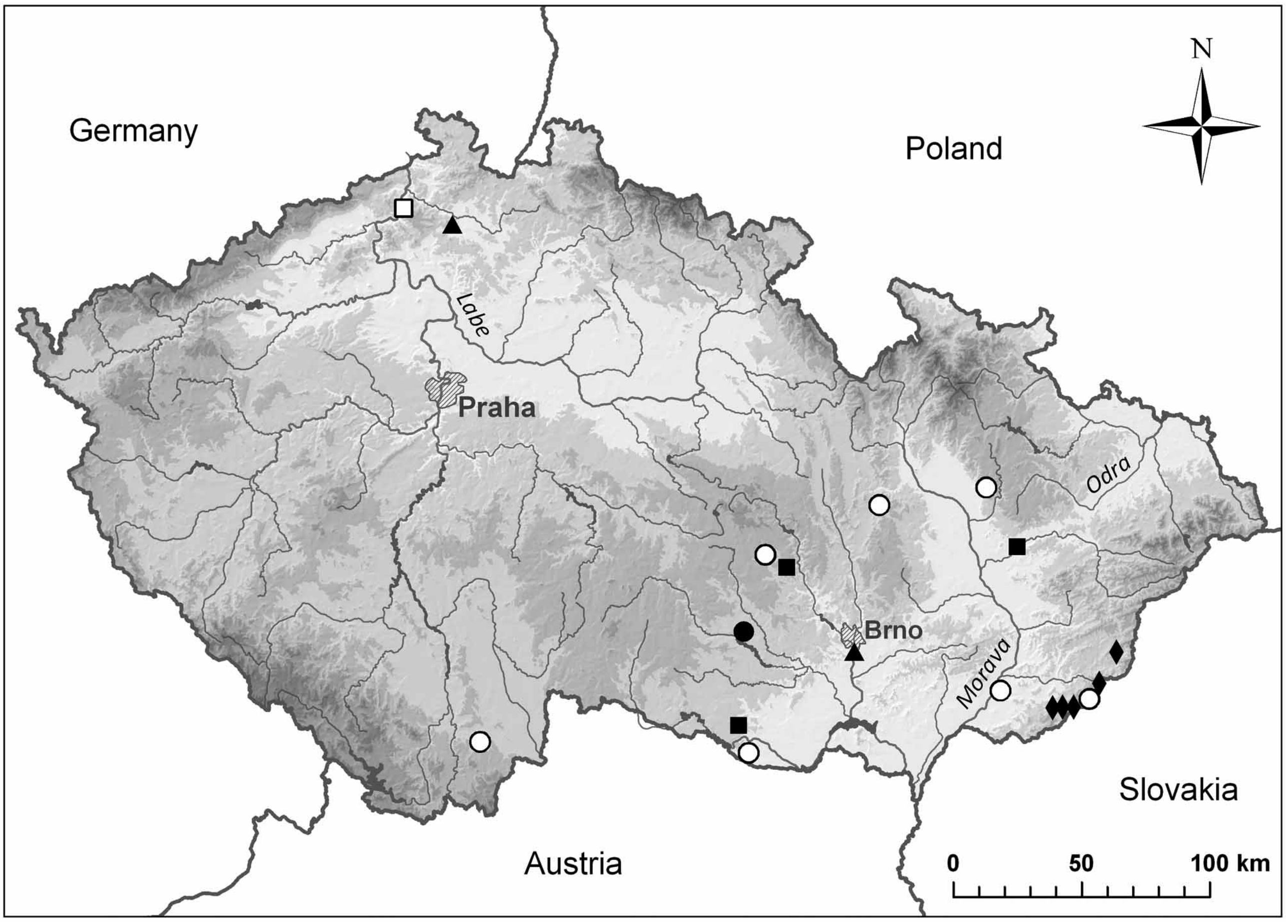

FIGURE 1. The distribution of ♦ *Trichodrilus strandi*, ■ *Pristina jenkinae*, □ *Pristina osborni*, ▲ *Rhyacodrilus subterraneus*, ○ *Aulodrilus limnobius* and ● *A. pigueti* in the Czech Republic.

Ecology. *T. strandi* is well adapted for this rather hostile environment because of a tiny flexible body that enables it to utilise small interstitial spaces, particularly those in tufa-covered substrates. In studied sites, it represented the eudominant species followed by 16 additional oligochaete taxa, mostly taxa in the family Enchytraeidae. Its main adaptation can be feeding on specific kinds of bacteria that colonise surfaces in mineral-rich waters (Tarmo Timm pers. com.). Although the environment in spring fens is rather stable with small temperature fluctuations, we recorded seasonal variations in the ratio between juveniles and adults from 50% of juveniles in spring to their prevalence (80%) in autumn. *T. strandi* is a monocyclic gatherer-collector burrowing in sediments of xenosaprobic streams, and it is considered a K-strategist (Šporka 2003).

Morphology. This species can be distinguished from other taxa in that all chaetae are bifid with tiny upper teeth, the male pores are located on conical porophores in IX, and spermathecal pores are present in X (Fig. 2). The positions of sexual pores in *T. strandi* (porophores in X, spermathecal pores in IX) are opposite of those in the genus *Stylodrilus*, with which young *T. strandi* can be confused. We have recorded variability in the spermathecae position of 200 individuals: 36% had spermathecae in X, 38% in between X and XI (crossing the septa) and 26% in XI. We did not observe any lateral wings in the genital region, but we identified only fixed individuals.

Distribution. The nearest known localities for *T. strandi* are in the Valča Brook—a tributary of the Turiec River in the Malá Fatra Mts. (Slovakia) (Šporka 1996a), which is approximately 80 km from our sites (Šporka 1996a), and the spring outflow of an ice cave in the Tatra Mts.—approximately 150 km from our sites (Hrabě 1942). European distribution is highly scattered with records from karstic or flysh areas in France (Giani 1979; Juget & Dumnicka 1986), Croatia (Karaman 1987) and Italy (Brinkhurst 1963). According to Fauna Europaea (Timm & Giani 2004), the species was found in the Czech Republic, but unfortunately no additional information for this report was provided, nor is available. These data most likely represent records reported from areas in what is now known as Slovakia prior to its split from the former Czechoslovakia. *T. strandi* as the stygophilous species, representative of the Lumbriculidae, is more limited in global distribution (Martin *et al.* 2008). This species is worth further investigation since its populations seem to be geographically separated and there is even the question if they represent the same species.

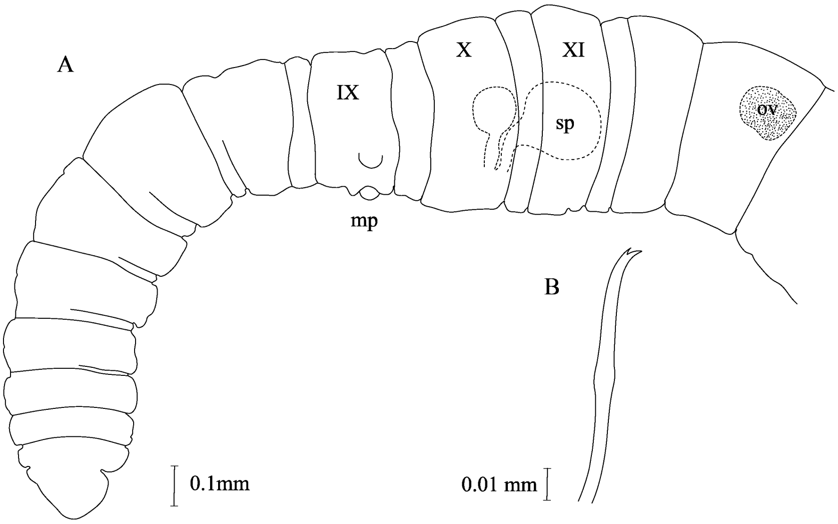

FIGURE 2. *Trichodrilus strandi*. A: Schematic drawing of reproductive organs in IX, X, XI segment, lateral view. mp: male pore on conical porophorus, sp: spermatheca, ov: ovarium. B: dorsal chaeta.

Family: Naididae

Subfamily: Pristininae

Pristina jenkinae (Stephenson, 1931)

Synonyms: *Naidium jenkinae* Stephenson, 1931; *Pristina idrensis* Sperber, 1948; *Pristinella jenkinae* (Stephenson, 1931)

Records. Teplá Brook, Věžná, *49°26'42"N / 16°16'33"E*, lgt. PP, det. PP (2003); Bečva River, Lipník-Osek, *49°29'48"N / 17°31'06"E*, lgt. KB, det. PP (2004); Gránický Brook, Znojmo, *48°51'60"N / 16°01'33"E*, lgt. PP, det. PP (2005); all specimens were immature.

Characteristics of sites. The species was found in two different habitats: epirhithral and hyporhithral streams. The epirhithral habitat was represented by two small brooks (2nd and 3rd Strahler order) with dominating stony-gravel substrates, natural channel morphology and forested catchments (catchment area up to 20 km^2). The hyporhithral habitat was a 30 m wide shallow reach of the river of 7th Strahler order (catchment area 1,526 km^2) with cobble-pebble substrate, which flows through extensively used farmland. A natural self-restoration of this reach of the river resulted from a big flood in 1997.

Ecology. *Pristina jenkinae*, a detritophagous oligochaete, was recorded in different freshwater habitats (Timm & Veldhijzen van Zanten 2002), including hyporheic zones (Strayer & Bannon-O'Donnell 1988; Giani *et al.* 2001; Wetzel & Taylor 2001), and in wet soil of a tropical rainforest (Collado & Schmelz 2001). In central Europe, it was reported from rhithral and potamal zones, with preferences from xeno- to beta-mesosaprobity (Hörner *et al.* 2002). The absence of any historic records of this species from the Czech Republic are likely attributed to difficulties in its identification (ecophenotypic variation in its chaetae, and status as a valid taxon) and its rare to occasional occurrence (Wetzel & Taylor 2001).

Morphology. This species can be distinguished from related European *Pristina* species by none or slight shortening of the upper teeth in ventral chaetae from the anterior to posterior end, parallel teeth of dorsal needles with upper teeth slightly (anterior most) or distinctly (posterior bundles) shorter and thinner (from 1/2 to 2/3), smooth hair chaetae, and gradual intestinal dilatation from ½ VI to VII (Collado & Schmelz 2001; Timm & Veldhijzen van Zanten 2002). A single spermathecal chaeta is present in VII in mature specimens, but we unfortunately did not find any sexually active individuals during this present study.

Distribution. *Pristina jenkinae* has a cosmopolitan distribution (Timm & Veldhijzen van Zanten 2002) and it has been reported from many other European countries: Germany, Poland, Slovakia, Moldova, Romania, Italy, France, Spain, Portugal, Norway, Sweden, The Netherlands, the UK, Greece, and Finland (Timm & Giani 2004). However, the absence of records in other European countries can be connected with identification difficulties and the unclear taxonomical status of this taxon. *P. jenkinae* was at first synonymised with *P. idrensis* by Kathman (1985) and recently redescribed and discussed as probably distinct species by Collado & Schmelz (2001).

Pristina osborni (Walton, 1906)

Synonyms: *Naidium osborni* Walton, 1906; *Naidium minutum* Stephenson, 1914; *Pristinella osborni* (Walton, 1906); *Pristina minuta* (Stephenson, 1914)

Records. Labe (Elbe) River, Děčín *50°42'34"N / 14°11'44"E*, lgt. JK, det. PP (2008); all specimens were immature.

Characteristics of site. The species was found in a single site, in the Labe (Elbe) River approximately 20 km far from the Czech/German boundary near Dečín (Tetschen). This river is the largest in the Czech Republic (8th Strahler order) and it is extensively used for shipping to the German port of Hamburg. The mean depth of this reach of the river was 2.4 m, the bottom substrate was predominantly sand (45%) and gravel (55%) and water quality is classified according to BOD (biochemical oxygen demand) in the beta-mesosaprobity (Table 3).

Ecology. *Pristina osborni* inhabits freshwaters including hyporheic waters and caves (Giani *et al.* 2001) and wet soil (Stout 1958). Its ability to inhabit also semi-terrestrial environments enhances the opportunity for freshwater populations of this species to persist in intermittent wetlands (Montalto & Marchese 2005). In tropical regions, *P. osborni* prefers areas with higher calcium content (Alves *et al.* 2008). Asexual reproduction occurs most commonly via paratomy (Timm & Veldhijzen van Zanten 2002). The species is not usually abundant within the oligochaete community (Giani *et al.* 2001; Alves *et al.* 2008).

Morphology. The species can be distinguished by the typical shape of its dorsal bifid chaetae, with the teeth diverting in a wide angle and the presence of only one needle and one smooth hair seta in the dorsal bundles. The budding zone begins from XII, and the stomach dilatation begins abruptly in segments VII–VIII. The morphology of the genital organs was described in detail by Erséus and Grimm (1998).

Distribution. *Pristina osborni* has a cosmopolitan distribution (Timm & Veldhijzen van Zanten 2002), and is absent only in the eastern Palaearctic region (Timm & Giani 2004). European records are limited to southern and western countries (Portugal, Spain, Italy, France, The Netherlands, and Germany; Timm & Giani 2004). The Labe (Elbe) River, which flows through north-eastern Germany to the Baltic Sea, likely serving as a migration corridor for this species—from its original distribution area in western Europe (as well as it was documented for aliens) into the Czech Republic. *P. osborni* could spread into this reach of the river, probably from a lower part of the watershed via upstream migration or dissemination by shipping. However, downstream dissemination into the Labe River—proven to be an alternative pathway of spreading of epipotamal species (Pařil *et al*. 2008)—is rather improbable for *P. osborni*.

TABLE 3. Summary of the characteristics of the aquatic oligochaete species *Trichodrilus strandi*, *Pristina jenkinae*, *Pristina osborni*, *Rhyacodrilus subterraneus*, *Aulodrilus limnobius* and *A. pigueti* sites in the Czech Republic.

Species	*Trichodrilus strandi*	*Pristina jenkinae*	*Pristina osborni*	*Rhyacodrilus subterraneus*	*Aulodrilus limnobius*	*Aulodrilus pigueti*
Sites:						
No of sites	5	3	1	2	7	1
No of records	15	3	1	2	8	1
River basin	Morava	Morava	Labe	Morava, Labe	Morava, Dunaj, Labe	Morava
Abundance	>10%	1–2%	1–2%	1–2%	2.1–5%	1–2%
Water body	spring brook/ helocrene	brook/river	river	brook/river	brook/river	fish-pond
Abiotic parameters						
Min.-max.:						
Altitude [m a.s.l.]	350–642	225–420	120	190–240	165–485	440
Strahler order	1	2–7	8	5–6	3–4	–
Ann. average discharge [$m^3 s^{-1}$]	–	0.001–15.3	309	1.2–5.2	0.01–0.8	–
Average stream width [m]	–	0.9–33.0	100	7.2–12.0	1.9–5.0	–
Chemical parameters						
Min.- max.:						
pH	7.3–8.2	6.0–9.0	8.8	7.3–8.5	6.0–9.1	9.5–9.8
Conductivity [$mS m^{-1}$]	406–599	91–1180	427	333–737	118–921	414–434
Ann. average water temp. [°C]	7.0–14.5	10.6–10.7	14.3	10.2–10.9	8.1–11.8	–
BOD [mg O_2 l^{-1}]	–	1.0–6.6	4	1.2–20.8	0.8–9.4	–
Dissolved oxygen [mg l^{-1}]	2.4–12.7	7.1–14.7	8.9	6.9–14.1	5.4–13.7	6.9–15.4

Subfamily: Rhyacodrilinae

Rhyacodrilus subterraneus Hrabě, 1963

Records. Robečský Brook, Česká Lípa, *50°40'05"N / 14°30'27"E*, lgt. IS, det. JS/PP (2007), immature specimens; Svitava River, Brno, *49°08'43"N / 16°37'48"E*, lgt. PH, det. PP (2006); one mature specimen.

Characteristics of sites. Although most published records refer to groundwater and hyporheic habitats (Erséus *et al.* 1999; Wetzel & Taylor 2001), we found *R. subterraneus* in surface waters. These surface habitats, however, likely receive groundwater influences. The Robečský Brook is situated in the north-western part of the Czech Republic in semi-natural landscape with numerous wetlands and fishponds. The sample was taken downstream of the Peklo Natural Reserve, where a brook flows through a narrow sandstone gorge with substrate formed of mud (40%), sand (30%) and gravel (30%). On the contrary, the second sampling site is situated downstream of Brno, the second largest city in the country, in the straightened and dyked Svitava River stretch (substrate: 20% mud, 20% sand, 30% gravel and 30% stones), which also serves as a recipient of sewage water from urban settlements.

Ecology. *Rhyacodrilus subterraneus* occurs in fresh and brackish water (Erséus *et al.* 1999); in reference to its name, especially in interstitial hyporheic zones and wet soil. Many records were reported from deeper hyporheic habitats (Strayer 2001) and caves (Wetzel & Taylor 2001; Ferreira *et al.* 2007); nevertheless, it was also found in species-rich oligochaete assemblages in eutrophic streams (Timm *et al.* 1997). Reproduction in this species is sexual (Timm & Veldhijzen van Zanten 2002), but in some regions (United States) only immature specimens have been found (Wetzel & Taylor 2001).

Morphology. *Rhyacodrilus subterraneus* can be distinguished from its congeners by straighter and two times longer upper tooth of anterior dorsal chaetae with intermediate teeth, and two times longer upper tooth in anterior ventral chaetae. Penial chaetae near male pores in XI segment of mature specimens are only single with a simple curved tip (Timm & Giani 2004).

Distribution. Prior to records collected during this present study, this rare Holarctic species had been found only in western European countries (France, Germany, Norway, Sweden, Spain, and The Netherlands; Timm & Giani 2004), and in the eastern and Midwestern United States (Strayer & Bannon-O'Donnell 1988; Strayer 2001; Wetzel & Taylor 2001; Kathman & Brinkhurst 1998).

Subfamily: Tubificinae

Aulodrilus limnobius Bretscher, 1899

Records. Gránický Brook, Znojmo, *48°51'60"N / 16°01'33"E,* lgt. PP, det. PP. (2005); Drietomice River, Starý Hrozenkov, *48°57'13"N / 17°52'29"E,* lgt. PP, det. PP (2005); Trusovický Brook, Jívová, *49°42'27"N / 17°21'40"E*, lgt. KB, det. PP; Olešná River, Zvole, *49°29'26"N / 16°09'44"E*, lgt. KB, det. PP (2002); Nectava River, Březinky, *49°39'24"N / 16°46'52"E*, lgt. KB, det. PP (2002); Okluky River, Uherský Ostroh, *48°59'38"N / 17°24'07"E*, lgt. H, det. PP (2002); Farský Brook, Trhové Sviny, *48°50'24"N / 14°37'55"E*, lgt. JZ, det. PP (2002); all specimens were immature.

Characteristics of sites. All records were from small and middle-sized brooks (3rd and 4th Strahler order, river width up to 5 m) with a bottom substrate dominated by sand, gravel, and stones. Most of these highland stretches had a natural morphology (with buffer strips) and extensively used (cropland, grassland), partially forested catchments. The brooks have good water quality (from oligo- to beta-mesosaprobity), with one exception of lowland stream (Okluky River, alpha-mesosaprobity).

Ecology. Being a detritophagous species, *A. limnobius* occurs from hyporhithral to potamal, and also has been collected from standing water areas including deeper, profundal habitats (Hörner *et al.* 2002; Šporka 2003; Alves *et al.* 2008). It can tolerate intermediate eutrophication (Verdonschot 2006) and organic pollution from oligo- to alpha-mesosaprobity (Hörner *et al.* 2002). Microhabitat preferences of fine substrates rich in organic material (pelal, psamal and argylal) have been reported (Šporka 2003; Alves *et al.* 2008). Among oligochaetes, *A. limnobius* belongs to K-strategists (Šporka 2003) with one reproductive cycle per year, mostly realised by asexual reproduction (architomy); mature specimens are rare (Timm & Veldhijzen van Zanten 2002). Worms burrow in sediment, where they build reinforced tubes of silt (Timm & Veldhijzen van Zanten 2002).

Morphology. *Aulodrilus limnobius* has characteristic bifid crotchets, with up to 10 chaetae per bundle (all with shorter upper teeth, and occasionally with wing-like dilations of the distal ends of the chaetae in posterior segments), and an unsegmented tail, serving as a respiratory organ—typical for this genus. It can be distinguished from its congeners known to occur in the Czech Republic—*A. pigueti, A japonicus*, and *A. pluriseta*—by a lack of hair chaetae (Timm & Veldhijzen van Zanten 2002; van den Hoek & Verdonschot 2005).

Distribution. This cosmopolitan species has been recorded in most of European countries, absent only in Austria, the UK, Croatia, Denmark, Greece, Slovenia, and Portugal (Timm & Giani 2004). According to Fauna Europaea (Timm & Giani 2004), the species was found also in the Czech Republic, but unfortunately, the source of this data is not available.

Aulodrilus pigueti Kowalewski, 1914

Records. Štěpánek Pond, Pozďatín, *49°13'22"N / 16°02'25"E*, lgt. JS, det. KP (2007); the single specimen collected during this study was immature.

Characteristics of sites. *Aulodrilus pigueti* was found at a single locality, the Štěpánek fishpond in the Czech-Moravian Highland Mountains (Českomoravská vrchovina), which is situated in the middle part of the Czech Republic. This fishpond is a small shallow pond (2.2 ha) exploited by intensive fish farming. The management of the fish stock composed of *Cyprinus carpio* Linnaeus and *Tinca tinca* Linnaeus involved supplementary feeding and application of manure, which caused nutrient enrichment. Macrophytes, mostly *Glyceria maxima* (Hartman) and *Typha latifolia* Linnaeus, covered only 5% of the littoral zone.

Ecology. *Aulodrilus pigueti* burrows in sediments where it forms tubes from detritus (Timm & Veldhijzen van Zanten 2002). Finogenova and Arkhipova (1994), Schloesser *et al.* (1995) and Šporka (1996b) reported that *A. pigueti* inhabits various freshwater environments, such as lakes, rivers, and marshes. It prefers water quality from oligo- to alpha-mesosaprobic and substrate pelal (Šporka 2003), and tolerates a lower amount of dissolved oxygen and acidification (Orciari & Hummon 1975; report EPA/600/3-90/073). The sample from which this single specimen of *A. pigueti* was identified was dominated by *Dero digitata* (Müller, 1774), more species in the genus *Limnodrilus*, and other Tubificinae.

Morphology. *Aulodrilus pigueti* can be distinguished from all other taxa by distal dilatations of bifid crotchets with shorter upper teeth (typical for the genus *Aulodrilus*). Dorsal crotchets beginning from VI–IX are replaced by oar-shaped pectinate chaetae with a rounded or sometimes slightly bifid tip. It is possible to distinguish it from congeners and all other Tubificinae by the presence of oar-shaped chaetae in dorsal bundles in its middle segments.

Distribution. *Aulodrilus pigueti* is a cosmopolitan species, often found in tropical countries (Timm & Veldhijzen van Zanten 2002; Arslan & Sahin 2003). The nearest record to the Czech Republic is from backwaters of the Morava River in Slovakia (Šporka 1996b); it has also been recorded from the neighbouring countries of Germany and Poland (Timm & Giani 2004). While *A. pigueti* is widely distributed in many other European countries it has not been reported from Austria, Hungary, Romania, Belgium, the UK, Denmark, Latvia, Switzerland, and Slovenia (Timm & Giani 2004).

Conclusions

This updated check-list of aquatic oligochaetes of the Czech Republic, based on historical and recent records, includes 95 species: Lumbriculidae (12 species), Naidinae (36 species), Pristininae (eight species), Tubificinae (22 species), Rhyacodrilinae (six species), Propappidae (one species), Criodrilidae (one species), Haplotaxidae (one species), and Lumbricidae (8 species). The Red list of Czech aquatic oligochaete species has been corrected and updated, and now includes nine endangered species (EN), 14 vulnerable species (VU), and one near threatened (NT). Special attention should be given to the protection and conservation of unique habitats from which these endangered and vulnerable species have been reported. In addition, it is important to establish and support monitoring programs for all aquatic habitats to document introduced and invasive species that are known to be spreading throughout Europe and elsewhere worldwide.

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Research Paper

Small-Scale Distribution of Aquatic Macroinvertebrates in Two Spring Fens with Different Groundwater Chemistry

key words: springs, fens, macroinvertebrate assemblages, within-site heterogeneity, seasonal variation

Abstract

We examined responses of macroinvertebrate assemblages to environmental and temporal variations along spring source-spring brook transects in two fen habitats, sharply differing in groundwater chemistry, and compared the patterns among individual taxonomical groups. We hypothesised a different importance of environmental heterogeneity and seasonal changes primarily linked to strong tufa precipitation, which causes stronger environmental filtering in the calcareous fen. In concordance, we observed that assemblages of the more homogenous calcareous fen primarily changed over time, due to seasonal shifts in source availability and favourable conditions. Their spatial distribution was determined by the amount of CPOM, tufa crusts and temperature variation, but a substantial part of the assemblage exhibited spatial uniformity (Plecoptera, Clitellata, and especially Trichoptera and Diptera). The assemblages of the more heterogeneous *Sphagnum*-fen were primarily driven by water pH and substrate and the season was a notably weaker predictor. We found that different macroinvertebrate groups can display various responses to the measured variables shaping the overall pattern obtained based on the whole community. Further, greater environmental heterogeneity can result in temporally stable species distribution patterns even at very small spatial scales within a single site.

1. Introduction

Small-scale distributions of macroinvertebrates in springs have been studied mainly in terms of the zonation patterns of assemblages along the springbrooks and spring source-springbrook gradients. These studies looked at different lengths of the spring fen gradient and different within-site heterogeneity and the composition of assemblages from different studies reflected different environmental factors such as discharge (Minshall, 1968; Chertoprud, 2006), temperature (Ward and Dufford, 1979; Resh, 1983; Von Fumetti *et al.*, 2007; Barquín and Death, 2011), and substrate (Ward and Dufford, 1979; Ferrington *et al.*, 1995; Ilmonen and Paasivirta, 2005). The distance from a spring-source was commonly

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interpreted as a proxy for rapid changes in the environment with decreasing influence of a stable supply of groundwater (*e.g.*, Von Fumetti *et al.*, 2007) and rapid changes of distributional patterns of macroinvertebrates at the species level and at higher taxonomic levels. These patterns have often been described by comparing the macroinvertebrate species occurring at different distances from the spring-source (*e.g.*, Resh, 1983; Williams and Hogg, 1988; Barquín and Death, 2011). Beside substrate, discharge and temperature regimes, groundwater chemistry may also influence macroinvertebrate distributions within a single spring site. It can be expected to be important in heterogeneous springs, where the input of groundwaters of different chemistry creates markedly different patches within a spring. Direct effects of pH, bases and metal content in water can alter the suitability of these microhabitats for macroinvertebrates since individual species and/or taxonomic groups are diversely sensitive to these factors, mainly due to physiological reasons (*cf.* Ford, 1989; Sutcliffe and Hildrew, 1989; Vuori, 1995). Glazier and Gooch (1987) and Glazier (1991) reported that alkalinity and pH had an important influence on spring assemblage compositions; similarly, Williams *et al.* (1997) found that water chemistry parameters, describing different levels of urbanisation, were important determinants of spring assemblages.

Benthic macroinvertebrate assemblages of springs are composed of species with different strengths of the linkage to the spring environment (Lindegaard *et al.*, 1998) and also with different biological and ecological traits (Williams, 1991; Wagner *et al.*, 1998). Therefore, the distribution of macroinvertebrates within a spring reflects not only the various habitat preferences of species (*e.g.*, Ilmonen and Paasivirta, 2005) and their requirements for different food sources (*e.g.*, McCabe and Sykora, 2000; Barquín and Death, 2006), but also their life histories (*cf.* Williams and Hogg, 1988; Barquín and Death, 2004). The responses of invertebrate orders and/or taxonomic groups to measured factors describing spring habitats can differ from one another (*e.g.*, Minshall, 1968; Resh, 1983; Williams and Hogg, 1988); thus, the whole macroinvertebrate assemblage may comprise taxa with different, even inverse, responses. However, there is a lack of studies directly comparing the responses of whole macroinvertebrate assemblages with responses expressed by the individual taxonomic groups comprising them.

In this study, we explored the within-site distribution of macroinvertebrates in two spring fens that significantly differ in water pH and mineral richness. The first site was a calcareous fen with differing degrees of tufa precipitation which provided an ecologically extreme habitat. The second site was a *Sphagnum*-fen characterised by different pH levels, mineral richness and different substrates. We aimed (i) to compare the spatial and temporal variations between the two complete macroinvertebrate assemblages and among individual taxonomic groups, namely Ephemeroptera, Plecoptera, Trichoptera, Diptera (except Chironomidae), Chironomidae, and Clitellata, and (ii) to analyse the influence of the measured variables on species composition of the assemblages. We hypothesised that temporal variation would be more important in the calcareous fen since precipitations of tufa can constrain the number of microhabitats and their diversity, while the spatial distribution of species would be more important in the environmentally more heterogeneous *Sphagnum*-fen and would be predominantly driven by gradients of pH and mineral richness.

2. Methods

2.1. Study Area and Sites

The studied spring fens are located in the Western Carpathian flysch zone, in the borderland between the Czech Republic and Slovakia. Flysch is characterised by a serial alternation of sandstone and claystone deposits with different calcium contents. Due to specific bedrock chemistry, spring fens with different mineral richness of groundwater occur in this area. Two spring fens representing the opposite ends of the pH/calcium gradient were selected for this study. The first site is a calcareous fen with strong tufa precipitation (the Bílé Potoky Nature Reserve; 49°06′47″ N, 18°01′24″ E) situated in the Bílé Karpaty Mountains; the second site is a *Sphagnum*-fen (the Obidová Nature Monument 49°31′03″ N, 18°31′24″ E) situated in the Moravskoslezské Beskydy Mountains. Both spring fens are composed of four helocrenes with springbrooks which drain the whole spring fen area and connect downstream. In each site, we chose a *ca.* 400 m long spring source-springbrook transect comprising three helocrenes of different groundwater chemistry and their springbrooks. The transect ran longitudinally through the spring fen and covered the whole site's heterogeneity.

The calcareous fen was situated on a steep meadow hillside which reaches an altitude between 380 and 500 m above sea level (m a.s.l.) and covers 8.8 ha. Great horsetail (*Equisetum telmateia* EHRH.) and butterbur (*Petasites hybridus* LINNÉ) were the dominant vascular plants; brown mosses (family Bryidae) dominated in the moss layer. However, no plants covered the bottom of the springbrooks. The pH of the water was alkaline (mean = 8.3 ± 0.2) and conductivity was very high (mean = 480 ± 43.0 μS cm^{-1}). The spring source-springbrook transect was characterised by an increasing discharge and an increasing variation in water temperature downstream (Table 1). An intense precipitation of calcium carbonate (tufa) formed strong crusts (mostly upstream) as well as small tufa grains (predominantly downstream). Beside tufa incrustations, the dominant types of bottom substrate were stones and dead leaves. Dead leaves dominated in the upper sections near the spring sources, while stones were mostly found in the lowermost section of the spring fen (Table 1).

The *Sphagnum*-fen was situated in a meadow with moderate slopes between 710 and 750 m a.s.l. and covered 7.3 ha. The spring source-springbrook transect was characterised by a sharp gradient in pH and mineral richness. The uppermost part of the spring fen was classified as a mineral-poor *Sphagnum*-fen characterised by an acidic pH (mean = 5.2 ± 0.6) and a high iron concentration (2620 μg l^{-1}). The remaining part of the *Sphagnum-fen* was classified as a mineral-rich *Sphagnum*-fen dominated by calcitolerant *Sphagna*. The mean pH was higher here than in the mineral-poor part of the *Sphagnum*-fen (pH 6.6 ± 0.7); the iron concentration remained very high in the middle part of the spring fen (mean = 2875 ± 650.9 μg l^{-1}) but this value decreased distinctly downstream (mean = 244 ± 72.7 μg l^{-1}). The conductivity was rather low (mean = 60 ± 14.2 μS cm^{-1}) along the whole *Sphagnum*-fen. The discharge was lower in the middle sections of the spring fen than in the upper and lower sections due to the high retention of water by peat in the middle sections (Table 1). The substrate characteristics changed considerably along the entire spring source-springbrook transect; fine detritus (FPOM) and *Sphagna* prevailed near the spring sources, whilst a coarse inorganic substrate (coarse gravel) dominated in the lower part of the *Sphagnum*-fen (Table 1).

2.2. *Field Sampling and Explanatory Variables*

Sampling was carried out three times: in spring (April–May), summer (July), and autumn (September) in 2006. Benthic samples were collected in five (calcareous fen) and six (*Sphagnum*-fen) sections of the springs which represented different abiotic conditions along the 400 m long transects from spring sources to the springbrooks draining each fen. The length of each section was approx. 50 m, and the distance between neighbouring sections was at least 20 m. In each section, a sample consisting of ten sampling plots (25 cm × 25 cm), which represented all of the habitats along the sampled spring section, was taken semiquantitatively with a hand net (mesh size 0.5 mm) by the kick-sampling method (FROST, 1971). After washing the sampled substrate several times in a bucket and removing the coarse inorganic substrate, the sample was elutriated through a net of a 0.5 mm mesh size and the remaining material was preserved in 4% formaldehyde. The samples were subsequently sorted and invertebrates were identified in the laboratory, mostly to the species and genus levels.

Water temperature, pH, conductivity, and dissolved oxygen were measured in the field using portable instruments (WTW Multi 340i/SET) in each sampling occasion. The annual variation of temperature of each section was calculated from individual measurements as the difference between maximal and minimal temperature values during the year. Discharge was estimated by sampling the spring water in a vessel for a defined period of time in each sampling. We recorded the percentage cover of stones, gravel, sand, coarse particulate organic matter (CPOM), fine particulate organic matter (FPOM), and tufa crusts in each section. For statistical analyses, the relative proportions of stones, gravel, and sand in each section were expressed by the phi coefficient (FURSE *et al.*, 1986). In autumn, water samples were collected and concentrations of soluble Na, K, Ca, Mg, Al, Fe, SO_4, NO_3, and PO_4 ions were measured

Table 1. Mean values of the physicochemical variables measured in the calcareous fen and the *Sphagnum*-fen in spring, summer, and autumn in 2006. The amount of inorganic substrate and FPOM did not change during the year, therefore concrete numbers of phi coefficient, tufa crusts, and FPOM are shown in the table instead of mean values. The samples are numbered from 1 to 5 and 1 to 6 and describe the increasing distance of the sampled sites away from the source of the fens. Var. in temperature – variation in temperature; CPOM – coarse particulate organic matter; FPOM – fine particulate organic matter.

	pH	Var. in temperature (°C)	Conductivity (µS cm^{-1})	Dissolved oxygen (mg l^{-1})	Discharge (ml s^{-1})	*phi*-coefficient	Tufa crusts (%)	CPOM (%)	FPOM (%)	Ca (mg l^{-1})	Fe (µg l^{-1})	Distance from helocrenes (m)
Calcareous-fen												
1	8.1 ± 0.12	3.7	523 ± 54.0	8.3 ± 1.9	377 ± 441	–3.2	30	43.3 ± 4.7	0	105	534	1
2	8.3 ± 0.05	4	474 ± 27.0	9.2 ± 2.3	377 ± 239	–1.9	30	53.3 ± 4.7	0	75.9	83	10
3	8.2 ± 0.12	4.8	497 ± 9.4	8.9 ± 2.2	767 ± 525	–3.1	20	33.3 ± 4.7	0	93.7	279	120
4	8.5 ± 0.08	7.4	495 ± 32.0	9.1 ± 2.5	933 ± 556	–0.775	20	30	0	92.9	130	220
5	8.4	7	483 ± 7.5	9.9 ± 3.2	933 ± 556	–2.95	20	10	0	82.5	164	300
Sphagnum-fen												
1	5.2 ± 0.6	6	55 ± 16.7	3.1 ± 3.0	233 ± 94	–1.41	0	0	60	9.3	2620	1
2	6.1 ± 0.6	5	74 ± 2.6	5.4 ± 1.3	63 ± 9	16.25	0	0	90	6.8	3660	1
3	6.5 ± 0.5	6	58 ± 4.3	6.8 ± 1.8	73 ± 61	–1.75	0	0	70	5.4	2090	10
4	6.6 ± 0.7	4	65 ± 16.2	7.5 ± 1.9	933 ± 634	–5.01	0	0	30	10	311	170
5	7.0 ± 0.3	5	47 ± 2.6	8.3 ± 2.0	300 ± 284	–3.55	0	0	40	6.3	143	200
6	6.8 ± 0.8	4	63 ± 14.5	7.8 ± 1.8	1067 ± 680	–4.83	0	0	5	10.4	278	300

in an accredited laboratory. These samples were collected in late autumn because of the relative stability of water chemistry in this period (*cf.* HÁJEK and HEKERA, 2004).

2.3. Statistical Analyses

We analysed responses of the entire benthic community first (*i.e.*, all taxa from all samples) and then from the whole array we chose the following taxonomic groups, which were the most species rich and abundant in studied sites: Ephemeroptera, Plecoptera, Trichoptera, Diptera except Chironomidae, and Clitellata. These datasets were analysed separately. The individual species-by-sample matrices were subjected to principal components analysis (PCA) due to the low species turnover within the studied transects. The length of the main gradient in the detrended correspondence analysis (DCA) did not exceed 3 units, indicating that ordination techniques based on the linear response model were most appropriate (LEPŠ and ŠMILAUER, 2003). The relationships between explanatory variables and site scores on the first four ordination axes of PCA were assessed using Spearman's rank correlations (r_s) for continuous variables and the Mann-Whitney U test for nominal variables. Partial redundancy analysis (pRDA) with the season as a covariable was used for testing the amount of variability explained by each physicochemical variable with a forward selection for each tested taxonomic group. The Monte-Carlo permutation test was used to test the significance of variables ($P = 0.01$; 1999 permutations). Prior to PCA and RDA, the species abundance data were log $(x + 1)$ transformed to reduce the effect of dominant taxa. The variation partitioning approach (BORCARD *et al.*, 1992) was used to determine the relative amounts of variation in species data explained by two different constraining groups of variables (physicochemical variables and season) in separate RDAs. Since the explained variation was also influenced by the number of explanatory variables (PERES-NETO *et al.*, 2006), we only used the three most powerful predictors in each group of variables that were significant in the separate RDAs. The CANOCO 4.5 software package (TER BRAAK and ŠMILAUER, 2002) was used for computing the ordinations and STATISTICA 8 (HILL and LEWICKI, 2007) was used for the univariate analyses. Sequential Bonferroni corrections of the significance levels (HOLM, 1979) were applied for multiple comparisons of explanatory variables.

3. Results

3.1. Macroinvertebrate Diversity and Abundance

Altogether, 138 and 163 taxa were represented by 54,424 and 45,988 individuals recorded in the calcareous fen and in the *Sphagnum*-fen, respectively (Appendix 1). An abundance of non-insect taxa prevailed at both sites. The amphipod *Gammarus fossarum* KOCH accounted for 73% and 30% of all individuals in the calcareous fen and in the *Sphagnum*-fen, respectively. After *G. fossarum*, the clitellate *Trichodrilus strandi* HRABĚ was the most abundant species in the calcareous fen and the snail *Bythinella austriaca* (FRAUENFELD) was the most abundant species in the *Sphagnum*-fen (Fig. 1). In contrast, insect fauna was more taxon rich than non-insect fauna with a high dominance of Diptera, which was particularly rich in the calcareous fen and comprised nearly 65% of all taxa recorded there (Fig. 2). There were large proportions of other insect taxa in the *Sphagnum*-fen (Trichoptera and Plecoptera) and Clitellata were also characteristic for this site (Fig. 2).

3.2. Differences in the Proportions of Spatial and Temporal Variations between Spring Fens

The PCA and variation partitioning in partial RDAs revealed significant differences in the role of physicochemical variables and season for structuring of assemblages in the study of spring fens (Table 2, Fig. 3). When looking at the whole benthic assemblage in the calcare-

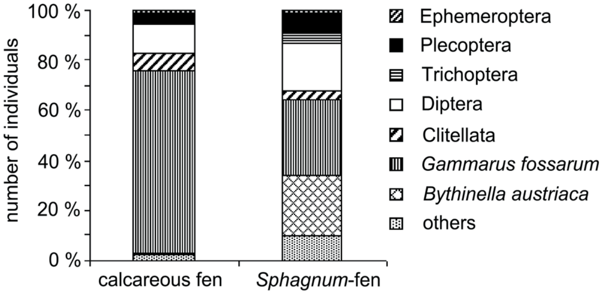


Figure 1. The relative abundance of macroinvertebrates at the studied sites.

ous fen, the first PCA axis (28% of the explained data variation) reflected the difference between spring, summer, and autumn samples, whereas the second ordination axis (18% of the explained data variation) expressed changes in physicochemical variables along the transect (Fig. 4). On the contrary, *Sphagnum*-fen assemblages revealed the opposite pattern; the first ordination axis (24.5% of the explained data variation) displayed the grouping of samples along the transect and the second PCA axis (17% of the explained data variation) was associated with the season (Fig. 5). The variation partitioning method revealed that physicochemical variables had a substantially greater influence (38%) than season (19%) in the *Sphagnum*-fen, while the difference in the variation explained by physicochemical variables and season was not so high in the calcareous fen (28.5% and 17%, respectively) when considering the whole assemblage (Fig. 3).

Furthermore, the differences in the effect of physicochemical variables and season between the individual taxonomic groups were also recorded. In the calcareous fen, the relative part of the species data variation explained by season was the highest for Trichoptera and Diptera (except Chironomidae) (32% and 28.5%, respectively), which corresponded well with the high significance of season in these groups in the PCA analysis (Table 2). Ephemeroptera was the group with the largest part of the variation explained by physicochemical variables in the calcareous fen (45.5%). In the *Sphagnum*-fen, the greatest influence of physicochemical variables (66%) and the lowest influence of season (3.7%) were characteristic for Ephemeroptera. The second highest proportion of the variation explained by physicochemical variables (43%) was recorded in the Trichoptera data set. Simultaneously, Ephemeroptera and Trichoptera were the only taxonomic groups in the *Sphagnum*-fen where the season was not significant on the first PCA axis (Table 2). In contrast, the smallest proportion of vari-

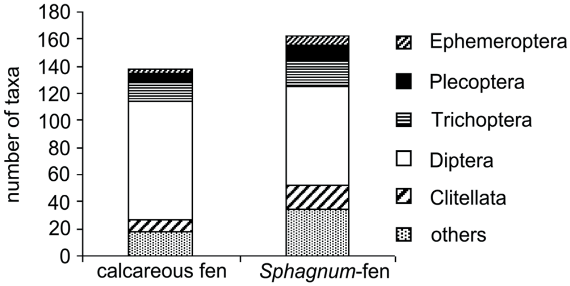


Figure 2. The taxonomic structure of macroinvertebrate assemblages at the studied sites.

Table 2. Relationships between PCA ordination site scores (first two axes), explanatory variables, and number of taxa and individuals in the calcareous fen and the *Sphagnum*-fen using Spearman's rank correlations (r_s) and the Mann-Whitney U test. Using Bonferroni corrections the current cut level for Spearman's correlations was $P < 0.003$ (in bold). Values at the significance level of $P < 0.01$ are in normal print. Diptera ex. Chir. – Diptera except Chironomidae; var. in temperature – variation in temperature; CPOM – coarse particulate organic matter; FPOM – fine particulate organic matter; ns – not significant.

calcareous fen	Ephemeroptera		Plecoptera		Trichoptera		Diptera exc. Chir.		Chironomidae		Clitellata		All taxa	
	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis
Explained variance	0.798	0.157	0.364	0.291	0.301	0.211	0.309	0.240	0.306	0.235	0.531	0.210	0.279	0.178
	r_s		r_s		r_s		r_s		r_s		r_s		r_s	
pH	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Var. in temperature	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.775**
Conductivity	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dissolved oxygen	ns	ns	**0.793**	ns	0.647	ns	ns	**0.832**	ns	**0.705**	ns	ns	ns	ns
Discharge	ns	ns	ns	ns	ns	ns	ns	ns	**–0.790**	ns	**–0.721**	ns	ns	ns
phi-coefficient	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Tufa crusts	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	–0.692
CPOM	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**–0.705**
Ca	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fe	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Distance from helocrenes	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**0.772**
Number of taxa	**0.885**	ns	–0.539	ns	ns	ns	0.686	ns	ns	ns	ns	ns	ns	ns
Number of individuals	**0.974**	ns	ns	**–0.75**	ns	ns	**0.9**	ns	**0.964**	ns	**0.985**	ns	**0.703**	ns
	P (U test)		*P* (U test)		*P* (U test)		*P* (U test)		*P* (U test)		*P* (U test)		*P* (U test)	
Season	0.03	ns	0.007	ns	0.005	ns	0.007	0.005	0.04	0.03	0.014	ns	0.002	ns
Sphagnum-fen	Ephemeroptera		Plecoptera		Trichoptera		Diptera exc. Chir.		Chironomidae		Clitellata		All taxa	
	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis	1. axis	2. axis
Explained variance	0.606	0.248	0.358	0.197	0.259	0.207	0.393	0.151	0.305	0.201	0.324	0.227	0.245	0.171
	r_s		r_s		r_s		r_s		r_s		r_s		r_s	
pH	ns	ns	**0.733**	ns	ns	ns	ns	**0.662**	0.621	ns	ns	**0.788**	ns	ns
Var. in temperature	**–0.882**	ns	ns	ns	**–0.866**	ns	ns	ns	ns	**–0.64**	ns	ns	–0.629	ns
Conductivity	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Dissolved oxygen	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	–0.604	ns	ns	ns
Discharge	**0.739**	ns	ns	ns	**0.769**	ns	**0.679**	ns	ns	ns	ns	ns	0.634	ns
phi-coefficient	**–0.894**	ns	ns	ns	**–0.699**	ns	ns	ns	ns	**–0.642**	ns	–0.636	**–0.674**	ns
FPOM	**–0.914**	ns	ns	ns	**–0.825**	ns	ns	ns	–0.636	ns	ns	–0.605	**–0.887**	ns
Ca	**0.673**	ns	ns	ns	**0.712**	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fe	**–0.733**	ns	ns	ns	–0.636	ns	ns	**–0.843**	ns	ns	ns	**–0.724**	**–0.761**	ns
Distance from helocrenes	**0.886**	ns	ns	ns	**0.863**	ns	ns	**0.683**	ns	ns	ns	**0.723**	**0.889**	ns
Number of taxa	**0.968**	ns	ns	ns	ns	ns	ns	ns	ns	**0.770**	ns	ns	ns	–0.589
Number of individuals	**0.96**	ns	**–0.834**	ns	ns	0.595	**0.721**	ns	ns	**0.746**	ns	ns	0.607	ns
	P (U test)		*P* (U test)		*P* (U test)		*P* (U test)		*P* (U test)		*P* (U test)		*P* (U test)	
Season	ns	ns	0.02	0.05	ns	0.04	0.01	ns	0.008	ns	0.009	0.05	ns	0.03

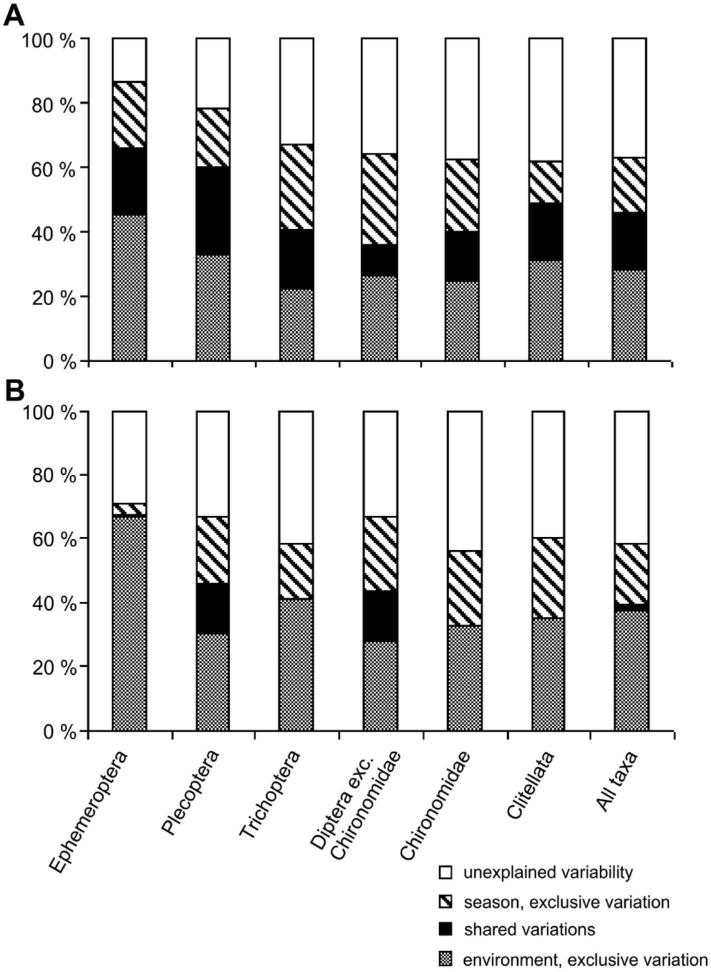


Figure 3. Comparison of the variance explained by physicochemical variables and season between the taxonomic groups of the calcareous fen (A) and the *Sphagnum*-fen (B) using the variation partitioning approach in separate RDAs.

ation explained by the physicochemical variables was detected in the Plecoptera (30%) and Diptera (28%) assemblages (Fig. 3).

3.3. Responses of Individual Taxonomic Groups to Environmental Gradients

Calcareous fen. According to the PCA, the main changes in the species composition of taxonomic groups were significantly associated with dissolved oxygen (Plecoptera and to a lesser degree Trichoptera) and discharge (Chironomidae and Clitellata) (Table 2). Numbers of individuals significantly correlated with the first PCA axes of all taxonomic groups (except Plecoptera and Trichoptera) (Table 2). Using the season as a covariable in partial RDA, variation in water temperature explained a large part of the variation in the whole

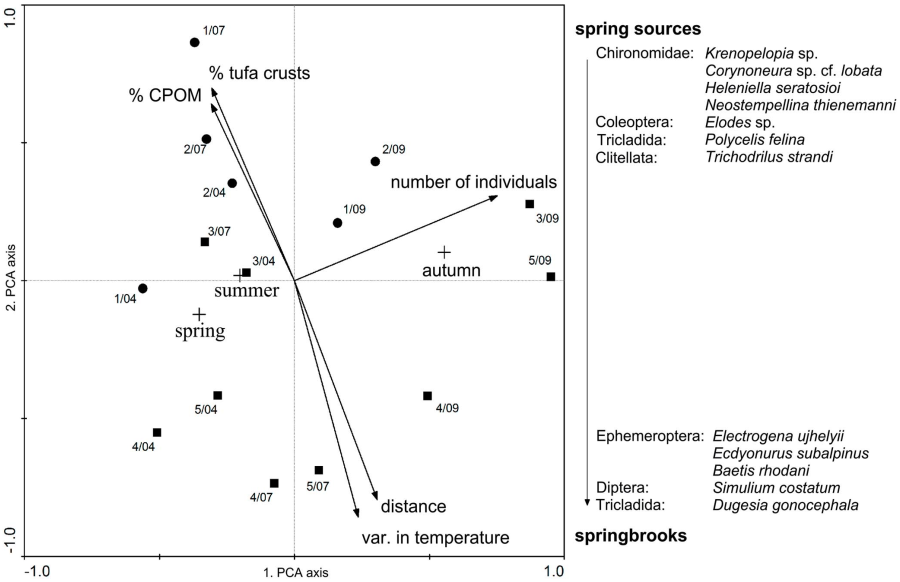
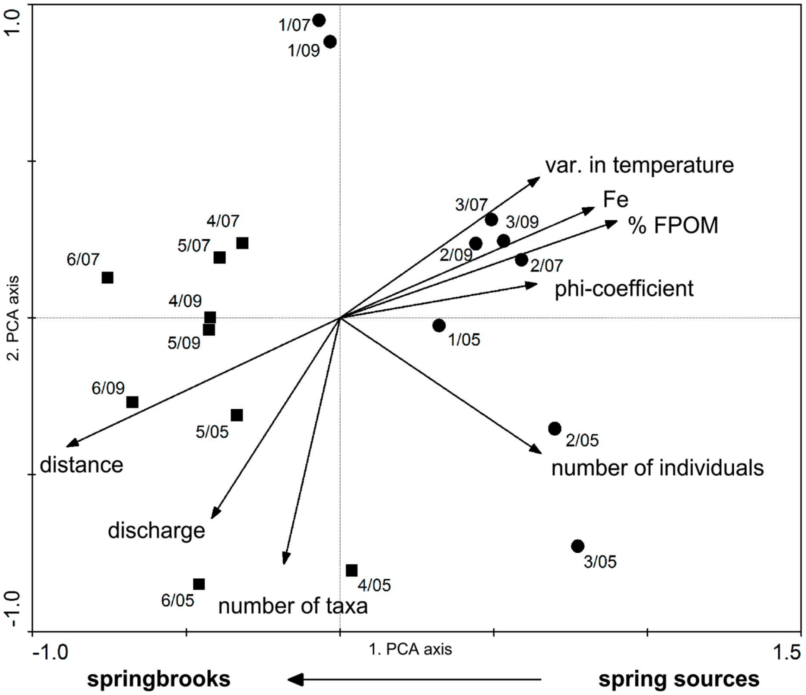


Figure 4. Principal component analysis (PCA) of all species data in the calcareous fen: ordination plot of sampled sites on the first two PCA axes with posteriori plotted abiotic variables. Only the variables significantly associated with the PCA axis at the level $P < 0.01$ are shown. The most important species which were characteristic of upstream and downstream sections are displayed. • – springbrooks in the upper part of the fen connected to their spring sources; ■ – downstream springbrooks formed by the joining of upstream springbrooks. Numbers from 1 to 5 before the slash describe the increasing distance of the sampled sites from the spring source of the fen; numbers behind the slash describe the month of sampling. Var. in temperature – variation in temperature; CPOM – coarse particulate organic matter; distance – distance from helocrenes.

assemblage data set. Looking at the whole assemblage, the most significant factors in the individual taxonomic groups were the amount of CPOM (Ephemeroptera), variation in temperature and Ca concentration (Plecoptera), and tufa crusts (Chironomidae) (Table 3). In the Clitellata assemblage, the pRDA results confirmed the importance of discharge, indicating its significant influence on this taxonomic group. However, no significant predictor was found in the Diptera assemblage (which did not include Chironomidae) (Table 3).

Sphagnum-fen. Changes in physicochemical variables along the spring source-springbrook transect played a more important role in the structuring of macroinvertebrate assemblages in the *Sphagnum*-fen than in the calcareous fen. Changes along the longitudinal transect were best displayed by the first PCA axis of the Ephemeroptera and Trichoptera assemblages, which were significantly correlated with nearly all of the physicochemical variables, reflecting changes along the transect (Table 2). Water pH was the only physicochemical variable that correlated with the first PCA axis of Plecoptera, reflecting a seasonal variation in this assemblage. However, the remaining three taxonomic groups showed a different pattern; they were more closely related to the physicochemical variables obtained on the second PCA axis of each taxonomic group (Table 2). On the basis of partial RDA analysis, FPOM was identified as the most important predictor and pH as the second most important when looking at the whole assemblage (Table 3). Likewise, the amount of FPOM controlled a large part of the variation in the Ephemeroptera, Plecoptera, and Chironomidae data sets. The main changes in species composition of the remaining groups were explained by Fe and/



Ephemeroptera:	*Ecdyonurus subalpinus*
	Habrophlebia lauta
	Baetis sp. juv.
Trichoptera:	*Silo pallipes*
Coleoptera:	*Elmis* sp.
	Limnius sp.
Megaloptera:	*Sialis fuliginosa*

Plecoptera:	*Leuctra nigra*
	Nemurella pictetii
Trichoptera:	*Beraea pullata*
Chironomidae:	*Paratendipes nudisquama*
	Rheocricotopus effusus
	Krenopelopia sp.
	Parametriocnemus stylatus
Mollusca:	*Bythinella austriaca*
	Pisidium personatum
	Pisidium casertanum
Clitellata:	*Erpobdella vilnensis*

Figure 5. Principal component analysis (PCA) of all species data in the *Sphagnum*-fen: ordination plot of sampled sites on the first two PCA axes with posteriori plotted abiotic variables. Only the variables significantly associated with the PCA axis at the level $P < 0.01$ are shown. The most important species which were characteristic of upstream and downstream sections are displayed. • – springbrooks in the upper part of fen connected to their spring sources; ■ – downstream springbrooks formed by the joining of upstream springbrooks. Numbers from 1 to 6 before the slash describe the increasing distance of the sampled sites from the beginning of the fen; numbers behind the slash describe the month of sampling. Var. in temperature – variation in temperature; FPOM – fine particulate organic matter, distance – distance from helocrenes.

Table 3. Results of the forward selection procedure in pRDA using the season as a covariable for species data in the calcareous fen and the *Sphagnum*-fen. The numbers from 1 to 9 express the order of factors according to the proportion of explained variation in each taxonomic group. Variables significant at $P = 0.01$ (Monte-Carlo test) were included in the model; these variables are in bold. Significance levels: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Diptera ex. Chir. – Diptera except Chironomidae; var. in temperature – variation in temperature; CPOM – coarse particulate organic matter; FPOM – fine particulate organic matter.

calcareous fen	Ephemeroptera		Plecoptera		Trichoptera		Diptera exc. Chir.		Chironomidae		Clitellata		All taxa	
Var. in temperature	5	2%	**2**	**11%****	8	3%	2	7%	6	4%	5	5%	**1**	**16%****
Dissolved oxygen	**3**	**7%****	4	3%	6	5%	1	13%	7	4%	6	4%	3	6%
pH	6	2%	6	2%	7	4%	6	4%	4	6%	4	6%	5	5%
Conductivity	8	1%	3	5%*	3	8%	8	4%	3	6%	7	2%	2	7%
Discharge	4	3%	8	2%	4	6%	4	5%	2	6%	1	14%*	8	3%
phi-coefficient	9	0%	5	2%	9	0%	7	4%	8	4%	8	1%	6	5%
Tufa crusts	7	1%	7	2%	5	5%	5	4%	**1**	**13%****	2	11%	4	6%
CPOM	**1**	**30%*****	9	0%	2	9%	9	0%	9	0%	9	0%	7	4%
Ca	2	8%*	**1**	**19%****	1	10%	3	6%	9	0%	3	7%	9	0%
Fe	9	0%	9	0%	9	0%	9	0%	5	6%	9	0%	9	0%
Total variance		**54%**		**46%**		**50%**		**47%**		**49%**		**50%**		**52%**

Sphagnum-fen	Ephemeroptera		Plecoptera		Trichoptera		Diptera exc. Chir.		Chironomidae		Clitellata		All taxa	
FPOM	**1**	**50%*****	**1**	**17%*****	3	7%	8	3%	**1**	**20%*****	**7**	3%	**1**	**22%*****
Var. in temperature	4	7%*	3	7%*	**1**	**20%*****	5	4%	2	7%	**2**	**10%****	4	6%
pH	7	1%	2	8%*	**2**	**16%*****	9	1%	8	3%	4	5%	**2**	**11%****
Conductivity	9	0%	7	3%	8	3%	7	3%	7	4%	5	4%	8	3%
Dissolved oxygen	8	1%	4	5%	7	5%	4	4%*	9	2%	9	3%	7	3%
Discharge	**2**	**11%****	5	4%	9	2%	3	4%	6	5%	6	4%	9	3%
phi-coefficient	6	4%	9	2%	5	6%	6	3%	4	6%	3	6%*	**3**	**7%****
Ca	5	7%*	6	4%	6	6%*	**2**	**10%*****	3	7%*	8	3%	**5**	**6%****
Fe	**3**	**8%****	8	3%	4	7%*	**1**	**14%*****	5	6%	**1**	**19%*****	6	5%
Total variance		**89%**		**53%**		**72%**		**46%**		**60%**		**57%**		**66%**

or Ca concentrations (Diptera except Chironomidae and Clitellata), water pH (Trichoptera), and variation in water temperature (Trichoptera and Clitellata) (Table 3).

4. Discussion

4.1. Spatio-Temporal Variations in Macroinvertebrate Assemblages

In the calcareous fen, the assemblages primarily changed in response to the changing seasons (Fig. 4, Table 2), whereas in the *Sphagnum*-fen, they were primarily influenced by substrate and water chemistry (Fig. 5, Table 2). On one hand, this could be connected to the different within-site heterogeneity of environmental conditions between the two spring fens, and/or on the other hand to seasonal shifts in available sources and favourable conditions. Strong tufa precipitation in the calcareous fen created a rather uniform environment, thus the main microhabitat differentiation was caused by discharge and the presence of leaf packets, which varied seasonally. In contrast, the seasonal variation was overridden by a sharp gradient of physicochemical variables (substrate structure, pH and mineral richness) in the *Sphagnum*-fen. This demonstrates the importance of spatial heterogeneity for the distribution of macroinvertebrates in springs, which was also previously pointed out by Ward and Dufford (1979), who recorded a significant effect of substrate heterogeneity on macroinvertebrate diversity and abundance in a springbrook-pond system. The pronounced shared effect of physicochemical variables and season expressed in the calcareous fen (Fig. 3) can be attributed in particular to the seasonal variability in discharge and the amount of leaf packets (*cf.* significant correlations of physicochemical variables in the PCA analysis and their non-significant contribution after including the season as a covariable in pRDA; Tables 2 and 3). It also suggests an important role of seasonal shifts in the sources and periods of favourable environmental conditions in the calcareous fen. Harsh conditions in the calcareous fen may mean that aquatic fauna is strongly dependent on the allochthonous input of organic matter in the form of leaves and wood. A substantial part of the assemblages was formed by univoltine species (especially many plecopterans and dipterans) with early spring and spring emergence. This is obvious because the life cycles of many shredders in temperate regions are synchronised with the input of riparian leaves in the autumn (*e.g.*, Malicky, 1989; Williams, 1991). They showed a major growth period in late autumn and winter when they reached high abundance and species richness in the samples (Table 2). On the contrary, the *Sphagnum*-fen was not influenced by the seasonal input of organic matter since it was surrounded by coniferous forest and was rich in organic matter due to the continual strong formation of peat. We recorded equal numbers of individuals collected during the seasons and the numbers of taxa recorded even decreased towards autumn (Table 2).

4.2. Spatial Distribution of Macroinvertebrates in the Calcareous Fen

The spatial distribution of macroinvertebrates was primarily determined by factors linked with the distance from the helocrene spring sources: tufa precipitation, organic matter input and temperature variation (Tables 2 and 3). The sections directly following the helocrenes were inhabited by unique crenophilic species from all macroinvertebrate groups that were not present downstream, whereas some species occurred exclusively in downstream sections. The contribution by particular groups to the total composition of the macroinvertebrate assemblages differed. A relative spatial uniformity of species composition (and the dominant influence of seasonal variation) was particularly observed for Trichoptera, Diptera (except Chironomidae), Plecoptera, and Clitellata. The spatial uniformity of the Trichoptera assemblage was most likely attributable to the tufa substrate, which is extremely unsuitable for many species. It forms small particles similar to sand grains, which are known to be an unstable and nutrient-poor substrate for caddis flies (SOLUK, 1985; COBB and FLANNAGAN, 1990; PHILLIPS, 2003), and/or strong compact crusts which hinder access to the deeper parts of the bottom substrate (ZOLLHÖFER *et al.*, 2000). Moreover, Trichoptera assemblages, even if they included many species restricted to springs, did not show fixed zonation patterns in a spring source-springbrook gradient, but these patterns had a strong seasonal component (MCCABE and SYKORA, 2000). Similarly, spatially uniform Diptera (except Chironomidae) assemblages varied more distinctly during the season, most likely because they consisted of many eurytopic taxa which are able to colonise a wide range of habitats (*cf.* BRINKMANN, 1991; WAGNER *et al.*, 1998; DE JONG *et al.*, 2008). On the other hand, there were several Trichoptera and Diptera taxa dominating in tufa zones which either have mechanisms to cope with tufa deposition or may actually benefit from tufa formation (MARKS *et al.*, 2006). We found several calciphilous species of Trichoptera and Diptera (*e.g.*, *Rhyacophila pubescens* PICTET, *Plectrocnemia brevis* MCLACHLAN, *Pericoma calcilega* FEUERBORN, *Thaumastoptera calceata* MIK) which could favour these conditions. However, they were evenly distributed along the studied transect. For Plecoptera, we found an effect of Ca concentration, which was difficult to interpret. The species composition of the Plecoptera assemblage was similar along the studied transect, but their abundance was higher in sections with lower Ca concentrations. We observed strong calcium carbonate encrustations on the thorax and tergites of *Nemoura* and *Protonemura* larvae sampled in the places with the strongest tufa precipitation. These encrustations might have adverse effects similar to those of Fe precipitates which cause the inhibition of motion and the smothering of aquatic invertebrates in humic waters (VUORI, 1995). A similar situation was observed in clitellate assemblages which were locally negatively affected by discharge and the presence of strong tufa crusts, because they are sensitive to physical disturbances (BARQUÍN and DEATH, 2004) and the reduction of interstitial spaces in the stream bed caused by tufa deposition (*cf.* RUNDIO, 2009). Clitellates assemblages have been documented as not being as closely related to seasonal changes as insect fauna in springs (BARQUÍN and DEATH, 2009). However, in our study, the seasonal variation was distinctive owing to the seasonal dynamics of the dominant species *Trichodrilus strandi*, which is monocyclic with spring-summer copulation, lying cocoons in summer (ŠPORKA, 2003; SCHENKOVÁ *et al.*, 2010), with juveniles in autumn increasing the number of individuals in samples.

A comparably lower influence of seasonal variation was observed in Ephemeroptera and Chironomidae assemblages. Their species composition was significantly associated with coarse organic matter and tufa crusts. They formed discrete assemblages in places near helocrenes (Chironomidae) or in the springbrook (Ephemeroptera). This pattern resulted from the dominance of crenobiontic and madicolous Chironomidae such as *Neostempellina thienemanni* REISS*, Krenopelopia* sp., *Krenosmittia cf. camptophleps* (EDWARDS) and *Parachaetocladius abnobaeus* (WULKER) (LINDEGAARD, 1995) near the helocrenes and rheophilous mayflies (*Ecdyonurus subalpinus* KLAPÁLEK, *Electrogena ujhelyii* (SOWA)) dwelling predominantly in the springbrook section of the fen.

4.3. Spatial Distribution of Macroinvertebrates in the Sphagnum-*Fen*

The spatial distribution of macroinvertebrates along the transect was governed by a joint influence of pH/mineral richness and substrate. Particular taxa were more or less sensitive to variations in pH and iron concentrations along the transect and/or the preferred substrate of different volumes of fine detritus. Water chemistry varying along the studied transect influenced Clitellata and Diptera (except Chironomidae) assemblages by the effects of acidity and high iron concentrations (Table 3). Clitellates showed a shift from acidotolerant species-dominated assemblages (Enchytraeidae) to eurytopic aquatic species-dominated assemblages

(Naididae and Lumbriculidae). Diptera (except Chironomidae) were characterised by an increased abundance of acid-benefiting Ceratopogonidae (Sommer and Horwitz, 2001) and by a very rare occurrence of species from the families Tipulidae and Limoniidae, which were documented as being limited by pH and heavy metals (Sasaki *et al.*, 2005).

Two groups, Chironomidae and Plecoptera, were predominantly associated with substrate, regardless of acidity and the distance from the helocrenes (Table 3). Many Chironomidae species showed clear preferences for either spring sections with a dominance of FPOM or with a high amount of inorganic substrate. These results correspond well to the findings of Ferrington (1998), who emphasised the importance of a predominant substrate type for the chironomid assemblage composition in springs. A joint influence of pH/mineral richness and substrate was observed in the Ephemeroptera and Trichoptera assemblages. As the mayflies are the most acid-sensitive aquatic insects (Braukmann, 2001), they were completely missing in the most acidic parts of the fen. Under episodically slight acidic and circum-neutral conditions, they were less abundant in sections with organic substrate and abundant in sections with a higher discharge and coarse sediment. This response was caused by the different degrees of acid sensitivity of mayflies. *Baetis vernus* Curtis, an “acid-benefitting” species, which can even increase their densities with increasing acidity (Tixier *et al.*, 2009), and the “slightly acid-sensitive” species *Baetis niger* (Linnaeus), which tolerates episodically acid events (Braukmann, 2001; Petrin *et al.*, 2007), inhabited sections with circum-neutral conditions that were episodically weakly acidic. In sections with neutral conditions, *i.e.*, springbrooks, the number of mayflies significantly increased and the assemblage included “acid-sensitive” species. The distribution in these sections was governed by the substrate, namely by the proportions of FPOM (preferred by, *e.g.*, *Baetis niger*) and stones and gravel (occurrence of *Ecdyonurus subalpinus* and *Habrophlebia lauta* Eaton and higher abundance of *Baetis vernus*). Contrary to Ephemeroptera, the Trichoptera taxa richness and abundance remained constant along the *Sphagnum*-fen. However, some species occurred exclusively in acidic sections (*i.e.*, acid-tolerant and/or crenophilous species, according to Braukmann, 2001; Graf *et al.*, 2008) or in acid-neutral sections (*i.e.*, slightly acid-sensitive and acid-sensitive lotic species; Braukmann, 2001; Graf *et al.*, 2008). Spring-dwelling species are generally more tolerant to low pH levels than rhitrobiontic species (see also Braukmann, 2001; Sasaki *et al.*, 2005), which appears to be an adaptation to special crenal conditions (Hahn, 2000). Simultaneously, the Trichoptera assemblage dwelling in this site comprised many species with pronounced substrate preferences which were distributed in relation to the fine detritus/inorganic particle proportions in the bottom substrate (*e.g.*, *Silo pallipes*, *Odontocerum albicorne*, and *Hydropsyche saxonica*).

5. Conclusions

Spatial heterogeneity in environmental conditions can be an important factor that controls macroinvertebrate within-site distribution. Higher heterogeneity can result in temporally stable species distribution patterns even at very small spatial scales, contrary to environmentally more homogeneous habitats where seasonal changes are of higher importance. Environmentally more heterogeneous habitats also promote variation in individual taxa responses to the measured variables, shaping the overall pattern obtained based on the whole community. In the more heterogeneous habitat, the response of the whole assemblage was a complex of different group responses, contrary to analogical or non-significant responses in a less heterogeneous habitat. This study stresses the importance of segregating the responses of individual macroinvertebrate groups when studying species distribution patterns even within a single habitat. Responses of individual groups can be highly variable or even reverse at environmentally more heterogeneous sites.

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Appendix 1. The list of taxa collected in the calcareous fen and *Sphagnum*-fen in 2006.

		calcareous fen	*Sphagnum*-fen
Platyhelminthes			
	Dugesia gonocephala (Dugés, 1830)	×	
	Polycelis felina (Dalyell, 1814)	×	
Mollusca			
	Ancylus fluviatilis O. F. Müller, 1774		×
	Bythinella austriaca (Frauenfeld, 1857)	×	×
	Galba truncatula (O. F. Müller, 1774)	×	×
	Pisidium personatum Malm, 1855	×	×
	Pisidium casertanum (Poli, 1791)	×	×
	Radix peregra (O. F. Müller, 1774)		×
Annelida			
	Achaeta sp.		×
	Cernosvitoviella sp.		×
	Cognettia glandulosa (Michaelsen, 1888)		×
	Cognettia sphagnetorum (Vejdovský, 1878)		×
	Eiseniella tetraedra (Savigny, 1826)	×	×
	Enchytraeus sp.	×	×
	Erpobdella octoculata (Linnaeus, 1758)		×
	Erpobdella vilnensis Liskiewicz, 1927		×
	Fridericia sp.	×	×
	Haplotaxis gordioides (Hartmann, 1821)	×	
	Henlea cf. *perpusilla* Friend, 1911		×
	Limnodrilus hoffmeisteri Claparéde, 1862		×
	Lumbriculus variegatus (O. F. Müller, 1774)		×
	Marionina sp.		×
	Mesenchytraeus armatus (Levinsen, 1884)	×	×
	Nais communis Piguet, 1906		×
	Pristina rosea (Piguet, 1906)		×
	Rhyacodrilus falciformis Bretscher, 1901	×	×
	Stylodrilus heringianus Claparéde, 1862		×
	Tubifex tubifex (O. F. Müller, 1774)	×	×
	Trichodrilus strandi Hrabě, 1936	×	
Crustacea			
	Gammarus fossarum Koch, 1835	×	×
Ephemeroptera			
	Baetis niger (Linnaeus, 1761)		×
	Baetis rhodani (Pictet, 1845)	×	
	Baetis vernus Curtis, 1834		×
	Ecdyonurus subalpinus Klapálek, 1907	×	×
	Electrogena ujhelyii (Sowa, 1981)	×	
	Habrophlebia lauta Eaton, 1884		×
	Paraleptophlebia submarginata (Stephens, 1835)		×
	Rhithrogena sp.		×
Odonata			
	Aeshna cyanea (O. F. Müller, 1764)		×
	Cordulegaster boltonii (Donovan, 1807)	×	
	Pyrrhosoma nymphula (Sulzer, 1776)		×
Plecoptera			
	Amphinemura standfussi (Ris, 1902)		×
	Diura bicaudata (Linnaeus, 1758)		×
	Isoperla cf. *tripartita* Illies, 1954	×	

Appendix 1. (continued)

		calcareous fen	*Sphagnum*-fen
	Leuctra braueri Kempny, 1898	×	×
	Leuctra inermis Kempny, 1899		×
	Leuctra nigra (Olivier, 1811)		×
	Leuctra prima Kempny, 1899	×	
	Nemoura cf. *avicularis* Morton, 1894		×
	Nemoura cambrica Stephens, 1836	×	×
	Nemoura cinerea (Retzius, 1783)		×
	Nemoura marginata Pictet, 1835	×	×
	Nemoura sciurus Aubert, 1949		×
	Nemurella pictetii Klapálek, 1900		×
	Protonemura lateralis (Pictet, 1835)	×	×
Heteroptera			
	Velia caprai Tamanini, 1947		×
Megaloptera			
	Sialis fuliginosa Pictet, 1836		×
Neuroptera			
	Osmylus fulvicephalus (Scopoli, 1763)	×	
Coleoptera			
	Anacaena globulus Ad. (Paykull, 1798)	×	
	Anacaena sp. Lv.	×	×
	Agabus guttatus Ad. (Paykull, 1798)		×
	Agabus paludosus Ad. (Fabricius, 1801)		×
	Agabus sp. Lv.	×	
	Deronectes platynotus Ad. (Germar, 1834)		×
	Deronectes sp. Lv.		×
	Elmis aenea Ad. (O. F. Müller, 1806)		×
	Elmis maugetii Ad. (Latreille, 1798)		×
	Elmis sp. Lv.		×
	Elodes sp. Lv.	×	×
	Esolus angustatus Ad. (O. F. Müller, 1821)		×
	Eubria palustris Lv. Germar, 1818		×
	Hydraena gracilis Ad. Germar, 1824		×
	Hydraena morio Ad. Kiesenwetter, 1849	×	
	Hydraena nigrita Ad. Germar, 1824	×	
	Hydraena saga Ad. d'Orchymont, 1930	×	×
	Hydrobius fuscipes Lv. (Linnaeus, 1758)		×
	Laccobius striatulus Ad. (Fabricius, 1801)	×	
	Limnius perrisi Ad. (Dufour, 1843)		×
	Limnius perrisi Lv. (Dufour, 1843)		×
	Limnebius truncatellus (Thunberg, 1794)		×
	Oreodytes sanmarkii Ad. (C. R. Sahlberg, 1834)		×
	Platambus maculatus Ad. Linnaeus, 1758	×	×
	Platambus maculatus Lv. Linnaeus, 1758		×
Trichoptera			
	Beraea pullata (Curtis, 1834)		×
	Beraea sp.	×	
	Ernodes articularis (Pictet, 1834)		×
	Ernodes articularis/vicinus	×	
	Chaetopteryx fusca Brauer, 1857		×
	Chaetopteryx fusca/villosa	×	
	Chaetopteryx major McLachlan, 1876	×	×

Appendix 1. (continued)

		calcareous fen	*Sphagnum*-fen
	Crunoecia irrorata (Curtis, 1834)	×	
	Hydatophylax infumatus (McLachlan, 1865)		×
	Hydropsyche saxonica McLachlan, 1884		×
	Limnephilus cf. *ignavus* McLachlan, 1865		×
	Limnephilus cf. *sparsus* Curtis, 1834		×
	Notidobia ciliaris (Linnaeus, 1761)		×
	Odontocerum albicorne (Scopoli, 1763)		×
	Oligotricha striata (Linnaeus, 1758)		×
	Parachiona picicornis (Pictet, 1834)		×
	Plectrocnemia brevis McLachlan, 1871	×	
	Plectrocnemia conspersa (Curtis, 1834)	×	×
	Potamophylax nigricornis (Pictet, 1834)	×	×
	Rhyacophila philopotamoides McLachlan, 1879	×	
	Rhyacophila pubescens Pictet, 1834	×	
	Rhyacophila sp.	×	×
	Sericostoma personatum (Kirby *et* Spence, 1826)	×	×
	Silo pallipes (Fabricius, 1781)		×
	Wormaldia occipitalis (Pictet, 1834)	×	×
Diptera			
Tipulidae	*Nigrotipula nigra* (Linnaeus, 1758)	×	
	Tipula bosnica Strobl, 1898	×	
	Tipula fulvipennis De Geer, 1776	×	
	Tipula goriziensis Strobl, 1893	×	
	Tipula lateralis Meigen, 1804	×	
	Tipula maxima Poda, 1761	×	
	Tipula rufina Meigen, 1818	×	
	Tipula unca Wiedemann, 1817		×
Limoniidae	*Cheilotrichia* sp.	×	
	Eloeophila sp.	×	×
	Ellipteroides alboscutellatus (von Roser, 1840)	×	
	Gonomyia lucidula Meijere, 1920	×	
	Gonomyia sp.	×	
	Helius sp.		×
	Molophilus sp.	×	
	Neolimnomyia filata (Walker, 1856)	×	×
	Orimarga sp.	×	
	Scleroprocta sp.	×	×
	Thaumastoptera calceata Mik, 1866	×	
Pediciidae	*Dicranota* sp.	×	×
	Pedicia sp.	×	×
	Tricyphona sp.	×	×
Ptychopteridae	*Ptychoptera lacustris* Meigen, 1830	×	
Psychodidae	*Berdeniella* sp.	×	
	Jungiella sp.	×	
	Pericoma calcilega Feuerborn, 1923	×	
	Pneumia sp.	×	
	Psychoda sp.	×	
	Threticus sp.	×	
	Ulomyia sp.	×	×
Dixidae	*Dixa maculata* Meigen, 1818	×	×
	Dixa submaculata Edwards, 1920	×	

Appendix 1. (continued)

		calcareous fen	*Sphagnum*-fen
Thaumaleidae	*Thaumalea* sp.	×	×
Ceratopogonidae	*Atrichopogon* sp.	×	×
	Ceratopogoninae	×	×
	Dasyhelea sp.	×	×
Chironomidae	*Apsectrotanypus trifascipennis* (Zetterstedt, 1838)	×	×
	Brillia bifida (Kiefer, 1909)	×	×
	Chaetocladius piger Gr.	×	×
	Conchapelopia sp.	×	×
	Corynoneura cf. *lobata* Edwards, 1924	×	×
	Diamesa cf. *tonsa* (Haliday, 1856)	×	×
	Eukiefferiella cf. *brehmi* Gouin, 1943		×
	Eukiefferiella brevicalcar (Kieffer, 1911)		×
	Eukiefferiella cf. *fuldensis* Lehmann, 1972	×	×
	Heleniella serratosioi Ringe, 1976	×	×
	Heterotanytarsus apicalis (Kiefer, 1921)		×
	Heterotrissocladius marcidus (Walker, 1856)		×
	Krenopelopia sp.	×	×
	Krenosmittia cf. *camptophleps* (Edwards, 1929)	×	
	Limnophyes sp.	×	×
	Macropelopia cf. *nebulosa* (Meigen, 1804)		×
	Metriocnemus hygropetricus Gr.	×	×
	Metriocnemus fuscipes Gr.	×	×
	Micropsectra sp.	×	×
	Microtendipes chloris Gr.		×
	Nanocladius parvulus/rectinervis		×
	Natarsia sp.	×	×
	Neostempellina thienemanni Reiss, 1984	×	
	Orthocladius lignicola Kieffer, 1914	×	
	Orthocladius rivicola Gr.	×	×
	Orthocladius rivulorum Kiefer, 1909		×
	Paramerina sp.		×
	Parametriocnemus stylatus (Sparck, 1923)	×	×
	Paraphaenocladius sp.	×	×
	Paratendipes nudisquama (Edwards, 1929)		×
	Phaenopsectra sp.		×
	Polypedilum convictum Gr.	×	
	Polypedilum laetum Gr.		×
	Polypedilum scalaenum Gr.	×	×
	Prodiamesa olivacea (Meigen, 1818)	×	×
	Parachaetocladius abnobaeus (Wülker, 1959)	×	×
	Rheocricotopus atripes (Kiefer, 1913)	×	×
	Rheocricotopus effusus (Walker, 1856)	×	×
	Rheocricotopus fuscipes (Kiefer, 1909)	×	×
	Rheocricotopus sp.		×
	Smittia sp.	×	
	Stempellinella ciliaris/flavidula	×	×
	Synorthocladius semivirens (Kiefer, 1909)	×	×
	Tanytarsus sp.		×
	Thienemanniella clavicornis/vittata		×
	Trissopelopia sp.		×
	Tvetenia bavarica Gr.	×	×
	Zavrelimyia sp.	×	×

Appendix 1. (continued)

		calcareous fen	*Sphagnum*-fen
Simuliidae	*Simulium argyreatum* Meigen, 1838	×	
	Simulium cf. *carpathicum* (Knoz, 1961)	×	×
	Simulium costatum Friederichs, 1920	×	×
	Simulium ornatum Meigen, 1818	×	
	Simulium trifasciatum Curtis, 1839	×	
	Simulium vernum Gr.	×	×
Sciaridae		×	×
Cecidomyidae		×	×
Stratiomyidae	*Beris clavipes* (Linnaeus, 1767)	×	
	Beris vallata (Forster, 1771)		×
	Nemotelus pantherinus (Linnaeus, 1758)	×	
	Oxycera meigenii Staeger, 1844	×	
	Oxycera pardalina Meigen, 1822	×	
	Oxycera pygmaea (Fallen, 1817)	×	
Tabanidae	*Chrysops caecutiens* (Linnaeus, 1758)		×
	Hybomitra sp.		×
	Tabanus autumnalis Linnaeus, 1761		×
	Tabanus sp.	×	
Rhagionidae	*Chrysopilus* sp.	×	
Athericidae	*Ibisia marginata* (Fabricius, 1781)	×	
Empididae	*Chelifera* sp.	×	×
	Clinocera sp.	×	
	Dolichocephala sp.	×	
Dolichopodidae		×	
Syrphidae	*Melanogaster hirtella* (Loew, 1843)		×
Sciomyzidae	*Pherbellia* sp.	×	×
Drosophilidae	*Scaptomyza* sp.		×

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PRIMARY RESEARCH PAPER

Species richness and composition patterns of clitellate (Annelida) assemblages in the treeless spring fens: the effect of water chemistry and substrate

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Abstract Spring fens are isolated treeless wetlands of a high conservation value. Their environmental conditions are strongly related to their groundwater chemistry, which controls species distribution within various groups of organisms. Clitellates, a dominant group of non-insect aquatic fauna, however, have never been studied in these habitats. It is unclear from previous studies to what extent the distribution of aquatic non-insect taxa reflects water chemistry rather than the substrate structure. We studied 34 spring fens sampled in 17 isolated sites in the Western Carpathian Mountains to determine mainly the effects of water chemistry and substrate structure on variation in species richness and composition of clitellate assemblages as examples of the non-insect fauna. A total of 34 taxa were found, with 3–15 taxa collected per sample. Species richness was negatively correlated with water mineral concentration measured as water electric conductivity ($r = -0.57$, $P < 0.001$) and positively with TOC ($r = 0.60$, $P < 0.001$). Surprisingly, the lowest number of taxa was found in calcareous fens and richness increased towards *Sphagnum*-fens. There was a species turnover related to changes in mineral richness and substrate characters. The main change of species composition was promoted by changes in substrate structure. The second gradient of species composition was linked with the amount of nutrients, moisture, and dominance of sphagna, and was associated with an increase of eurytopic species in fens with high nutrient availability. It was difficult to separate the effects of water chemistry and substrate on clitellate species distributions owing to the fact that variation in tufa precipitation and vegetation was driven by water chemistry changes. This study presented the first quantitative data on fen clitellate assemblages, which appear to have an unusual pattern of species richness. In contrast to plants and molluscs, calcareous fens appeared to be a harsh environment for clitellate species. Only few specialized species, mainly *Trichodrilus strandi*, were able to establish viable populations. The significant effect of water chemistry on clitellate distribution patterns raises questions about the direct influence of water chemistry on non-insect aquatic taxa, which have previously been considered to be mostly determined by substrate characteristics.

Keywords Spring fens · Oligochaeta · Hirudinida · Poor–rich gradient · Diversity pattern

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Introduction

Identification and explanation of non-random patterns of species richness and composition is one of the key factors in understanding the underlying processes of community ecology. However, the patterns observed can vary between different habitats and they are also dependent on the length of the gradient and the groups of organisms studied. Thus, it is usually not possible to apply results of studies in one type of habitat or taxonomical group directly to another habitat or group. Knowledge of the ecology of particular taxonomical groups is often highly uneven across different habitat types.

For example, clitellates (sensu Erséus, 2005), which include oligochaetes and leeches, have been studied in detail in various types of running waters, lakes, and artificial habitats (e.g. Šporka, 1998; Verdonschot, 2001; Nijboer et al., 2004; Dumnicka & Boggero, 2007; Dumnicka et al., 2007). However, there are only few studies of this group in mire habitats. These studies dealt with oligochaetes of ombrotrophic bogs and moorlands (i.e. mires that receive all of their water and nutrients from precipitation) and bog streams (Svendsen 1957a, b; Cragg, 1961; Smith, 1986; Smith & Kaster, 1986; van Duinen et al., 2006), which showed to have very low species richness. The only abundant species inhabiting these nutrient-poor and acidic mires, *Cognettia sphagnetorum*, is also the only thoroughly studied species in terms of growth, survival, and feeding habits (e.g. Springett & Latter, 1977; Latter & Howson, 1978). Habitats slightly influenced by alkaline groundwater (lagg zones, transitional mires, and bog brooklets) tend to have higher species richness because of higher availability of nutrients (van Duinen et al., 2006).

There is only sketchy information on clitellate assemblages of treeless spring fens (i.e. minerotrophic mires, wetlands supplied by mineral-rich groundwater). Available studies on fens deal with the production of soil oligochaetes (Erman & Erman, 1975) and species composition of assemblages in degraded fenland area in the Netherlands affected by eutrophication (Verdonschot, 1984) and in ditches and canals of drained fens in Germany (Langheinrich et al., 2004). Clitellates of undisturbed, nutrient-limited spring fens have never been studied.

The habitat diversity of well-preserved fens is primarily determined by the chemistry of the groundwater supplying them, especially the calcium concentration and water pH (e.g. Hájek et al., 2006). A sharp gradient of mineral richness from acid mineral-poor fens to extremely mineral-rich calcareous fens was originally described in relation to vegetation as the poor–rich gradient (e.g. du Rietz, 1949; Malmer, 1986; Hájek et al., 2006). Five vegetation types of increasing complexity along the gradient clearly describe the diversity of fens (for details, see Hájek et al., 2006). Three of the vegetation types are *Sphagnum*-fens (mineral-poor, moderately mineral-rich, and mineral-rich fens) and two are brown-moss fens (extremely mineral-rich and calcareous fens). Conspicuous changes in species richness and composition have also been observed along this gradient in molluscan (Horsák & Hájek, 2003), algal (Poulíčková et al., 2003; Fránková et al., 2009), and testacean (Opravilová & Hájek, 2006) assemblages. However, the question whether aquatic macroinvertebrates respond significantly to this ecological gradient is yet to be studied, because most existing ecological studies have not covered the entire length of the gradient (e.g. Erman & Erman, 1975; van Duinen et al., 2006; Suren et al., 2008).

Previous studies that have included water chemistry were restricted to rather uniform mire habitats, which is the probable reason why these studies found that the variation in macroinvertebrate assemblages was governed by physical habitat attributes at the within-site scale and by various basic ecological differences among wetland types at the between-site scale. Glazier (1991) assumed there was an influence of mineral richness and pH on the composition of macroinvertebrate assemblages of springs, because non-insect taxa highly dominated in assemblages of hard-water springs and insect taxa dominated in soft-water springs, whereas insects usually prevail in other lotic habitats regardless of their pH or alkalinity (Glazier & Gooch, 1987; Glazier, 1991). Glazier (1991) proposed a complex influence of the adverse effects of low pH and alkalinity on crustaceans and molluscs and the benefits of physical constancy in the habitats he studied which favoured non-emergent species with high population densities. Subsequent research confirmed the importance of habitat stability, especially in terms of minimal fluctuation in flow and temperature fluctuations (Barquín & Death, 2004), and supported the existence of a weak association between aquatic insects and spring water only in hard-water springs (Virtanen et al., 2009) and tufa-forming spring fens (Bojková & Helešic, 2009). However, the influence of water chemistry on the distribution patterns of the aquatic non-insect fauna has not been studied to date in mires.

The aims of this study were to (i) determine the effect of water chemistry on species richness and composition of clitellate assemblages, (ii) show the pattern of species distribution along the main environmental gradients, and (iii) provide the first quantitative data on aquatic and semi-aquatic oligochaetes and leeches of spring fens. We have chosen the Western Carpathian flysch zone (Czech Republic and Slovakia) as our study area because they vary from calcium-poor acidic fens with a tendency towards ombrotrophy (about 2 mg l^{-1} of calcium) to extremely calcium-rich tufa-forming fens (up to 300 mg l^{-1} of calcium).

Materials and methods

Study area and sites

The study area was located on the western margin of the Western Carpathians (48°56′–49°32′N, 17°44′–18°51′E), in the border region between the Czech Republic and Slovakia (Fig. 1). It was chosen because of the variable chemistry of the local aquifers and, therefore, the groundwater supplying the wetlands within it, as well as because of the overall the similarities in hydrological characteristics, origin, and age of spring fens within the area (Rybníčková et al., 2005). The bedrock of this area is formed by alternating claystone and sandstone with variable chemistry, especially differing in calcium and magnesium content. Marls, claystone, limestone, and calcareous sandstone prevail in the south-western part of the study area, where groundwater is extremely rich in calcium and magnesium, which causes formation of cold water travertine (tufa) in most cases. Towards the north-east, the groundwater is still rich in calcium, but has a lower content of magnesium and higher contents of sodium, potassium, and iron. The northern part of the study area is characterised by decalcified, iron-cemented sandstone. It has the lowest calcium concentration within the entire study area (Rapant et al., 1996; Hájek et al., 2002).

Seventeen sites of treeless, mostly sloping fens supplied by permanent springs, were chosen to cover the complete mineral-richness gradient (Fig. 1). The sites were selected on the basis of previous comprehensive studies of spring fen vegetation in order to cover all main vegetation types that mirror main ecological types of spring fen habitats (Poulíčková et al., 2005; Hájek et al., 2006). To obtain a balanced set of these structural habitat types, six sites were selected within calcareous fens with a different degree of calcium carbonate precipitation (*Caricion*

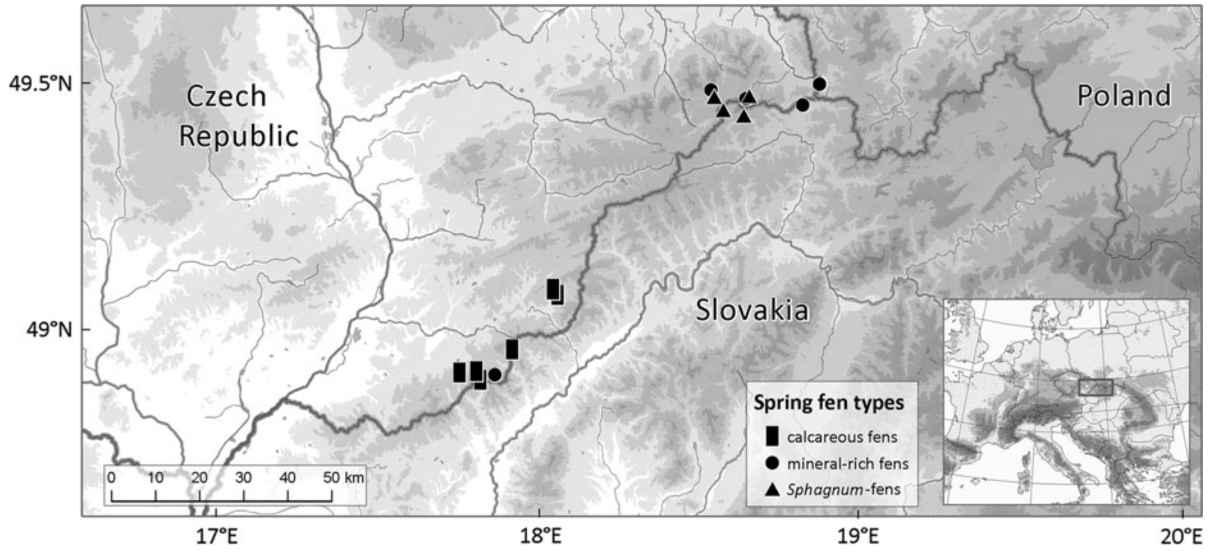


Fig. 1 Location of the study area and sites

davallianae), six within mineral-rich fens without calcium carbonate precipitation (*Caricion davallianae*), and five sites within *Sphagnum*-fens of different mineral richness and acidity of water (*Sphagno warnstorfii–Tomenthypnion*, *Caricion fuscae*, and *Sphagno recurvi–Caricion canescentis*). The altitudes of the studied fens varied from 450 to 750 m a.s.l.

Field sampling and explanatory variables

Samples of clitellates were collected three times a year (May, July, and September 2006), from 17 sites in two different habitats (altogether 102 samples), i.e. the part of the spring fen with strongest flow and the part with slow-flowing or almost standing water. For statistical analyses, samples from individual seasons were merged to provide 34 samples from two habitats at 17 sites. Each sample was a plot delimited by a metal frame of 25 × 25 cm (625 cm^2), where vegetation and bottom sediment were gathered to the depth of 5 cm. It was elutriated through a net of 250 μm mesh size, and substrate was kept in 4% formaldehyde solution. Clitellates were extracted by hand-sorting under a stereomicroscope in the laboratory. Oligochaetes were permanently mounted on microscope slides. The keys of Hrabě (1954, 1981), Nielsen & Christensen (1959, 1961, 1963), Timm (1999), and Timm & Veldhijzen van Zanten (2002) were used for identification of oligochaetes. Hirudinida were identified according to Neubert & Nesemann (1999) and Košel (2001). Species nomenclature follows Siddall et al. (2001), Erséus (2005), and Erséus et al. (2008).

Water conductivity, temperature, dissolved oxygen, and pH were measured in situ by portable instruments (WTW Multi 340i/SET). Water conductivity represents the approximation of total water mineral concentration; it strongly correlates with Ca + Mg concentration (Hájek et al., 2005) and it can be used as a reliable proxy of the calcium concentration (Sjörs & Gunnarsson, 2002; Horsák, 2006). At each sampling date, 100 ml of substrate was extracted from next to each sampling plots for measuring total organic carbon (Shimadzu TOC-V_{CPH}). For characterising each site, a one-shot sample of substrate and water was taken in August 2006. Substrate samples were sampled from a plot of 25 × 25 cm to the depth of 5 cm. These plots always lay next to the places where samples of clitellates were taken and reflected the same structural microhabitat type. Particulate organic matter (POM) was elutriated from these samples using an elutriation apparatus (Omesová & Helešic, 2004), sieved through a net of 1 mm mesh size, and dried at 70 °C. Coarse particulate organic matter (CPOM; fraction >1 mm) was sorted according to the origin (sphagna, brown mosses, vascular plants, and wood/leaves). All fractions were weighed and described as the proportions of the total weight of CPOM. Remaining inorganic substrate was dried at 80 °C and used for grain size analysis. Median diameter of particle size (D_{50}) was used for describing the substrate particle size (Giere, 1993). Water samples were collected in autumn due to relative stability of water chemistry (Hájek & Hekera, 2004) and the content of ions Ca^{2+}, Mg^{2+}, Fe, NO_3^-, and PO_4^{3-} were measured (Table 1).

Two environment characteristics of sites (moisture and nutrients) were estimated based on the vegetation composition using the Ellenberg indicator values (EIV) in the JUICE program (Tichý, 2002). The indicator value for nutrients referred to the availability of three major nutrients (N, P, K) to plants and it strongly correlated with plant biomass production (Schaffers & Sýkora, 2000). There is a general lack of correlation between soil or water nutrient concentrations and Ellenberg value for nutrients (Schaffers & Sýkora, 2000). The species composition of vascular plants was recorded in the 4 × 4 m (16 m^2) plots placed in the central part of each site. The cover of each plant species was estimated using the Braun-Blanquet nine-grade scale (van der Maarel, 1979), and an EIV for plant species was calculated (for details see Schaffers & Sýkora, 2000; Horsák et al., 2007). EIVs have proved to be very useful in studies of animal ecology, yielding accurate estimates of variables difficult to measure directly in the field (Horsák et al., 2007).

Statistical analyses

All explanatory variables were subjected to the Principal Components Analysis (PCA) on the correlation matrix (centred and standardised) to reveal the main gradients of environmental variation and to show the relations among variables. The species abundance data were log-transformed as $Y = \log$

Table 1 Descriptive statistics for the variables used in the analyses

	Calcareous fens				Mineral-rich fens				*Sphagnum*-fens			
	MEAN	MAX	MIN	SD	MEAN	MAX	MIN	SD	MEAN	MAX	MIN	SD
pH	7.9	8.2	7.3	0.3	7.3	8.4	6.3	0.6	5.4	6.9	2.8	1.1
Conductivity (μS cm^{-1})	485.8	600.0	406.0	60.3	288.3	599.0	48.0	169.8	62.9	114.0	30.0	19.5
Discharge (ml s^{-1})	188.3	533.3	46.7	162.5	78.9	130.0	43.3	30.7	120.0	433.3	23.3	157.7
TOC (g kg^{-1})	18.9	65.8	0.0	18.0	103.0	271.8	18.4	68.8	214.6	319.0	76.3	81.0
PO_4^{3-} (mg l^{-1})	0.1	0.5	0.0	0.2	0.2	0.3	0.0	0.1	0.3	0.7	0.0	0.3
NO_3^{-} (mg l^{-1})	9.3	20.5	0.2	6.2	1.0	2.5	0.1	0.9	0.5	1.3	0.1	0.4
Fe (μg l^{-1})	305.7	816.0	40.0	280.3	2925.5	3600.0	169.0	846.4	1400.4	3660.0	46.0	2327.6
Ca (mg l^{-1})	88.8	112.0	60.6	19.8	48.7	107.0	9.3	33.2	6.0	9.2	3.7	1.9
Mg (mg l^{-1})	11.7	18.6	2.4	5.8	9.3	15.5	1.2	5.5	1.9	3.1	0.7	0.9
D_{50} (mm)	3.4	11.6	0.2	3.8	0.3	0.9	0.1	0.3	0.1	0.1	0.1	0.0
POM (%)	9.8	42.1	0.2	11.1	28.0	82.2	1.6	24.4	58.3	99.1	30.2	19.0
Leaves/wood (%)	58.8	100.0	20.0	36.3	46.5	90.0	3.0	43.5	0.0	0.0	0.0	0.0
Vascular plants (%)	18.0	24.0	14.0	4.3	48.8	72.0	6.0	25.3	54.0	80.0	24.0	23.0
Brown-mosses (%)	64.3	80.0	55.0	11.1	48.0	94.0	10	28.5	14.0	26.0	2.0	12.0
Sphagna (%)	0.0	0.0	0.0	0.0	24.0	24.0	24.0	0.0	62.0	100	18.0	28.7

($n + 1$) to reduce the undue influence of dominant species. Detrended Correspondence Analysis (DCA) was used to study relationships between species composition and explanatory variables. Spearman rank correlations (r_s) and Mann–Whitney U test were used to examine possible relationships between explanatory variables and site scores on the ordination axes. Bonferroni corrections of the significance level were used for multiple comparisons of explanatory variables (Holm, 1979).

The variation partitioning approach (Økland, 1999) was used to determine the relative amount of variation in clitellate data explained by two different constraining groups of variables (water chemistry and substrate characteristics) in separate Canonical Correspondence Analyses (CCA) using the forward selection procedure and the Monte Carlo test with 1,999 permutations (Lepš & Šmilauer, 2003). Since explained variation is also influenced by the number of explanatory variables (Peres-Neto et al., 2006), we used only variables that were significant in two CCAs where all water chemistry variables and all substrate characteristics were included (not shown in the Results). The CANOCO 4.5 package (ter Braak & Šmilauer, 2002) was used for ordination techniques and STATISTICA 8 (Hill & Lewicki, 2007) for the other (uni-dimensional) analyses.

Results

Major environmental gradients and relationships among measured variables

Many of the explanatory variables were inter-correlated, as shown in the PCA of 18 explanatory variables (Table 2). PCA found one important environmental gradient within a set of explanatory variables, and the majority of these variables were significantly associated with the first PCA axis. This axis reflected the gradient of mineral richness (conductivity, Ca^{2+}, Mg^{2+}), pH, NO_3^{-} concentration, substrate structure (D_{50}, TOC, POC, wood/leaves, sphagna), moisture, and discharge (Table 2). The second PCA axis was significantly correlated with nutrients, Fe concentration, and vascular plants. The third PCA axis was correlated with PO_4^{3-} concentration and brown-mosses. The fourth axis explained a very small part of the total variation, and no significant correlations with the analysed variables were observed.

Major gradients of species composition

A total of 34 taxa of clitellates of 7,299 specimens were found in the studied spring fens (Table 3).

Table 2 PCA of 18 explanatory variables

	PCA 1 r_s	PCA 2 r_s	PCA 3 r_s	PCA 4 r_s
Eigenvalues	0.413	0.143	0.108	0.078
Water chemistry				
pH	**−0.922**	−0.004	−0.044	0.149
Conductivity	**−0.817**	−0.025	−0.323	0.049
Fe	0.465*	**0.555**	−0.127	0.288
Ca^{2+}	**−0.819**	0.199	−0.242	0.179
Mg^{2+}	**−0.736**	0.031	0.142	−0.217
NO_3^-	**−0.758**	−0.404	−0.017	−0.008
PO_4^{3-}	0.278	0.071	**−0.529**	0.450*
Substrate				
TOC	**0.865**	0.230	0.148	−0.166
Moisture	**0.609**	−0.002	−0.466*	0.093
Nutrients	−0.246	**0.620**	−0.042	0.083
D_{50}	**−0.777**	−0.335	0.012	0.118
POM	**0.802**	0.305	0.078	−0.317
Leaves/wood	**−0.806**	−0.498*	−0.285	0.201
Vascular plants	0.323	**0.612**	−0.183	−0.471*
Brown-mosses	0.099	0.447*	**0.662**	0.454*
Sphagna	**0.721**	−0.364	0.057	−0.178
Others				
Average discharge	**−0.606**	−0.222	0.342	−0.081
Depth	0.275	0.436	−0.009	−0.287

Eigenvalues of axes and correlations of explanatory variables with the first four PCA axes (r_s) are shown. Significant correlations at the level of $P < 0.01$ are marked by asterisks and those significant after Bonferroni correction ($P < 0.002$) are in bold

Numbers of taxa ranged from 3 to 15 per sample and from 6 to 27 per site; the median number of species per sample and site was 7 and 15, respectively. The clitellate assemblages consisted mostly of taxa belonging to families Enchytraeidae (14 taxa) and Naididae (11 taxa). Both Lumbriculidae and Lumbricidae were represented by three taxa and Haplotaxidae by a single species. Two species of Hirudinida were recorded. Dominant oligochaete species, *Trichodrilus strandi* and *Stylodrilus heringianus* (both Lumbriculidae), represented 31.7 and 28.7%, respectively, of all specimens recorded (Table 3).

In the DCA, the first axis accounted for 22.4%, the second for 8.2%, the third for 5.6%, and the fourth 3.3% of the total variance of species data. This analysis revealed two major gradients in species data; the first gradient expressed on the first ordination axis reflected the influence of mineral richness and substrate characteristics on the composition of clitellate assemblages (Fig. 2). Variables displaying significant correlation ($P < 0.002$) with the sample scores on the first DCA axis were those describing chemistry of spring fen water (i.e. pH, conductivity, NO_3^-, Ca^{2+}, and Mg^{2+}) and characteristics of the bottom substrate (i.e. D_{50}, TOC, proportions of POM and wood/leaves in substrate sample) (Table 4). Flow parameters (water discharge and depth) were not significantly correlated with the first DCA axis (Table 4). The first DCA axis showed the shift in species composition from calcareous fens with different degree of tufa precipitation towards *Sphagnum*-fens and, in several cases, towards mineral-rich fens (Fig. 2). This gradient represented the compound effect of mineral richness and substrate character. According to the position of individual species on the first DCA axis (Fig. 3), a stygophilous species *T. strandi* was the only species with a strong affinity to calcareous fens. In the middle part of the axis, semiaquatic taxa *Fridericia* spp., *Eiseniella tetraedra*, *Mesenchytraeus armatus*, *Cognettia sphagnetorum*, and *Rhyacodrilus falciformis* were present. Common aquatic oligochaete species, as well as a leech *Erpobdella vilnensis*, had the highest scores on the first axis. The second ordination axis expressed the second important gradient in species data, the gradient of moisture and nutrients as indicated by EIV values and the dominance of sphagna. Along this gradient, the group of *Sphagnum*-fens (upper right part of the diagram) and mineral-rich fens (lower right part of the diagram) were clearly separated (Fig. 2). *Cognettia sphagnetorum*, *C. glandulosa*, and *Lumbriculus variegatus* were associated with *Sphagnum*-fens, which was indicated by their higher scores on the second axis. Predominantly, eurytopic aquatic species (e.g. *Limnodrilus hoffmeisteri*, *Tubifex tubifex*, and *Erpobdella vilnensis*) were characteristic of a group of mineral-rich fens on the opposite side of the axis (Fig. 3). The number of taxa was significantly correlated with sample scores on the first DCA axis that reflects main changes in species composition (Table 4). Abundance did not significantly vary along both axes. Species richness significantly increased with decreasing conductivity ($r = -0.57$, $P < 0.001$) and with increasing TOC ($r = 0.60$, $P < 0.001$). The number of taxa was lowest in calcareous fens and

Table 3 List of recorded taxa

Names of taxa	Calcareous fens (12)	Mineral-rich fens (12)	*Sphagnum*-fens (10)
Oligochaeta			
Enchytraeidae			
Buchholzia sp.	0	0	0.02/1
Cernosvitoviella sp.	0.21/1	0.05/1	0.09/4
Cognettia glandulosa (Michaelsen, 1888)	0.09/1	0.56/3	2.71/7
Cognettia sphagnetorum (Vejdovský, 1878)	1.03/3	7.67/5	10.03/10
Enchytraeus albidus Henle, 1837	0	0.05/1	0
Enchytraeus cf. *buchholzi* Vejdovský, 1879	0.15/1	0	0
Enchytraeus spp.	0.96/3	0.38/1	0.19/4
Fridericia spp.	10.22/7	3.19/8	2.91/7
Henlea spp.	1.09/5	0.12/2	0
Marionina argentea (Michaelsen, 1889)	0.04/1	0	0
Marionina sp.	0.10/2	0.27/2	0
Mesenchytraeus armatus (Levinsen, 1884)	0.31/3	1.86/4	0.48/5
Mesenchytraeus sp.	0	0	0.03/5
Stercutus niveus Michaelsen, 1888	0.17/1	0	0
Naididae (sensu Erséus et al. 2008)			
Aulodrilus limnobius Bretscher, 1899	0	0.18/1	0
Limnodrilus udekemianus Claparède, 1862	0.05/1	0.88/4	0.14/2
Limnodrilus hoffmeisteri Claparède, 1862	0	1.10/5	0.66/3
Nais communis Piguet, 1906	0	0.34/4	1.26/3
Pristina bilobata (Bretscher, 1903)	0	0.23/2	0.43/4
Pristina rosea (Piguet, 1906)	0	0	0.02/1
Rhyacodrilus coccineus (Vejdovský, 1875)	0	0	0.05/1
Rhyacodrilus falciformis Bretscher, 1901	0.26/2	1.54/6	0.09/3
Tubifex ignotus (Štolc, 1886)	0	0.05/1	0
Tubifex tubifex (Müller, 1774)	0.38/3	0.95/1	1.71/3
Tubificidae juv.	0.39/3	1.64/3	1.35/5
Lumbriculidae			
Lumbriculus variegatus (Müller, 1774)	0	4.09/3	12.77/7
Stylodrilus heringianus Claparède, 1862	0.09/1	13.64/8	59.88/10
Trichodrilus strandi Hrabě, 1936	77.55/12	8.13/2	0
Lumbricidae			
Eisenia fetida (Savigny, 1826)	0	0	0.07/1
Eiseniella tetraedra (Savigny, 1826)	6.41/12	22.67/12	1.52/6
Lumbricidae juv.	0	0	0.06/2
Haplotaxidae			
Haplotaxis gordioides (Hartmann, 1821)	0.50/2	0.38/1	0
Hirudinida			
Erpobdlellidae			
Erpobdella vilnensis (Liskiewicz, 1925)	0	29.62/8	3.54/4
Trochaeta bykowski Örley, 1886	0	0.40/2	0

The average abundance per sample/the frequency in samples are given. Numbers of samples are given in parentheses

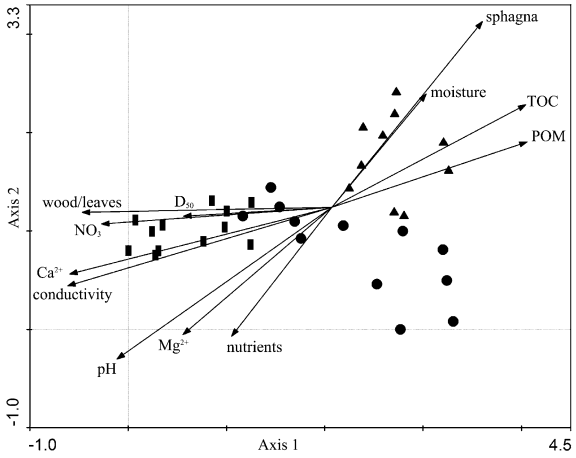


Fig. 2 DCA ordination plot of samples on the first two ordination axes. Variables that significantly correlated with the first and the second ordination axes were posteriori plotted. Classification of investigated sites was based on the vegetation units: *rectangles* calcareous fens, *circles* mineral-rich fens, *triangles Sphagnum*-fens

increased towards *Sphagnum*-fens. The number of specimens per sample was significantly correlated with moisture ($r = 0.54$, $P < 0.001$).

The position of samples from two different habitats (flowing and almost still water) differed in the ordination only in the case of calcareous fens, where sample scores differed significantly on the first axis (Mann–Whitney *U* test, $P < 0.05$); samples collected in the higher flowing habitats were situated on the left part of the DCA diagram. These flowing water samples were the most species poor of all samples (highly dominated by *T. strandi*), probably because the formation of tufa was the strongest in these habitats. Samples taken in slow-flowing habitats had a high portion of enchytraeids and therefore they were placed along the first axis close to samples from mineral-rich fens.

Individual effects of two different variable groups

In the CCA, which included all explanatory variables, conductivity, vascular plants, moisture, TOC, and PO_4^{3-} were the variables explaining the most variation in species data (Table 4). It suggested the importance of both water chemistry and substrate, although conductivity was the strongest single predictor of assemblage structure. Flow characteristics (mean discharge and depth) were not significant in the model. The variation partitioning revealed that water chemistry explained slightly more variability in species data than characteristics of the substrate did (Fig. 4). Of the 16% of variation explained by water chemistry alone, 10% was explained by conductivity and PO_4^{3-}. Moisture and TOC were responsible for the majority (9%) of the variability explained by substrate characteristics (13%). The amount of shared variability (18%) was higher than each partial effect, showing a strong correlation between bottom substrate character and mineral richness. Conductivity, that directly reflects the mineral richness of water, was significantly correlated with TOC ($r_s = -0.73$, $P < 0.001$), D_{50} ($r_s = 0.62$, $P < 0.001$), and proportions of wood/leaves and sphagna in the substrate sample ($r_s = 0.64$ and $r_s = -0.67$, $P < 0.001$). Conductivity was not significantly correlated with vascular plants ($r_s = -0.26$).

Discussion

Putting aside the widely studied effects of pollution on clitellates (e.g. Brinkhurst & Cook, 1974; Uzunov et al., 1988; Lang, 1998), main factors controlling the species richness and composition of clitellate assemblages in different freshwater habitats are linked with substrate composition, organic matter content, food quality of the organic component, current velocity (e.g. Montanholi-Martins & Takeda, 1999; Verdonschot, 1999, 2001; Dumnicka, 2006; Syrovátka et al., 2009). To date, there have been only two studies examining the effect of mineral richness of water on clitellate assemblages (Martínez-Ansemil & Collado, 1996; Schenková et al., 2001). Both reported a significant influence of water mineral richness on clitellate assemblages on a large geographical scale. However, the reported patterns showed different regional diversity of clitellates, partly interfering with different geology, rather than real effects of water chemistry. In both studies, some doubt remained about the direct effect of mineral richness on aquatic clitellate assemblages.

Our results suggest that both mineral richness and substrate are important influences of the species richness and composition of clitellate assemblages in the Western Carpathian spring fens. Flow conditions of these sites were less important (Table 4). The

Table 4 Spearman correlations (r_s) between explanatory variables and sample scores on the first three DCA axes

	DCA				CCA		
	DCA 1	DCA 2	DCA 3	DCA 4	Order in forw. sel.	Explained variation	
	r_s	r_s	r_s	r_s		%	P
Water chemistry							
pH	**−0.780**	−0.500*	−0.411	**0.514**	11	3	n.s.
Conductivity	**−0.837**	−0.390	−0.376	0.406	1	21	<0.001
Fe	0.424	0.104	0.014	**−0.528**	10	3	n.s.
Ca^{2+}	**−0.750**	−0.447*	−0.316	0.357	8	3	n.s.
Mg^{2+}	**−0.512**	−0.498*	−0.386	0.487*	12	2	n.s.
NO$_3^-$	**−0.612**	−0.309	−0.053	0.421	6	4	n.s.
PO$_4^{3-}$	0.062	0.191	0.395	**−0.658**	5	4	<0.05
Substrate							
TOC	**0.610**	0.410	0.346	−0.411	4	5	<0.01
Moisture	0.195	**0.532**	0.013	−0.346	3	7	<0.01
Nutrients	−0.279	**−0.610**	−0.209	−0.042	7	4	n.s.
D$_{50}$	**−0.649**	−0.347	−0.110	0.492*	16	1	n.s.
POM	**0.623**	0.372	0.123	−0.248	13	2	n.s.
Leaves/wood	**−0.795**	−0.164	−0.035	−0.141	16	1	n.s.
Vascular plants	0.371	−0.304	−0.061	−0.313	2	7	<0.01
Brown-mosses	0.166	−0.075	−0.043	0.028	15	2	n.s.
Sphagna	0.501*	**0.702**	0.108	0.456*	17	1	n.s.
Others							
Discharge	−0.165	−0.438*	−0.461*	0.313	9	4	n.s.
Depth	0.197	−0.139	0.097	0.247	14	2	n.s
Number of species	**0.660**	0.200	0.247	−0.294	–	–	–
Number of specimens	0.084	0.410	−0.271	−0.076	–	–	–

Significant correlations at the level of $P < 0.01$ are marked by asterisks and those significant after Bonferroni correction ($P < 0.002$) are in bold. Percentages of species data variance explained by all variables. Orders of variable selection in forward selection in CCA and the significance (Monte Carlo test) are given

influences of both factors were complex, owing to direct as well as indirect influence of mineral richness on particular substrate characteristics. The precipitation of tufa on all submerged objects directly changes the environment, creating harsh conditions for clitellates. Thus, the sites with the strongest tufa formation were the most species poor. The effect of mineral richness was often indirectly mediated by vegetation, the structure, and composition of which are strongly influenced by mineral richness (Hájek et al., 2002; Rozbrojová & Hájek, 2008). Along the major mineral richness gradient both the amount and the quality of organic matter in fen substrates changed (Tables 1, 2), which created different habitat conditions for species strongly preferring specific substrates.

Species turnover clearly reflected the traits of species that dominated particular assemblages along the mineral richness gradient: a stygophilous species, semiaquatic sediment burrowers, aquatic sediment burrowers, and surface active species (Fig. 3). A very rare stygophilous species *Trichodrilus strandi* (cf. Dumnicka, 2001) occurred exclusively on tufa-forming (calcareous) fens and reached high abundance also in sites with such strong tufa precipitation that the occurrence of other species was constrained. Tufa habitats are in direct contact with groundwater due to a weak formation of peat and stable high inflow of groundwater (Hájková et al., 2004). Further along the gradient of decreasing mineral richness, weaker tufa precipitation (leading to its absence) changed

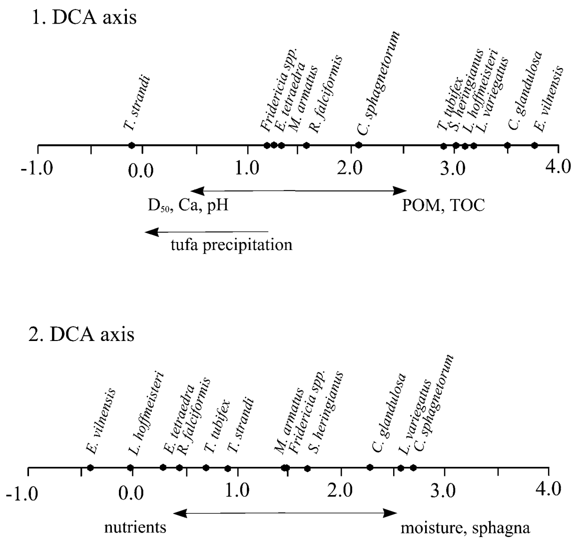
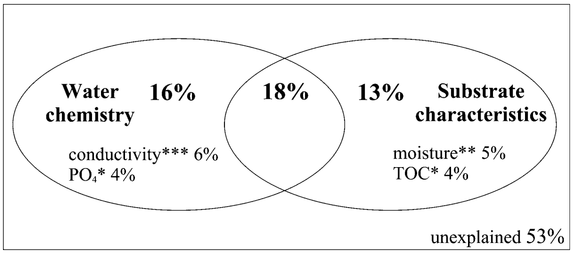


Fig. 3 Position of species along the first two ordination axes. Only species with a fit in the model above 10% are shown



* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Fig. 4 Variation partitioning. Independent and shared variability of water chemistry and substrate characteristics were expressed as percentages of the total inertia in partial CCAs. Only significant variables were included (see Table 2). Significance of independent variability explained was tested by the Monte Carlo test

assemblage composition towards species not restricted to surface sediments (mostly Enchytraeidae; Fig. 3). The wider vertical distribution of enchytraeids is connected with the ability to penetrate to deeper sediment layers, especially during periods of drought and/or higher water temperature, and to inhabit substrates of variable moisture (e.g. Springett et al., 1970; Lundkvist, 1982). These sediment-burrowing species have been reported from sediments in various freshwater and terrestrial habitats as well. For example, *Cognettia sphagnetorum* occurs in coniferous forest soils (e.g. Lundkvist, 1982; Graefe & Schmelz, 1999; Schlaghamerský, 2002), as well as in ombrotrophic and slightly minerotrophic mires (e.g. Erman & Erman, 1975; van Duinen et al., 2006). *Rhyacodrilus falciformis* inhabits mainly different running water habitats (Juget, 1987), but there were also several records from terrestrial habitats—moist soils in grasslands, meadows, and deciduous forests which were not permanently waterlogged (Graefe & Schmelz, 1999; Schlaghamerský & Kobetičová, 2005). These species from the middle part of the gradient of mineral richness are likely not fully confined to the aquatic environment. In contrast, the end of the mineral richness gradient was characterised by the dominance of aquatic sediment burrowers and surface dwellers (e.g. oligochaetes *Limnodrilus hoffmeisteri*, *Tubifex tubifex*, *Stylodrilus heringianus*, and a leech *Erpobdella vilnensis*) which were not recorded in the previous fen types or were scarce there.

The species richness of clitellates increased towards more acidic sites and increasing organic matter content, which is the opposite pattern than other organisms strongly responding to the mineral richness gradient. Species richness of vascular plants, diatoms, and molluscs increased towards mineral-rich fens (Hájek et al., 2002; Horsák & Hájek, 2003; Fránková et al., 2009). Chiefly, clitellates were species-poor at sites where the above-mentioned organisms reach their high or maximal species diversity. However, it should be stressed that this study did not include extremely mineral-poor and permanently acidic sites, simply because they are not present in the study area. These types of acidic mires are known to be the most species-poor due to a low-nutrient availability for clitellates (van Duinen et al., 2006). If these sites are included, a skewed unimodal response of clitellate species richness towards *Sphagnum*-dominated acidic mires is expected, because the highly acidic conditions of bogs were proved to limit the occurrence of many clitellate species.

Beside mineral richness, the pattern of clitellate occurrence also reflected different hydrological regimes and productivity of the fen habitats because clitellate assemblages were influenced by moisture and fertility of the substrate (Fig. 2; Table 4). The seasonal decrease of water level and desiccation of the upper soil layer causes nutrients to mineralise. For that reason, the species composition of the vegetation shifts from sedge-moss dominance to forb-rich vegetation with plant species that use more nutrients (Hájek et al., 2006; Rozbrojová & Hájek, 2008).

Consequently, the formation of peat is lower, and the structure of organic sediments is different in nutrient-enriched sites. It was observed that these changes caused the decrease of plant and mollusc fen specialists and their simultaneous replacement by ubiquitous species (Hájek et al., 2006). In clitellates, we observed a similar effect with an increase of eurytopic aquatic species resulting in their dominance in the assemblage. The species change could be explained by the influence of different peat amount and its structure. Nutrient-poor sites (associated with the presence of sphagna and higher moisture) were dominated by oligochaete species fed on decomposing leaves and vegetation (*Lumbriculus variegatus*; Moore, 1978; Brinkhurst & Gelder, 1991) and on older decomposing litter of *Eriophorum* and *Calluna* (*Cognettia sphagnetorum*; Latter & Howson, 1978). As these oligochaetes are acidotolerant and reproduce by fragmentation, they often reach high densities in nutrient-poor ombrotrophic mires and coniferous forest soils (Springett & Latter, 1977; Schlaghamerský, 2002; van Duinen et al., 2006). On the other hand, nutrient-rich sites were dominated by detritovorous oligochaetes (Tubificinae and Rhyacodrilinae) because the higher availability of nutrients leads to greater production of plant biomass amounts of decomposing organic matter in than in nutrient-poor sites.

Conclusion

The results of our study showed the importance of mineral richness, substrate, and nutrient availability in constituting the clitellate assemblages in the spring fens. The effect of water chemistry was roughly equivalent to the effect of substrate as proportions of an independent variation in species data explained by water chemistry and substrate characteristics were significant and comparable. However, water chemistry and substrate characteristics are inter-correlated. This is the result of tufa precipitation in calcareous fens and vegetation changes along the mineral-richness gradient, as well as related to seasonal decrease of water level in some fens. These factors determined the substrate character by the formation of peat and the structure of organic sediments. We also showed that response of species richness to the same ecological gradient may be reversed for different taxonomic groups. Contrary to previous studies on various taxonomic groups in fens, the species richness of clitellates increased towards more acid sites owing to more favourable substrate conditions. Nevertheless, when including the most acidic sites into the study, a unimodal response could be expected.

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Environmental impact of heated mining waters on clitellate (Annelida: Clitellata) assemblages

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Abstract: Mining is a relatively highly monitored industry. While chemical pollutants (toxic ions, radionuclides, etc.) have mostly been eliminated from mining waters, other types of environmental pollution (temperature regime alterations, high concentrations of various anions, etc.) can affect benthic invertebrates. In this study, we focused on the effect of mining water effluent on the diversity and density of aquatic Clitellata. Four sampling sites were selected. Three sites in a natural stream (the Nedvědička River, Czech Republic), one upstream and two downstream from the mining effluent, and one site on the mining waters were sampled monthly during 2008–2009. Environmental variables were recorded in and samples were collected from two types of habitats – riffles and pools. The response of clitellate assemblages was evaluated using principal component analysis and generalised estimating equations. The results indicated that the mining effluent caused partial species exchange and had negative effects on clitellate taxa richness and abundance. These responses were specific to both the habitat (riffle/pool) and species sampled. In each of the different taxa studied, we observed one of four typical clitellate responses: (a) elimination of stenotherm species; (b) reduction of clitellate species followed by quick recovery; (c) neutral response; or (d) positive influence. We found that aquatic clitellates, which are considered to be eurytopic with broad ecological valences, are also sensitive to even slight environmental pollution.

Key words: aquatic Clitellata; oligochaeta; thermal pollution; mineralised heated water; pool and riffle habitats

Introduction

Freshwater ecosystems are threatened by various human activities. Among the strongest reported threats are alterations of temperature regime and water pollution. Unlike global climate changes, heated mining waters can cause a high, stable, and long-term increase in water temperature, which is often accompanied by increases in various ion concentrations.

Temperature influences the metabolism and life history of organisms through its effect on both physical and chemical processes, and it is a key determinant of invertebrate community composition in most environments (Hogg & Williams 1996; Živić et al. 2006; Haidekker & Hering 2008). Thermal water effluent causes disturbances in the natural state and can lead to the elimination of stenothermic species and the establishment of thermophilous species (Nedeau et al. 2003). The second aspect of mining or general industrial water inflow is the increase in the concentration of heavy metals and various ions, which change water pH, conductivity, and hardness (Maret et al. 2003; Nedeau et al. 2003; Miliša et al. 2010). The final stress factor for benthic organisms downstream from the operations is elevated radiation; radionuclides accumulate primarily in bottom sediments and can alter the life histories of benthic invertebrates. However, in common conditions where radiation doses in the vicinity of a mine are under local government control, the direct effect on benthic invertebrates has not yet been shown (Thomas & Liber 2001).

In the Czech Republic, a unique opportunity to study the environmental impact of heated waters exists in the Nedvědička River, which is a recipient of treated mining water from the last underground uranium mine in Central Europe. Fortunately, the discharge into the Nedvědička River does not significantly increase downstream from the effluence, which enables the study of the effect of only the heated mining water, without changes in stream morphology. Besides temperature, mining water differs from stream water in that it has higher pH, conductivity, and inorganic carbon values, as well as lower oxygen and nutrient values, which makes the influence on stream biota more complex. The concentration of uranium, which is another chemical variable that could affect benthic invertebrates, was very low in the Nedvědička River at the point of the mining water inflow ($U_{\text{nat}} = 3.4$ mg L^{-1}), and it decreased rapidly towards the most distant downstream sampling point ($U_{\text{nat}} = 0.010$ mg L^{-1}). Both values were well below 24 mg L^{-1}, as required by Directive 2008/105/EC of the European Parliament (Hudcová et al. 2012). As the mutagenicity of the water sediments analysed by the Ames Test (Ames et al. 1975) was not previously recorded in either the mining waters or the Nedvědička River (Hudcová et al.

2012), this variable was not recorded in the presented study.

Of the variables we studied, water temperature was expected to have the greatest effect on local benthic communities. A moderate increase in water temperature, similar to those observed in the Nedvědička River, was tested by Hogg & Williams (1996) on benthic arthropods. They reported decreases in their densities, as well as alterations in their life cycles. Other parameters in our study, pH and oxygen concentration, are supposed to have a lower impact when altered. In such cases, when pH varies within a relatively small range around neutral (6.8–8.9) and oxygen concentrations are relatively high (6.5–12.2 mg L^{-1}), their influence on the composition of the benthic community is not significant (Quinn & Hickey 1990). In contrast, higher water conductivity, which reflects an increase in total ionic content, was associated with decreased invertebrate community diversity (Batty et al. 2005). Unfortunately, recent reports on the effects of altered thermal regime and changes in ion concentration on benthic macroinvertebrates have focused either on Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa (Voelz et al. 1994; Lakly & McArthur 2000) or on arthropods (Lehmkuhl 1972; Hogg & Williams 1996; Wellborn & Robinson 1996). Even if all components of the benthic community are considered, clitellates are treated as a single taxon or its several families (Saltveit et al. 1994; Batty et al. 2005; Taylor & Dykstra 2005, Mililša et al. 2010), due to difficulties associated with their identification.

For our study, we selected clitellate (Annelida: Clitellata) assemblages, as oligochaetous clitellates are generally considered to be the group of benthic invertebrates most resistant to various disturbances, although their sensitivity to disturbances still differs among species. Oligochaetous clitellates represent a group of permanent fauna, in which limited mobility is characteristic. The slow, upstream movement of oligochaetous clitellates such as Naidinae is accomplished by swimming or burrowing into the substratum, as is the case for non-swimming taxa, while their downstream movement is accomplished by drift (e.g., Cellot & Juget 1998). Once present at a site with altered thermal regime and water chemistry, they are continually exposed to the new environment. Forasmuch as clitellate species exhibit a wide range of ecological valences, including eurythermic and stenothermic species, their assemblage composition is likely to respond to thermal alterations.

Surprisingly, there is no consensus in the literature with respect to the response of the clitellates to increased temperature. In running waters, where the amount of dissolved oxygen is higher due to the continuous movement, an increase in clitellate density under an effluent of heated water was recorded, whether the water was heated naturally (Taylor & Dykstra 2005; Živić et al. 2013) or artificially (Nedeau et al. 2003). However, in stagnant waters, the diversity of clitellates was reduced to only a few of the most resistant species in very low densities (Krodkiewska 2005). Conductivity has been found to be one of the basic variables that induce changes in clitellate assemblages, with a decrease in diversity being caused by increasing conductivity (Bojková et al. 2011). While results have been recorded for conductivity resulting from Ca^{2+} and Mg^{2+} ion concentration, there have been no previous reports about the effect of SO_4^{2-} ion concentration on clitellate assemblage changes. Although water mineralisation resulting from the combination of various inorganic ion concentrations is known to be a significant variable affecting the structure of the clitellate community (Martínez-Ansemil & Collado 1996; Schenková et al. 2001), it is not known if it has a positive or negative effect on species diversity and density.

The poor understanding of the effect of heated mineralised water effluence on clitellate assemblages and their associated species, as well as discrepancies in reported results regarding the effects (both positive and negative) of thermal alteration on clitellate diversity and abundance, led us to propose three null hypotheses. First, the abundance and taxa richness of clitellates as a group that generally tolerates environmental pollution will not be influenced by the input of mining waters. Second, any changes in clitellate assemblages due to thermal and chemical alteration will be the same for pool and riffle assemblages. Third, clitellates, as a rather uniform group (e.g., feeding strategy, body shape) of invertebrates, will show no species-specific answer to thermal and chemical pollution.

Material and methods

Study site

The Nedvědička River is a second order stream with a length of 28.5 km and an 84.3 km^2 catchment area located in the Bohemian-Moravian Highlands in the Czech Republic. It runs through the village of Rožná, where uranium has been mined since 1958. Decontaminated mining water is used as a coolant in a chemical uranium ore processing plant. The cooling water is discharged into the Nedvědička River at 16.9 km from its spring (49°28′53″ N, 16°14′00″ E).

To evaluate the clitellate response to heated mining water, four sampling sites were chosen, three on the Nedvědička River and one on the mining water tributary. The first site (US: upstream) is on the Nedvědička River about 200 m upstream from the mining water inflow, the second site (MW: mining water) is on the mining water, the third site (DS1: downstream1) is on the Nedvědička River immediately downstream of this inflow, and the fourth site (DS2: downstream2) is on the Nedvědička River about 800 m downstream of the inflow site (Fig. 1). Sampling sites on the Nedvědička River only (US, DS1, and DS2) are included in all statistical analyses. The MW samples allowed us to determine background information about the chemistry of and the clitellate species pool in the mining water. The mining water runs in a brook approximately 1.5 m wide with a substratum composed of gravel in riffles and mud, and sand and gravel in pools. All sampling sites were well canopied with *Alnus* sp., *Salix* sp., *Fraxinus* sp., and *Sambucus nigra.* Sampling sites DS1, DS2, and MW did not freeze over in the winter due to the warm water inflow, in contrast to the US site, which froze to the depth during the winter months.

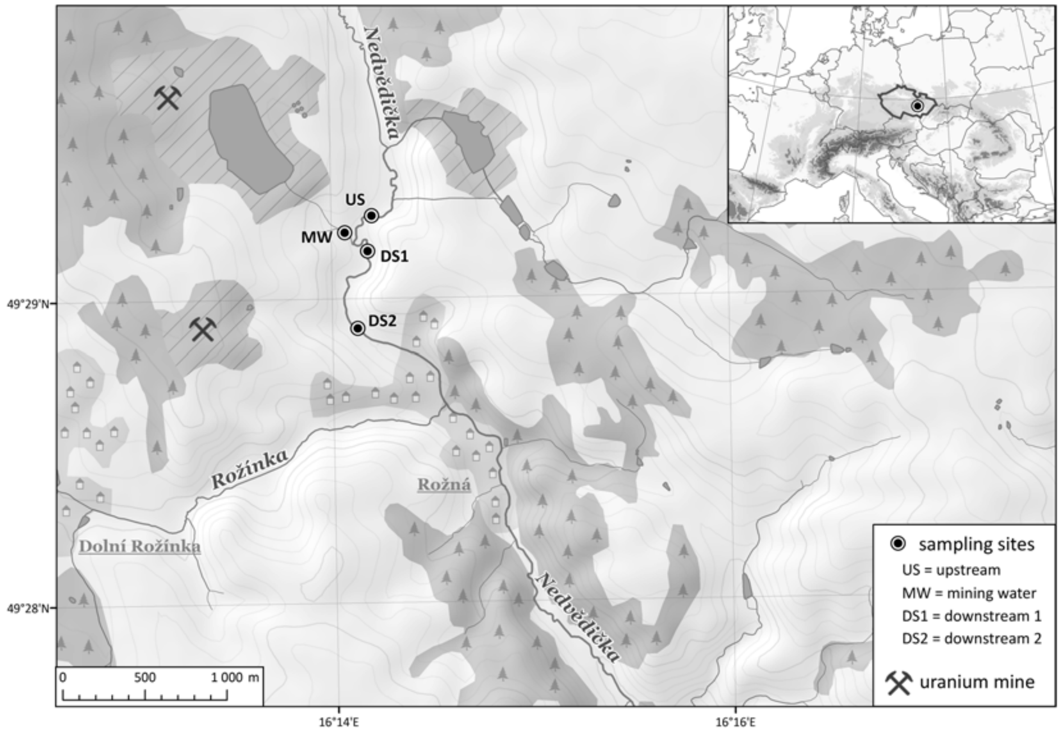

Fig. 1. Location of sampling sites.

Species data

Samples of macroinvertebrates were taken monthly from March 2008 to June 2009 (sampling in January was omitted) at each of the four sampling sites. Macroinvertebrates were collected quantitatively using a Hess stream bottom sampler (0.08 m^2) fitted with a 256-μm-mesh collection net. Two samples from each of the pools and two from each of the riffles were pooled separately at each sampling site. A total of 120 samples were obtained. The collected material was fixed in 4% formaldehyde in the field. In the laboratory, the clitellates were sorted and classified by species or genus level (in the case of juveniles).

Physical and chemical measurements

Basic physicochemical parameters were recorded at each of the sampling sites (Table 1). For each sampling site, one value each of conductivity, pH, dissolved oxygen, and oxygen saturation was measured (HACH HQ40d) during each monthly sampling in the field. Concentrations of NH_4^+, NO_3^-, PO_4^{3-} (VIS spectrometer HELIOS δ), and SO_4^{2-} ions (capillary electrophoresis) and total nitrogen (TN), total carbon (TC), total inorganic carbon (IC), and total organic carbon (TOC) (Shimandzu) were measured from each water sample in the laboratory. During each sampling, two values for depth, velocity at 40% of the depth from the bottom, and substrate composition were recorded separately for pools and riffles. Temperature data loggers were submersed at all sampling sites, and water temperature was recorded every 15 minutes during the entire sampling period. To evaluate the hydrodynamic characteristics of the sampling sites, the Froude number (Fr) was calculated using $\mathrm{Fr} = U/\sqrt{gD}$ (D: depth, g: acceleration due to gravity, and U: current velocity at 0.4 depth). The phi value (Hynes 1970) was used to evaluate substrate coarseness.

Statistical analyses

Principal component analysis (PCA) was used to search for the main gradients in the environmental data and to identify the relationships between the variables. Seasonal changes in the environmental data were separated from differences among sampling sites using special covariables that represented relative seasonality (for details, see Straka et al. 2012). The collected temperature data were scaled by day and month by means of these values and were used in the PCA. Environmental data were fitted linearly into the two-dimensional ordination space, and only significant variables (based on 999 permutations) were shown in the PCA diagram. Variables (Froude number and phi) related to the habitats were not included in the PCA because they were measured twice, for riffles and pools separately, while only one measurement per sampling site was taken for all the other variables.

The differences in Froude number and phi among the specific sampling sites were tested using the Wilcoxon matched pairs test; habitats were treated separately. The same test was used to find the differences in physicochemical variables between US and MW.

PCA was also used to study the relationships between species composition and explanatory variables. Seasonal changes in species data were separated from differences among sampling sites using the same covariables as in PCA on environmental data (see above). The species data were $\log(x+1)$ transformed to reduce the undue influence of dominant species. Samples containing fewer than five individuals were excluded from the analysis. To explain the ecological meaning of species data gradients, environmental variables were fitted linearly into the two-dimensional ordination space and only significant variables (based on 999 permutations) were shown in the PCA diagram.

Because the samples showed temporal dependence, we used the generalised estimating equations (GEE) with autoregressive (AR1) correlation structure and Poisson distribution to evaluate differences in abundance and taxa richness between particular sampling sites. The same method and test was used for testing the mining water effect on

Table 1. Minimum, maximum, and median values of the measured environmental variables at sampling sites.

Site	US			MW			DS1			DS2		
	Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
Variable												
Conductivity (μS cm^{-1})	202	318	410	987	1395	1580	400	604	930	415	583	789
pH	7.3	7.6	8.4	7.8	8.2	8.9	7.5	8.0	8.5	7.2	8.0	8.8
Dissolved O_2 (mg L^{-1})	8.7	11.4	12.7	7.6	8.8	10.1	8.1	10.2	12.3	8.2	10.3	12.0
O_2 saturation (%)	86	96	118	78	98	116	81	98	120	78	97	119
Mean monthly temperature (°C)	2.9	11.7	15.4	12.6	17.9	19.9	4.8	13.4	16.4	4.8	13.3	16
Min. monthly temperature (°C)	0.1	7.4	13.3	8.0	13.5	17.2	1.9	9.1	15.0	1.7	9.0	14.8
Max. monthly temperature (°C)	5.8	15.0	19.0	18.1	21.2	24.1	7.4	16.8	20.6	7.4	16.3	20.7
NH_4^+ (mg L^{-1})	0.4	0.7	1.6	0.0	0.6	1.6	0.0	0.5	1.1	0.0	0.5	1.3
NO_3^- (mg L^{-1})	8.6	29.5	41.9	0.4	12.9	30.5	13.8	22.0	41.1	13.6	21.3	41.5
PO_4^{3-} (mg L^{-1})	0.0	0.1	0.4	0.0	0.0	0.1	0.0	0.1	0.5	0.0	0.1	0.3
SO_4^{2-} (mg L^{-1})	44	48	66	430	500	690	80	170	280	81	145	270
TN (mg L^{-1})	0.6	7.8	15.5	0.2	1.7	8.4	0.2	6.0	14.6	0.8	5.7	14.6
TC (mg L^{-1})	11.8	21.3	64.8	16.9	32.8	63.9	12.9	20.5	38.9	12.7	20.5	37.9
IC (mg L^{-1})	4.4	11.3	23.2	11.2	23.6	46.1	7.6	13.3	27.8	6.4	15.9	27.3
TOC (mg L^{-1})	3.0	8.7	55.4	0.6	6.6	19.4	1.1	8.2	12.9	1.1	7.8	20.3
phi value (riffles)	–7.0	–4.6	–1.7	–7.0	–3.4	–1.7	–7.4	–5.8	–3.2	–7.0	–4.4	–1.3
phi value (pools)	–4.4	–0.7	2.4	–4.2	1.4	5.1	–5.1	–1.8	2.0	–4.5	–1.5	4.5
Froude number (riffles)	0.17	0.36	0.70	0.17	0.38	0.70	0.06	0.44	0.89	0.06	0.26	0.51
Froude number (pools)	0.01	0.08	0.17	0.01	0.11	0.17	0.01	0.05	0.12	0.01	0.06	0.14

Explanations: US – unaffected locality upstream from the tributary, MW – heated mining water, DS1 – first influenced locality downstream from the tributary, DS2 – second influenced locality downstream from the tributary, TN – total amount of nitrogen, TC – total amount of carbon, IC – amount of inorganic carbon, and TOC – total amount of organic carbon.

six of the most abundant and ecologically important taxa.

All analyses were performed in R (version 2.12.0; R Development Core Team, 2010) using the 'vegan' (Oksanen et al. 2011) and 'geepack' (Hojsgaard et al. 2006) packages.

Results

Environmental data

Mining water differs from water in the Nedvědička River along several parameters, the most important of which, for this study, are its elevated water temperature, higher conductivity, and lower organic pollution (Table 1). MW differed from the unaffected US most significantly in water temperature ($P < 0.001$), which ranged 8–24°C in MW. Heated mining water caused an average increase of 1.8°C in water temperature in DS1 when compared to US. Differences in temperature between the downstream sites (DS1 and DS2) were small (about 0.2°C; Fig. 2C). Significantly higher conductivity ($P < 0.001$), which reached values above 1,000 μS cm^{-1}, was caused by increased SO_4^{2-} ions. Inorganic carbon and pH were significantly higher in MW compared to US ($P < 0.01$), and dissolved oxygen and nutrients (NO_3^- and PO_4^{3-}) were lower ($P < 0.01$). Lower organic pollution in mining water (Table 1) was due to the presence of the decanting plant, which processes mining water upstream from its inflow into the Nedvědička River.

Mining water is discharged continuously, without fluctuation in volume (0.06 m^3 s^{-1}). The Nedvědička River displayed an unaltered character in the sampled stretch, with a width of 2.7–3.5 m, a mean discharge volume of 0.25 m^3 s^{-1}, a mean pool depth ranging from 0.24 m (US) to 0.37 m (DS2), a mean riffle depth ranging from 0.16 m (DS1) to 0.21 m (DS2), a mean velocity in pools ranging from 0.12 m s^{-1} (DS1) to 0.14 m s^{-1} (US), and a mean velocity in riffles ranging from 0.41 m s^{-1} (DS2) to 0.54 m s^{-1} (DS1). Coarse gravel and gravel were the dominating substratum in riffles, and gravel and sand were the dominating substratum in pools. Phi values were similar within particular habitats (riffle/pool), especially in pools (Fig. 2A). In the riffles, DS1 had significantly coarser substrate than that found in DS2 and US (Wilcoxon paired test, $P < 0.05$). In the pools, the US Froude number was significantly higher than in both downstream sites (Fig. 2B). In the riffles, DS1 had a significantly higher Froude number when compared to DS2 ($P < 0.05$), but not when compared to US.

The main gradients for the environmental data were identified using PCA. The first PCA axis explained 34.3% of the variance. The main gradient represented these measured variables (i.e., conductivity, temperature, pH, and dissolved oxygen), which differed significantly between the mining water tributary and the natural water sites on the Nedvědička River. The unaffected sampling site (US) was well separated from both affected sites (DS1 and DS2; Fig. 3). The differences between DS1 and DS2 were small; groups of points highly overlapped each other in ordination space. The second PCA axis explained 17% of the variance in the data and was associated with the amount of nutrients, specifically, total organic carbon and nitrate ions. General differences among sampling sites along this nutrient gradient were small.

Clitellate community

A total of 2,715 individuals representing 44 clitellate

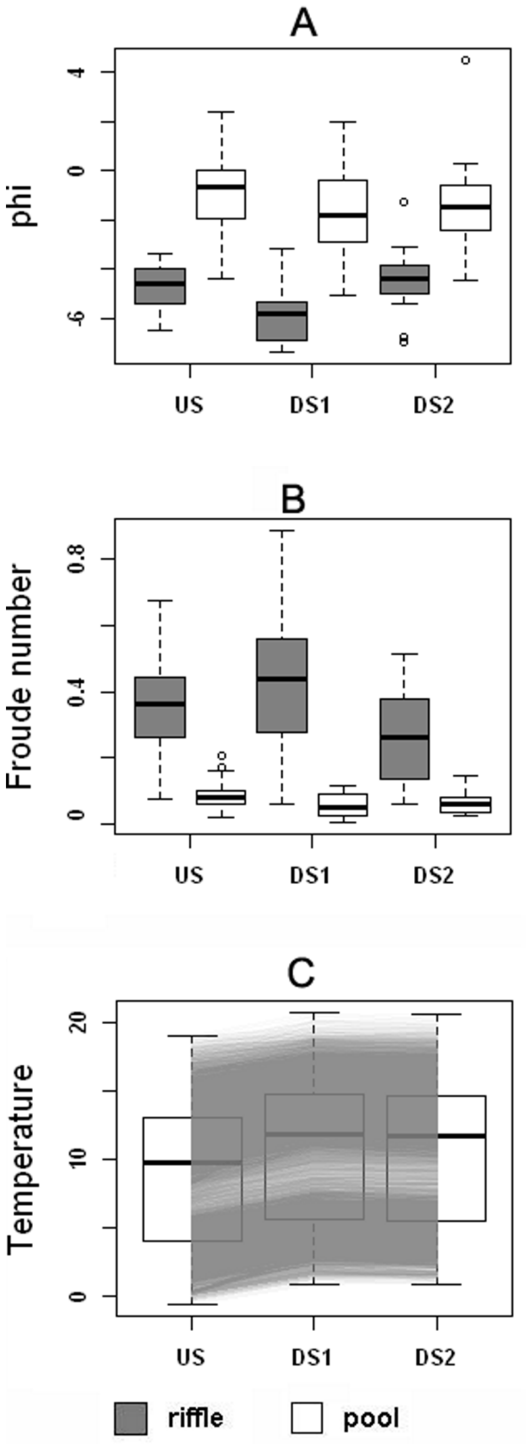

Fig. 2. Box-plots of environmental variables: phi values (A), Froude number (B) (habitats considered separately), and measured temperature (C), lines join daily means. US – site upstream from the mining water inflow, DS1 – first site downstream from the mining water inflow, and DS2 – second site downstream from the mining water inflow.

taxa were collected from the study sites in the Nedvědička River. The numbers of taxa per sampling site were 35 from US, 30 from DS1, and 24 from DS2. Total abundance was 1,710 individuals from US, 279 from DS1, and 942 from DS2. In the mining water tributary, 1,446 individuals from only 17 taxa were observed.

In PCA based on biological data, the first axis explained 33.9% of the variance (Fig. 4). Although points of particular sampling sites overlap, the main gradient in faunal data representing community change along the extent of sampling site disturbance (from US through DS2 to DS1) is clearly visible. This gradient was best expressed by fitted environmental variables: increase of conductivity, temperature, IC, and pH in the most affected DS1 on the one side, and higher amount of dissolved oxygen and organic carbon associated with unaffected US on the other side. The second gradient, explaining 11.1% of variability, is related to the substrate coarseness phi.

A comparison among sampling sites (both habitats evaluated together) in the number of taxa showed a significant ($P < 0.05$) decrease downstream from the tributary (Fig. 5A). However, the difference in number of taxa between DS1 and DS2 was insignificant. In addition to detailing the elimination of some taxa downstream from the effluent, species replacement was also monitored. Some of the taxa we observed upstream from the mining water effluent site were replaced either by new taxa or by taxa coming from the mining waters (Table 2). In addition, the number of individuals within each taxa was significantly lower downstream from the tributary (Fig. 5B) in both DS1 and DS2 ($P < 0.05$).

Separate evaluations of habitats revealed a significant decrease in the number of taxa between the pools in the US and both downstream sites ($P < 0.05$), but no difference between DS1 and DS2. The DS1 riffles had significantly fewer taxa compared to US and DS2 ($P < 0.005$); there was no difference between US and DS2. The number of individuals in pools was significantly higher in US compared to both DS sites ($P < 0.05$); DS1 had significantly fewer individuals than DS2 ($P < 0.05$). Similarly, the DS1 riffles had significantly fewer individuals compared to US and DS2 ($P < 0.005$), but there was no difference between US and DS2.

Comparing the habitats (riffles/pools) within sampling sites showed that they were populated differently by clitellates. Significantly higher taxa richness in pools was recorded for DS1 ($P < 0.001$; Fig. 5A), and significantly higher abundances in the pools were recorded for US and DS1 ($P < 0.05$).

Selected species responses

Genus *Stylodrilus* was the most abundant taxon found in the three sample sites on the Nedvědička River (US, DS1, and DS2), and genus *Limnodrilus* was the most abundant in MW (Table 2). Other species that were prevalent in particular sampling sites include *Haplotaxis gordioides* in US, *Nais bretscheri* in DS1, and *Aulodrilus japonicus* in DS2. Three species, *Bothrioneurum vejdovskyanum*, *Limnodrilus udekemianus*, and *Psammoryctides albicola*, were found exclusively in MW. We focused our study on the response to thermal and chemical alteration of six taxa (*Aulodrilus japonicus*, *Haplotaxis gordioides*, *Limnodrilus* spp., *Lumbriculus variegatus*, *Spirosperma ferox*, and

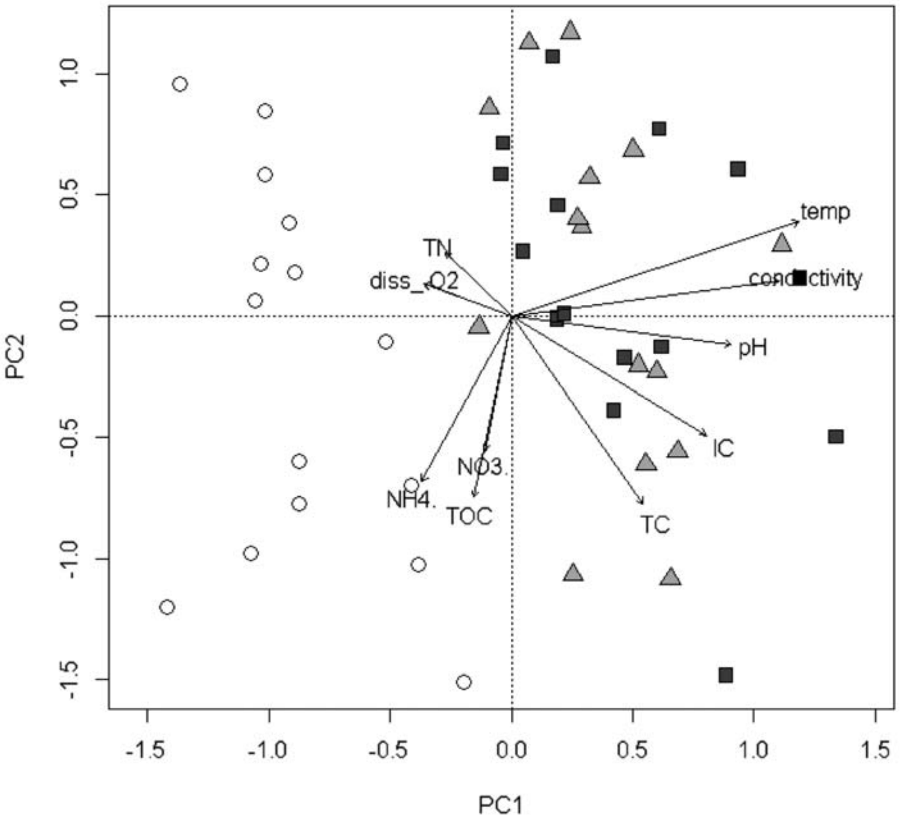

Fig. 3. PCA ordination plot of environmental variables. Circle – US, square – DS1, triangle – DS2. Black arrows represent fitted variables. Only significant variables are shown ($P < 0.05$, based on 999 permutations).

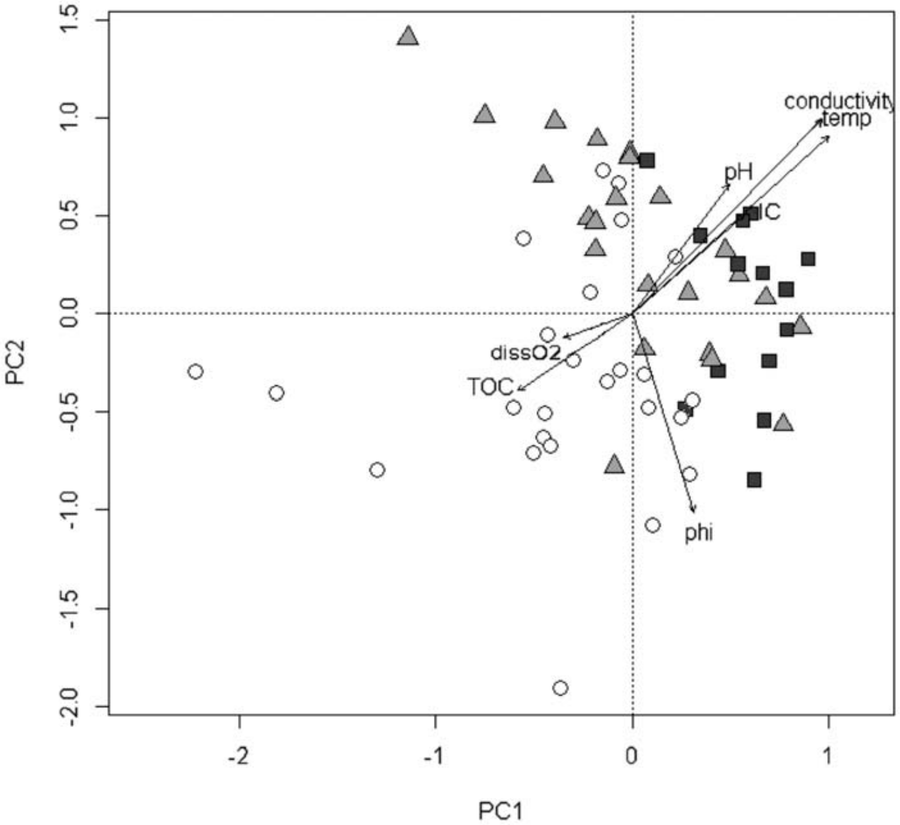

Fig. 4. PCA ordination plot of species data. Samples are displayed. Circle – US, square – DS1, triangle – DS2. Black arrows represent fitted variables. Only significant variables are shown ($P < 0.05$, based on 999 permutations).

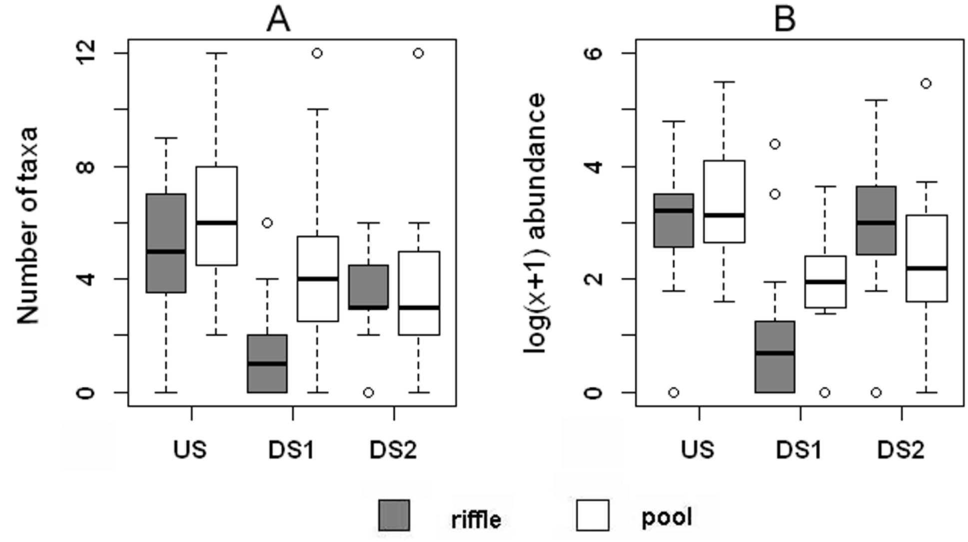

Fig. 5. Box-plots of taxa number (A) and logarithmic transformed abundance values (B) for three sampling sites. The habitats were treated separately. US – site upstream from the mining water inflow, DS1 – first site downstream from the mining water inflow, and DS2 – second site downstream from the mining water inflow.

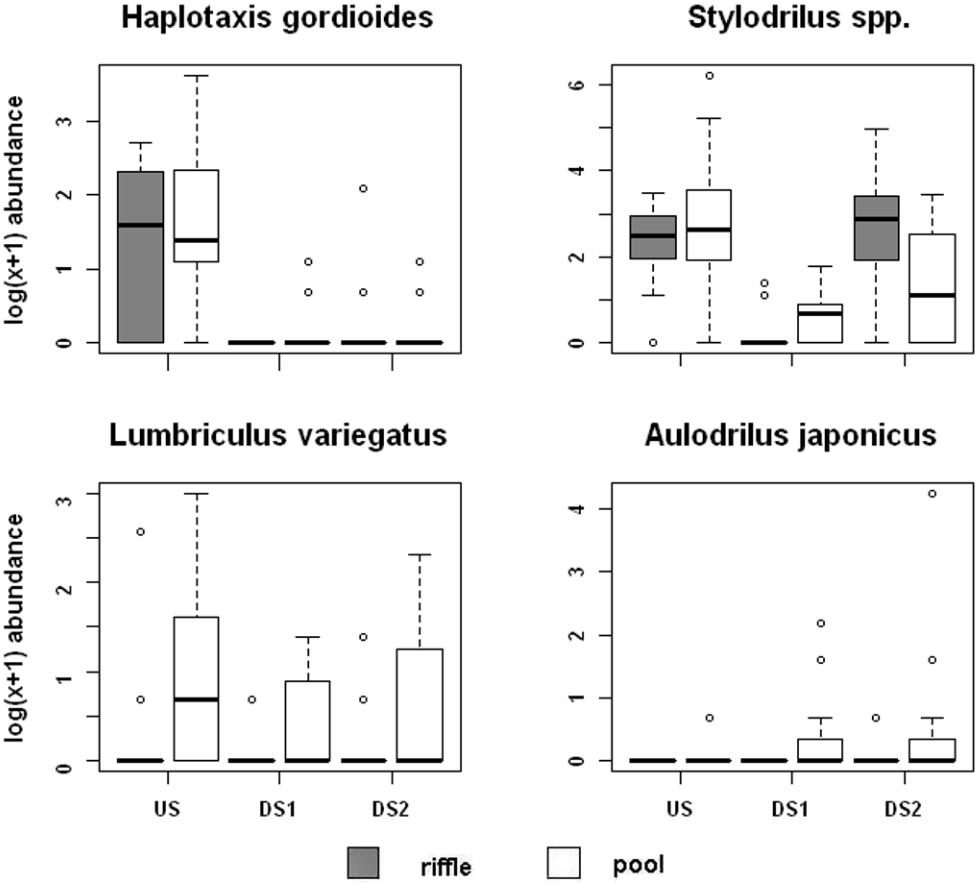

Fig. 6. Box-plots of logarithmic transformed abundances of selected species for three sampling sites; habitats treated separately. US – site upstream from the mining water inflow, DS1 – first site downstream from the mining water inflow, and DS2 – second site downstream from the mining water inflow.

Stylodrilus spp.) with the highest abundances and regular occurrences in the sample sites from the Nedvědička River. We observed four types of responses by the species to the mining water effluent (Fig. 6):

I) The decrease in abundance of *Haplotaxis gordioides* in both DS sites was significant ($P < 0.001$). In addition, this species was nearly eliminated downstream of the tributary, with only a few occasional occurrences (Fig. 6A).

Table 2. Total number of individuals of clitellate species recorded in sampling sites.

Site Name	US-R	US-P	MW-R	MW-P	DS1-R	DS1-P	DS2-R	DS2-P
NAIDINAE								
Nais alpina (Sperber, 1948)	3					5		
Nais barbata (O.F. Müller, 1773)	69	3			8			
Nais bretscheri (Michaelsen, 1899)	8	3			21	6	24	19
Nais communis (Piguet, 1906)	3					1		1
Nais elinguis (O.F. Müller, 1773)	6	7			3	2	27	2
Nais pseudobtusa (Piguet, 1906)	12				3	2		
Nais simplex (Piguet, 1906)	2							
Nais sp. juv.					74	23		
Nais variabilis (Piguet, 1906)	2							
Ophidonais serpentina (O.F. Müller, 1773)	2	2				1		
Slavina appendiculata (d'Udekem, 1855)		2			1			
Uncinais uncinata (Oersted, 1842)		8				5		2
PRISTININAE								
Pristina jenkinae (Stephenson, 1932)	1							
RHYACODRILINAE								
Bothrioneurum vejdovskyanum (Štolc, 1888)			1	25				
Rhyacodrilus falciformis (Bretscher, 1901)						5		
TUBIFICINAE								
Aulodrilus japonicus (Yamaguchi, 1953)		1		17		14	1	75
Aulodrilus limnobius (Bretscher, 1899)					1			
Aulodrilus pluriseta (Piguet, 1906)				9			1	3
Limnodrilus claparedeanus (Ratzel, 1869)				15		1		6
Limnodrilus hoffmeisteri (Claparède, 1862)		9	1	439		12	2	9
Limnodrilus udekemianus (Claparède, 1862)				5				
Limnodrilus sp. juv.	1	4	6	656		10	3	24
Psammoryctides albicola (Michaelsen, 1901)				2				
Psammoryctides barbatus (Grube, 1861)			1			2		2
Spirosperma ferox (Eisen, 1879)	8	26				16	9	50
Tubifex tubifex (O.F. Müller, 1774)		2		36		1		15
Tubificidae juv.	1	4	2	153		3	11	53
ENCHYTREIDAE								
Cognettia glandulosa (Michaelsen, 1888)			1	1			3	
Enchytraeus albidus (Henle, 1837)								1
Enchytraeus sp.	1	1				1		
Fridericia sp.		2	2		2	1	1	3
Henlea sp.	1		4		1	2	1	
Mesenchytraeus armatus (Levinsen, 1884)	1							
PROPAPPIDAE								
Propappus volki (Michaelsen, 1922)		32						
HAPLOTAXIDAE								
Haplotaxis gordioides (Hartmann, 1821)	79	126				3	9	4
LUMBRICIDAE								
Eiseniella tetraedra (Savigny, 1826)	5	20	24	6	7	1	4	16
LUMBRICULIDAE								
Bythonomus lemani (Grube, 1880)	1							
Lumbriculus variegatus (O.F. Müller, 1774)	14	46	3	31	1	11	5	26
Stylodrilus brachystylus (Hrabě, 1928)	38	157				4	67	18
Stylodrilus heringianus (Claparède, 1862)	8	13			3			
Stylodrilus sp. juv.	156	803		6	5	12	359	84
ERPOBDELLIDAE								
Erpobdella octoculata (L., 1758)	2	5			1	3		1
Erpobdella vilnensis (Liskiewicz, 1927)	2	4						
Erpobdella sp. juv.		2						
GLOSSIPHONIIDAE								
Glossiphonia complanata (L., 1758)		1						
Glossiphonia concolor (Apathy, 1888)					1			
Glossiphonia nebulosa (Kalbe, 1964)								1
Helobdella stagnalis (L., 1758)	1							
Total	427	1283	45	1401	131	148	527	415

Explanations: US – site upstream from the mining water inflow, MW – heated mining water inflow, DS1 – first site downstream from the mining water inflow, DS2 – second site downstream from the mining water inflow, R – riffles, P – pools.

II) While *Stylodrilus* spp. and *Lumbriculus variegatus* abundances decreased in DS1, further downstream in DS2, they increased again to almost the quantity found in US (Fig. 6B, C). The decrease of abundance in DS1 and the increase in DS2 were significant in both *Stylodrilus* spp. and *Lumbriculus variegatus* ($P < 0.001$). Furthermore, an exchange of preferred habitat for *Stylodrilus* spp. was observed. It showed a preference for pools in US and DS1, while it showed a preference for riffles in DS2.

III) A neutral response to the mining water effluent was observed in two taxa, *Spirosperma ferox* and *Limnodrilus* spp.; abundances in all sampling sites were almost the same.

IV) An increase of abundance in *Aulodrilus japonicus* was observed in the downstream sites (Fig. 6D). The increase in DS1 and DS2 compared to US was significant ($P < 0.05$). A significantly higher abundance in DS2 compared to DS1 was also observed ($P < 0.05$).

Discussion

The Clitellata assemblages in this study, represented mainly by oligochaetes and partly by leeches, proved to be a good tool for the evaluation of aquatic ecosystem degradation caused by mineralised and heated mining water. Their diversity and density strongly reflected the changes in water environment.

The environmental variables that were altered the most below the mining water tributary included conductivity, temperature, pH, IC, and dissolved oxygen concentration (arranged in order of goodness of fit in the multivariate analysis), and we assume them to be responsible for changes in the clitellate assemblage composition. This assumption was confirmed when we fitted all environmental variables into a species data ordination plot. The main gradient of community changes corresponded with the gradient of environmental variables altered by MW inflow. There was no opportunity to segregate the effects of individual variables, but their combined impact was detectable. A significant decrease in species richness and abundance below the mining water tributary was observed (Fig. 4), despite the fact that clitellates are considered to be the most tolerant group to various ecological disturbances (e.g., Brinkhurst & Cook 1974; Uzunov et al. 1988). Contrary to our results, Nedeau et al. (2003) observed an increased abundance of oligochaetes and chironomid larvae under heated industrial effluence, but a simultaneous increase in water current and resulting changes in the stream morphology probably affected the results.

Two mechanisms likely explain the negative effect of mining water input on clitellate assemblages. First, the effect of changes in water chemistry and the resulting increase in conductivity causes a clitellate osmoregulation problem that is balanced by active seeking of optimal osmotic conditions (Preston 2009) more distantly downstream. Some species, such as *Propappus volki*, show a strong negative correlation with water hardness, which is often tightly associated with conductivity (Dumnicka & Pasternak 1978), and we observed that this species was completely eliminated below the mining water tributary in our study (Table 2). Osmoregulatory capacity differs among oligochaete species (Generlich & Giere 1996), and *Propappus volki* is probably highly sensitive to osmotic stress. Second, the effect of increased water temperature was remarkable. A small increase (1.8 °C on average) resulted in water remaining unfrozen during the winter, a feature to which cold water species (e.g., *Haplotaxis gordioides* and *Stylodrilus heringianus*; Dumnicka 2000), which naturally inhabit part of the stream above the tributary, are not adapted. The drop in their numbers below the tributary was significant (Fig. 6A, B). Hence, we can reject the hypothesis that running water clitellates are such a resistant group of organisms that the mining water input does not change their densities and taxa richness.

Stream morphology has been reported to be significant for clitellate distribution (Neadeau et al. 2003), which was confirmed by evaluating the riffle and pool samples separately. The clitellate assemblage composition of each habitat (riffle/pool) was distinctly affected (Fig. 5). The decrease in the number of taxa observed downstream from the mining water tributary was higher in the riffles than in the pools. Six taxa inhabiting only riffles upstream were excluded downstream from the tributary, whereas the three taxa inhabiting pools upstream were excluded downstream (Table 2). Rheophilous taxa, which often inhabit the upper parts of streams, for which low nutrients and higher oxygen content are typical (Schwank 1981), are often more vulnerable to decreased water quality (Verdonschot 2006). Contrary to our results, an identical diversity with different qualitative and quantitative composition was reported in another study below thermal water inflow for all macroinvertebrates, with only a decrease in overall abundance being reported (Živić et al. 2006). Nevertheless, the species exchange as the response to the thermal inflow (Živić et al. 2006) was analogous to our results. Similarly, changes in water chemistry, which are often accompanied by a higher trophic state, result not in decreased clitellate species diversity, but in changeover of the proportion of particular species with different ecological valences (Krodkiewska & Michalik-Kucharz 2009). We recorded species exchange below the mining water tributary and changes in the abundance of particular taxa, but overall, we observed a decrease in diversity and density. The reason for this result might be the cumulative effect of the simultaneous influence of high conductivity and increased temperature. Nevertheless, all of these changes were distinct for riffles and pools, and therefore, we can also reject the hypothesis that species will respond in the same manner to these changes in different habitats. In our study, we observed habitat-specific responses of the clitellates to the various disturbances.

The collective response of the entire clitellate community to the mining water inflow can be explained by analysing the responses of particular species to the changes in their environment. We found that the level of influence was not the same at the species level.

The elimination of the oligo-stenotherm species *Haplotaxis gordioides*, one of the most abundant species in the upstream site, explains its life strategy as stygobiont, meaning that it inhabits cool water springs and small streams (Schwank 1981; Dumnicka 2000). We believe that the increased water temperature and absence of winter freezing affected the downstream population negatively. The reduction of *Stylodrilus* spp. abundance was due to its preference for higher altitudes and, therefore, lower temperatures and higher oxygen concentrations (Dumnicka 2000; Verdonschot 2006). Its quick recovery is due to its ecological plasticity, expressed by a broad flow condition valence (Syrovátka et al. 2009). However, the analogous response of *Lumbriculus variegatus* is explained differently, by its preference for lower pH (Nijboer et al. 2004), which can result in its movement into parts of the stream with optimal pH values. Some taxa with broad ecological valence, such as *Limnodrilus* spp., were not influenced by changes in environmental conditions because of their high tolerance to low oxygen levels, increased temperatures, and high levels of conductivity caused by sulphate ions (Korn 1963; Dumnicka & Galas 2006). The input of mining waters was even favourable for *Aulodrilus japonicus*, which is intolerant of water pollution (Dumnicka & Pasternak 1978), especially nutrient loading and higher conductivity. Therefore, it was found only in small numbers in the mining water tributary and in riffles and pools downstream (DS1), but its abundance increased further downstream (DS2), where it found a compromise between conductivity and water quality levels.

Other taxa of the 44 recorded clitellates would also probably fit into one of these strategies, but they were present in such low numbers that they showed random distributions.

In contrast to the Nedvědička River, the mining water assemblages showed distinctly lower species diversity, comprising a subset of the species recorded in the other sampling sites (Table 2). Only three species that inhabited the mining water were excluded from the other, "better" sites. These species were *Bothrioneurum vejdovskyanum*, a rare species preferring either large rivers or smaller streams in lowlands (Hrabě 1981; Schenková et al. 2006);*Limnodrilus udekemianus*, which prefers organically enriched sites, but not those under poor conditions (Brinkhurst & Cook 1974); and *Psammoryctides albicola*, a lowland stagnant water species (Hrabě 1981).

To summarize, Clitellate assemblages, similar to other aquatic invertebrate taxa, are threatened by mining water pollution. This pollution not only lowers their diversity and density, but also causes species changeover. Therefore, analyses of clitellate assemblages must always be conducted at the species level. The problem is the exclusivity of the types of disturbances discussed in this report under natural conditions and, hence, worse plausibility of their mutual comparisons.

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The response of Clitellata (Annelida) to environmental gradients in spring fens

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Abstract

Patterns in aquatic Clitellata assemblage composition are known to be driven by several environmental gradients, with water chemistry and substratum characteristics being particularly important. In this study we explored 54 isolated spring fens across the eastern Czech Republic and Slovakia. These fens varied in calcium and magnesium concentrations, forming a sharp and well defined environmental gradient running from calcium-poor acidic fens to extremely calcium-rich tufa-forming fens. We found that the main changes in clitellate species composition were controlled by this gradient, and/or total organic carbon content, over a wide area, including fen sites differing in other environmental conditions and historical development. However, this pattern was weakened in sites with a high organic matter content, which represented a second driver of change in assemblage composition along with water temperature. Three main types of fens were determined using cluster analysis based on clitellate assemblage composition. However, only the first type, which included tufa-forming fens, was found to fit with the previously established spring fen types based on vegetation (i.e. extremely mineral-rich fens with a tufa, brown-moss mineral-rich fens, mineral-rich *Sphagnum* fens and mineral-poor *Sphagnum* fens). The second clitellate type included sites with low temperatures and occasional desiccation, while the third type was characterised by high temperatures and trophy. Using eight environmental predictors, we were able to significantly explain changes in the population abundances of all 12 common species (i.e. recorded at 15-plus sites). The results from individual species modelling also suggests that an increase in organic matter content can trigger compositional shifts towards assemblages of common eurytopic tubificid species. Thus, human-induced eutrophication and negative changes in spring fen hydrology, mainly drying up, can represent a serious threat for species-specific assemblages of aquatic clitellates, especially at alkaline sites due to their isolated and spatially limited nature.

Keywords: Clitellata, spring fens, environmental gradients, indicator species, species responses

1. Introduction

Spring fens represent scattered treeless wetlands fed by groundwater that host nutrient-limited vegetation (Hájek et al., 2006). Helocrene wetlands are characterised by highly stable environments, less prone to natural disturbances than other types of aquatic environments (Lake, 2000). They also tend to include various habitat types, both lotic and lentic, forming heterogeneous mesohabitat complexes (Lindegaard, 1995). They vary in water chemistry from calcium-poor acidic fens with a tendency towards ombrotrophy to extremely calcium-rich tufa-forming fens, thus forming a so called poor-rich gradient (Malmer, 1986; Hájek et al., 2006). The water chemistry of wetlands is generally extremely important for clitellates, with very acidic sites inhabited only by asexually reproducing species (van Duinen et al., 2006), while high mineral richness can favour spring fen specialists (Bojková et al., 2011). Spring fens also cover a broad variety of substrate types, known to be one of the most important environmental gradients determining clitellate species distribution (e.g. Šporka, 1998; Montanholi-Martins and Takeda, 1999; Verdonschot, 2001; Syrovátka et al., 2009).

As existing studies on aquatic clitellate assemblage diversity have shown, their broad-scale patterns can be explained mainly by water chemistry, substrate characteristics and organic pollution (Martínez-Ansemil and Collado, 1996; Schenková et al., 2001; Nijboer et al., 2004; Verdonschot, 2006). As such, spring fens seem to be an appropriate model system for deepening our understanding of the main responses of clitellate assemblages, as well as individual species responses. Further, there currently exists only limited data dealing with clitellates in headwater habitats, covering acidic conditions of ombrotrophic peatlands (van Duinen et al., 2006), bog streams (Smith, 1986; Smith and Kaster, 1986), limnocrene karstic springs (e.g. Dumnicka, 2006; Mori and Brancelj, 2006) or cave springs (e.g. Dumnicka, 2000; Achura and Rodriguez, 2008; Achurra et al., 2015). Some studies have explored several spring fens over a relatively spatially limited area (Erman and Erman, 1975; Bojková et al., 2011). However, virtually no data exist on clitellate diversity patterns from wetland headwater habitats over a broader spatial scale. Patterns of response in individual species have also been mostly neglected. Only a few studies have addressed this topic, exploring a species response to a single or only a few variables. For instance, organic matter content (Uzunov et al., 1988), current velocity and substratum (Martínez-Ansemil and Collado, 1996; Syrovátka et al., 2009), ecological quality, altitude and total phosphorus (Verdonschot, 2006) and other chemical variables (Timm et al., 2001) have been studied in running waters.

In this study we aim to analyse the effect of water chemistry and physical variables on the diversity of clitellate assemblages over a large spatial scale using an extensive dataset, collected from 54 isolated hydrologically uniform fen sites in the Western Carpathians Mountains (Czech Republic and Slovakia). Low variation in water temperature, as well as discharge makes water supply across the spring fens comparable (Rádková et al., 2014). We made sure to explore a high number of sites, covering a wide variety of ecological settings along several environmental gradients. The study sites belong to four main types based on water chemistry and vegetation (Hájek et al., 2006): (i) extremely mineral-rich fens with a tufa formation dominated by the moss *Cratoneuron commutatum*, (ii) brown-moss mineral-rich fens without a tufa formation dominated by the moss *Drepanocladus revolvens* agg., (iii) mineral-rich *Sphagnum* fens with calcitolerant *Sphagnum* species and (iv) mineral-poor *Sphagnum* fens dominated by *S. recurvum* agg. Each of the four spring fen types were spread throughout the entire study area with none of them locally aggregated. As various groups of invertebrates are strongly determined by the poor-rich gradient (Testacea: Opravilová & Hájek, 2006; Mollusca: Horsák & Hájek, 2003; Clitellata: on small scale, Bojková et al., 2011; Diptera: Omelková et al., 2013), we focused on spring fen clitellate assemblages inhabiting a large number of localities. Clitellates form neither cases nor solid cuticle structures to protect their soft and flexible bodies. Therefore, the effect of the calcium and magnesium concentration gradient is considered to be indirect (Martínez-Ansemil and Collado, 1996). Nevertheless, we expect to see a different species composition at sites with high inorganic particles compared to sites dominated by organic materials, such as wood, leaves and particulate organic matter (Verdonschot, 2001; Bojková et al., 2011). It has been shown that organic substrate is used by clitellates as a source of food (e.g. Lafont, 1984; Verdonschot, 2006) and can serve as a shelter.

The objectives of this study are to (i) determine the main gradients of clitellate assemblage composition at isolated fen sites distributed over a broad spatial scale, (ii) identify the main fen types based on clitellate assemblage composition and compare these clitellate-based types with the types defined based on chemistry and vegetation and (iii) determine the habitat preferences of common species using predictive models of species abundance as a linear function of environmental variable(s).

2. Material and methods

2.1. Study area

The study area was located in the Western Carpathian Mountains of the Czech and Slovak Republics (Fig. 1), covering an area of 223 km west to east and 74 km north to south. The area consists of three main parts: the Moravian-Slovakian borderland on flysh bedrock (i.e. alternating claystone and sandstone layers), the Orava region, which is the eastern extension of the flysh zone, and the Inner Western Carpathians with geological structures formed by cores of Paleozoic and crystalline schists overlaid by Mesozoic shale-sandstone and carbonate lithofacies (Poprawa and Nemčok, 1988; Hájek and Hekera, 2005; Horsák et al., 2007). This area was selected for this study because of the frequent occurrence of well-preserved spring fens with contrasting water chemistry, spanning from calcareous fens to acidic *Sphagnum* fens, within each of the three geologically distinct parts.

Fifty four permanent treeless spring fens, fed by groundwater and developed mostly on gentle slopes, were chosen for this study to cover as wide a gradient of groundwater chemistry (pH 3.6–8.4) as possible. The study sites can be classified into four main types based on water chemistry and vegetation (Hájek et al., 2006): (i) extremely mineral-rich fens with tufa (25 sites), (ii) mineral-rich fens without tufa (15 sites), (iii) mineral-rich *Sphagnum* fens (7 sites) and (iv) mineral-poor *Sphagnum* fens (7 sites). The uneven representation of the site types corresponds with the variation in the bedrock chemistry and a high frequency of alkaline sloping fens. The elevation of the study fens varied from 400 to 800 m a.s.l.

2.2. Field sampling

From 2006 to 2012, samples of aquatic macroinvertebrates were collected in spring and autumn from each study site. To cover the whole spring fen habitat heterogeneity, samples from three contrasting mesohabitats were collected at each site. Two types of mesohabitats, i.e. with flowing and standing water, were sampled from the spring mouth, each from a quadrate defined by a metal frame (25x25 cm). All the material taken up to the depth of 5 cm was elutriated using a hand net (mesh size 500 µm). A spring brook was the third type of mesohabitat, sampled using a hand net (mesh size 500 µm) from 10 25x25 cm sampling plots distributed along an approximately 20 m long stretch starting immediately downwards of the spring mouth. Altogether, 324 samples were obtained. To cover the whole seasonal and spatial variability of clitellate assemblages we merged samples from both seasons and from three mesohabitats into a single sample per site. Samples were preserved by 4% formaldehyde in situ and clitellates were hand-sorted in the laboratory under a stereomicroscope and identified after mounting into the Canadian Balsam. For the identification of oligochaetous clitellates, keys from Hrabě (1954, 1981), Timm (2009), Pižl (2002) and Schmelz and Collado (2010) were used, and for leeches keys from Neubert and Nesemann (1999) and Košel (2001) were used. Current taxonomy of clitellates follows Siddall et al. (2001), Erséus (2005) and Erséus et al. (2008).

2.3. Environmental variables

The following eight variables were established for each study site, as they have already been proven, or are likely to be, important drivers of aquatic clitellate assemblages and species population size (Martínez-Ansemil and Collado, 1996; Timm et al., 2001; Verdonschot, 2001; Verdonschot, 2006; Bojková et al., 2011; Rádková et al., 2014). Water temperature and dissolved oxygen were measured in the field using portable instruments (WTW Multi 340i/SET) at each sampling occasion. GPS positions were directly measured in the field and used to determine mean annual air temperature by using overlays of plot locations with a digital elevation model and climatic maps, based on Tolasz (2007), in the ArcGIS 8.3 program (ESRI, 2003). Of the intercorrelated variables altitude and mean annual air temperature, we selected the latter because of the independence of geographical position. Water samples for the laboratory analysis of Ca^{2+} and Mg^{2+} (combined, Ca+Mg) and NO_3^- ion concentrations were taken in autumn, when the chemistry of spring fens is the most stable (Hájek and Hekera, 2004). Total organic carbon (TOC) was measured from a 100 ml sample of substrate (Shimadzu TOC VCPH), which was taken next to the sample of the clitellates. This variable is closely bound to spring fen type categorisation, reaching only 5% in extremely mineral-rich fens, but significantly increasing in fens of low mineral content (poor fens) (Hájek et al., 2002). Substrate samples were collected during the autumn sampling from a 25x25 cm plot up to the depth of 5 cm. Substrate was collected from patches adjacent to the plots used for clitellate sampling and reflected the same structural microhabitat type. Organic matter (ORG) was obtained from substrate samples by elutriation (Omesová and Helešic, 2004), using a net with a mesh diameter of 100 µm, dried at 80 °C and weighed. The remaining inorganic substrate was dried at 80 °C and used for the grain size analysis (Giere, 1993), from which the amount of inorganic particles smaller than 500 µm was determined (INORG) and expressed as a percentage of the total weight of inorganic matter.

2.4. Statistical analyses

Relationships among explanatory variables were inspected using Spearman correlations and multi-panel scatter plots. The environmental variables were not found to be highly intercorrelated (rho<0.50), except for total organic carbon (TOC) and Ca+Mg concentration (rho=-0.57). These two variables were; however, used as they are likely to constitute ecologically different responses of clitellate species. Under a high Ca+Mg concentration, in alkaline sites, a strong precipitation of calcium carbonate notably changes substrate conditions, while TOC increases in both acidic and productive sites. Descriptive statistics of the eight explanatory variables used in the statistical analyses are summarised in Table 1.

Principal Coordinate Analysis (PCoA), also known as MDS (Metric Multidimensional Scaling), on Bray-Curtis distances was used to explore and visualise variation in the species composition of clitellate assemblages (command “cmdscale” in the “vegan” package). Species abundances were transformed as y=ln(x+1) prior to the analysis to reduce the effect of dominant species. The importance of ordination axes was evaluated using a broken-stick model (Peres-Neto et al., 2003), which is a permutation-based technique comparing the real eigenvalues of ordination axes with those eigenvalues generated randomly by the model. Based on this method, only the first and second axes were selected as important and meaningful for further interpretation of assemblage compositional variances. To associate the main changes in species composition with the environmental predictors, we used multiple regressions between the predictors and site scores on the first and second ordination axes. The significance was tested by 9999 permutations using the “envfit” function in the “vegan” package. Distance based Redundancy Analysis (dbRDA) using Bray-Curtis distances was performed to test the significance of the effect of each predictor on the species composition variance and to identify a parsimonious set of environmental predictors which could account for the maximum variation in the clitellate species data. Forward selection procedures and Monte Carlo permutation tests with 4999 runs were used.

Procrustes analysis with 9999 permutations (Gower, 1975; Jackson, 1995) was used to test the congruence between site scores on the first two PCoA and dbRDA axes.

Cluster analysis, followed by Indicator Species Analysis (Dufréne and Legendre, 1997), was performed to determine the main types of fens based on clitellate assemblage composition and to identify the clitellate species characteristics for these fen types. Bray-Curtis dissimilarity on log-transformed abundance data, y=ln(x+1), and Ward's method were used for the grouping. Indicator Species Analysis (Dufréne and Legendre, 1997) was performed using the "multipatt" function from the "indicspecies" package (De Cáceres and Legendre, 2009), with significance tested using a permutation test with 999 runs. Fen types determined based on clitellates were compared with the main types of fens defined based on vegetation (Hájek et al., 2006). Differences in environmental variables among the three main clitellate types of fens were compared using a one-way ANOVA, and a Tukey post hoc test (multiple comparison tests) was used to determine the significant differences between group pairs.

Generalized Linear Models (GLM; McCullagh and Nelder, 1989) with quasi-Poisson distribution of errors, i.e. corrected for overdispersion, were used to model related clitellate species abundances as a linear function of the eight environmental variables. Prior to modelling, we assessed the distribution of all variables to look for potential outliers. We transformed some of the predictors to straighten up their left-skewed distribution and to linearise the relationships between response variables and predictors. Ca+Mg, inorganic particles and total organic carbon were square root transformed, nitrate concentration and water temperature were transformed by natural logarithm, while the other variables were kept untransformed. Modelling was performed only for the 12 common species, i.e. those recorded at 15 or more sampling sites. First, the response of species abundance to eight predictors was searched visually in case of a potential unimodal response (found only for Ca+Mg and annual air temperature) we also tested the effect of the quadratic term along with a linear term, included for all predictors. To find the best model for each species we used a guided step-wise forward selection with *F* statistic. We started with a null model and the predictors were sequentially added into the model and at each step the current model was tested against a set of more complicated and simpler models. The selection process stopped once the inclusion of another term did not cause a significant improvement in the current model and, simultaneously, the omission of any term would lead to a significant worsening of the model. For all final models we examined residuals for residual patterns with respect to fitted values and to individual predictors and we also checked residuals for normality, homogeneity of variance and identified influential observations using Cook's distances (Cook, 1977).

All statistical analyses were performed in R (R Core Team, 2014) using the "vegan" (Oksanen et al., 2011), "PerformanceAnalytics" (Carl and Peterson, 2010) and "indicspecies" (De Cáceres and Legendre, 2009; De Cáceres, 2013) packages.

3. Results

3.1. The response of clitellate assemblages to environmental gradients

A total of 34,122 individuals collected from 54 study sites, were assigned to 42 clitellate taxa (mostly species, except for some genera of the family Enchytraeidae). On average 632 individuals and 13 species were collected per site. The highest abundances were obtained for Lumbriculidae, in contrast the highest taxa-richness was found for Naididae *sensu* Erséus et al. (2008) (Table 2).

Using Principal Coordinate Analysis (PCoA), we observed two main gradients of species composition variance, which covered 31.1% and 16.0% of the entire variation respectively. The main compositional change, expressed on the first PCoA axis, was related mainly to the variation in total organic carbon (TOC) in the substratum and also partly to Ca+Mg concentration and annual air temperature (Table 3, Fig. 2). Mineral poor *Sphagnum* fens, having a high content of poorly decomposed organic matter (recorded as a high TOC), were occupied by *Cognettia glandulosa*, *Lumbriculus variegatus* and *Nais communis*, plotted in the right part of the diagram. In contrast, extremely mineral-rich fens with a tufa formation and very low TOC in the substratum were inhabited by a rich population of *Trichodrilus strandi* (left part of the diagram). The second main direction of species compositional variance was mostly associated with the amount of organic matter vs. inorganic matter and the gradient of water temperature (Table 3, Fig. 2). Fen sites with a high amount of food and a high water temperature were plotted in the upper part of the diagram, harbouring rich populations of generalist species (e.g. *Tubifex tubifex* and *Limnodrilus hoffmeisteri*) and predators (*Glossiphonia nebulosa*, *G. concolor, Erpobdella vilnensis* and *Haemopis sanguisuga*). Several variables, such as concentration of calcium and magnesium, dissolved oxygen concentration and mean annual air temperature, expressed almost an equal association to both PCoA axes.

The distance based Redundancy Analysis (dbRDA) showed very similar results as the PCoA (Table 3), with TOC, Ca+Mg ions and annual air temperature being the most important environmental predictors of clitellate assemblages. Each of these variables separately explained more than 10% of the total variance in the species data and two of them were included in the most parsimonious model in the forward selection (Table 3). The Procrustes analysis showed a strong significant correlation between the position of samples in the ordination spaces of the PCoA and dbRDA (Procrustes R=0.854, *P*<0.001).

3.2. Clitellate assemblage types

Based on the cluster analysis we determined three main types of fens differing in clitellate assemblage composition (Fig. 3). In the first division, the sites split up into extremely mineral rich fens with a tufa precipitation (type A) and the other fens (type B and C). However, the following division reflected more complex environmental differences (Table

2, Fig. 3). The sites of the type A fens were characterised by high concentrations of Ca+Mg ions, low TOC and high annual air temperature (Fig. 4) and *Trichodrilus strandi* was identified as the indicator of this group. Based on field observations, all these sites were pristine calcareous fens. Type B included spring fens with cold water and also the lowest concentration of Ca+Mg ions and intermediate values of TOC (Fig. 2, Fig. 4). *Stylodrilus heringianus* was found to be an indicator species of this fen type. This group included both calcareous fens and the most acidic fens, defined as vegetation type 1-2 and 4. Type C was the most broad fen type in respect of clitellate distribution, with 12 species characteristic for this type of fen (Table 2). The sites of this fen type had the highest water temperatures and were also enriched by organic matter, resulting in significantly lower oxygenation compared with the other two types (Fig. 4). Their mineral richness was highly variable as they belonged to all four types defined by vegetation (Fig. 3).

3.3. Environmental preferences of studied species

We succeeded in building a significant model between the abundances of all the 12 dominant species and the eight environmental predictors using a GLM (Table 4). Based on the final parsimonious model, variables linked with mineral richness gradient (i.e. combined Ca and Mg concentration and TOC in the substratum) were the most important predictors for six species. Three of these species (all with a total explained variation >50%) were also among those species selected based on Indicator Species Analysis as characteristic for one of the three main fen types defined based on clitellate assemblage composition. The abundance of these species increased mostly in acidic fens, but *Trichodrilus strandi* increased in mineral-rich tufa fens as the indicator of type A. In contrast, *Rhyacodrilus falciformis* abundances expressed a unimodal response to concentrations of Ca^{2+} and Mg^{2+}. Water temperature, dissolved oxygen, organic matter and concentration of nitrates were found to be the main drivers for the other five species. Responses to water temperature both positively and negatively separated oligostenothermic species, such as *Pristina bilobata* and *Mesenchytraeus armatus*, from those species preferring high temperatures, such as *Tubifex tubifex*. Nevertheless, responses to high organic matter were positive not only for *Tubifex tubifex* but also for *Pristina bilobata* populations. The other predictors were less important in the models, yet significant responses were found in several species, having both positive and negative effects on individual species abundances.

4. Discussion

4.1. The response of clitellate assemblages to environmental gradients

Based on an extensive dataset of spring fen habitats, distributed over a large spatial scale and covering a broad ecological variety of spring fens, we found that nutrient limited ecosystems harbour clitellate assemblages of relatively high species richness, with a total number of 42 taxa (mostly species) and an average of 13 taxa per sampling site, recorded at

54 sampling sites. In comparison with other spring ecosystems, the diversity in the studied spring fens was almost as high as those of the karstic systems in southern Europe, with a total of 47 species and a range of 2–19 taxa per sampling site (Achura and Rodriguez 2008). Clitellate diversity in fenland areas and raised bogs appears to be even lower than that of fens, as Verdonschot (1984) reported 33 species and van Duinen et al. (2006) only 7–13 species in acidic conditions. It seems that spring fens are well utilized by species of Lumbriculidae, as they reached the highest mean abundances per sampling site in all three clitellate fen types (Table 2). *Trichodrilus strandi* dominated in extremely mineral-rich fens with tufa precipitation, *Stylodrilus heringianus* dominated in spring fens with cold water and low Ca+Mg ion concentrations and *Lumbriculus variegatus* dominated in fens with a high content of organic carbon in the substratum.

The importance of mineral richness on compositional variation of clitellate assemblages at spring fens has already been observed in the data collected in the Outer Western Carpathians (Bojková et al., 2011), which in many cases did not exceed 700 years (Hájková et al., 2012). However, the distribution of passively dispersing clitellates was found to be highly influenced by spatial processes, especially among habitat specialists (Milbrink, 1999; Rádková et al., 2014) and it is likely that there was not enough time for all species to colonise all the favourable sites studied by Bojková et al. (2011). Therefore, in this study we explored a higher number of sites located across the entire Western Carpathians, half of which were older than 2.500 years. There were several sites with continuous development over the whole Holocene (Horsák et al., 2015). The gradient of mineral richness was still highly significant for both compositional changes of clitellate assemblages and also for the responses of individual species. However, the highest predictive power for the overall variation in assemblage composition was found for TOC in the substratum. This can be explained by the confounding effect of water chemistry and TOC in the substratum, due to the accumulation of organic matter in acidic fens (Hájek et al., 2006). Further, several clitellate species are known to prefer substrate with a high amount of organic matter (e.g. Uzunov et al., 1988). Along this combined gradient of water chemistry and substrate characteristics a complete species composition turnover was observed. Acidic fens, i.e. with a high amount of accumulated organic carbon (TOC) in the sediment, were inhabited mainly by *Lumbriculus variegatus*, *Cognettia glandulosa*, *C. sphagnetorum* and *Nais communis*, which are known as acid-tolerant/acidophilus species (Graefe and Schmelz, 1999; van Duinen et al., 2006) that feed mainly on plant remains. The opposite end of the gradient is represented by alkaline sites with assemblages dominated by *Trichodrilus strandi*, a spring fen specialist/stygophilous species (Hrabě, 1942; Dumnicka, 2001; Bojková et al., 2011). We hypothesise that such harsh conditions at these preserved calcareous spring fens with tufa precipitations can be linked to the reduction of the interstitial spaces. This change has a negative effect on most oligochaetes (Rundio, 2009), but it is preferred by *T. strandi*, as it is a stygophilous species (Dumnicka, 2001) that probably specialised on feeding on microorganisms associated with a high calcium content (Timm, pers. com.).

4.2. The clitellate assemblage types

Using the classification of the study sites based on vegetation (Hájek et al., 2006) only the extremely mineral-rich fens with a tufa formations were fitted with a clitellate assemblage type. Clitellate type A almost exclusively occurred in extremely mineral-rich fens with a tufa formation (vegetation type 1), with only three sites containing brown-moss mineral-rich fens (vegetation type 2). *Trichodrilus strandi* was proven to be a specialist of pristine, oligotrophic, cold and highly mineralized spring and underground waters, as it was found to be a significant indicator of clitellate type A. Similar habitat preferences have already been reported for this species in the literature (Hrabě, 1981; Juget and Dumnicka, 1986; Giani et al., 2001; Achurra and Rodriguez, 2008; Bojková et al., 2011; Achurra et al., 2015). This species showed the highest indicator value of all species analysed (Table 2). *Rhyacodrilus falciformis* was also associated with type A, though not as an indicator, and is reported both as a crenophilous stygobiont of running water as well as a soil species (Schwank, 1981; Juget, 1987; Dumnicka, 2000; Schlaghamerský and Kobetičová, 2005). Such a relatively wide habitat tolerance can be; however, explained by its unimodal relationship to Ca+Mg concentrations, combined with a preference for low organic matter (Table 4), which explained 23.8% of the variance in its abundance data.

The second fen type defined based on clitellate assemblage composition (type B) included cold water fens with variable concentrations of Ca+Mg ions (Fig. 4). The high abundance of *Stylodrilus heringianus*, which was the only indicator species from this fen type, was explained by Ca+Mg ion concentration, which had a strong negative effect on population densities. Likewise, *Cognettia sphagnetorum* is a typical inhabitant of acidic soils (Graefe and Schmelz, 1999), and acidic wetlands up to ombrotrophic bogs (van Duinen et al., 2006), and was associated with this fen type. This species strongly and positively responded to decreases in Ca+Mg concentrations, which explained 47% of the variance in its abundance data (Table 4). However, another species associated with type B, *Mesenchytraeus armatus,* did not show any relationship to the poor-rich gradient and its abundances were predicted mainly by mean annual temperature, with a preference for colder waters. The other two dominant clitellate species from type B are characterised by their tolerance to desiccation; *Stylodrilus heringianus* prefers habitats with stony substrates and intermittent stream flow (Nijboer et al., 2004) and *Cognettia sphagnetorum* is able to survive 20 days of drought, probably in a desiccation tolerant stage (Maraldo and Holmstrup, 2009). In addition, the other species associated with this group were either Enchytraeidae (generally soil species) or semiaquatic species (the leech *Trocheta cylindrica*). The presence of these species might suggest an important common environmental feature of the fen sites included into type B, which is occasional desiccation. This was partly observed in the field, but it was not directly measured.

The highest species diversity and highest number of indicator species was associated with the third clitellate fen type, type C (Table 2). The presence of many generalist species was in the accordance with low oxygenation, high water temperature, high variability, but overall high TOC and also highly variable mineral richness. *Tubifex tubifex* and *Limnodrilus hoffmeisteri*, whose dominance can indicate waters with high organic pollution (e.g. Brinkhurst and Jamieson, 1971; Uzunov et al., 1988), were abundant in sites with either a high amount of organic matter content or total organic carbon content and nitrate concentration respectively. Another two indicator species, *Lumbriculus variegatus* and *Cognettia glandulosa*, with 40% and 27% of their variation in abundance explained by TOC were characteristic for most acidic sites in this group, preferring high water temperatures and high inorganic substratum respectively. It is worth noting that several sites included in this group showed vegetation classification favourable for *Trichodrilus strandi*. However, due to a human-made increase in organic matter, as a result of horse or cow pasture (indicated in the field), *Trichodrilus strandi* was most likely replaced by the eurytopic species *Tubifex tubifex* and *Limnodrilus hoffmeisteri*. Therefore, *Tubifex tubifex* could have a hypothetical competitive advantage, and also other eurytopic species, over the habitat specialists. This might be supported by the fact that eurytopic species did not show any association with characteristics of microhabitats, such as vegetation or substrate coarseness, if sufficient food in form of organic matter was available (Prenda and Gallardo, 1992).

To summarise, we confirmed that clitellate distribution is strongly connected with the gradient of spring fen groundwater chemistry, which remains the same across a large spatial area including isolated fen sites of highly variable age. Although we expected a significant imprint of dispersal, based on processed compositional variance (as found by Rádková et al., 2014), it seems that the effect of environmental conditions is extremely important. Our study also stresses that pristine and highly endangered spring fen habitats, hosting unique species-rich clitellate assemblages, can be easily destroyed by an increase in organic matter, either by human-made eutrophication or also negative changes in hydrological conditions (Hájek et al., 2006). Our results, based on individual species response modelling, suggest that these negative alterations in habitat conditions can trigger compositional shifts towards assemblages of common eurytopic tubificid species. A higher risk is expected for assemblages of calcareous spring fens, as acidic sites and their surroundings are less suitable for pasture and require more space, while alkaline fens develop on spatially limited groundwater outlets.

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Table 1 Descriptive statistics of environmental variables for the three types of fens (A, B, C; Fig. 3): Ca+Mg, the sum of concentrations of calcium and magnesium ions in the groundwater; O_2, dissolved oxygen; NO_3^-, the amount of nitrate; TOC, total organic carbon; ORG, total organic matter; INORG, % of inorganic particles smaller than 500 µm from total weight of the inorganic particles.

	Type A				Type B				Type C			
Variable	MIN	MEAN	MAX	SD	MIN	MEAN	MAX	SD	MIN	MEAN	MAX	SD
Ca+Mg (mg l^{-1})	25.40	70.29	120.60	29.08	4.40	32.27	79.10	21.84	8.50	40.30	101.40	26.86
O_2 (mg l^{-1})	2.91	6.85	10.00	1.74	2.50	6.85	10.00	1.88	1.43	4.90	7.83	1.70
NO_3^- (mg l^{-1})	<0.01	3.51	12.00	3.65	<0.01	3.83	21.66	5.80	<0.01	1.83	12.64	3.21
TOC (mg l^{-1})	<0.01	51.58	137.00	43.62	2.12	131.00	266.80	82.48	30.32	169.00	327.40	98.14
ORG (g)	18.65	77.95	129.70	30.61	18.08	71.50	146.50	44.03	33.20	106.20	186.10	35.64
INORG < 500 µm (%)	13.00	33.50	59.00	12.34	11.00	40.44	71.00	14.52	15.00	40.44	53.00	10.49
Water temperature (°C)	8.8	12.9	23.5	3.3	7.7	10.9	15.0	2.0	11.8	14.5	20.2	2.4
Annual temperature (°C)	5.1	6.8	8.3	0.8	4.5	5.8	7.2	0.8	5.2	6.1	7.3	0.6

Table 2 List of all recorded taxa arranged according to the results of the Indicator Species Analysis. The indicator value of a species represents the maximal indicator value of the species among the three types of fens (A, B, C; Fig. 3). Significant indicator species and *P* values are in bold. The assemblage type, with the maximal indicator value, is highlighted in grey for each of the indicator species. Average abundances per sample from the sites where the species was found and the number of these sites are shown before and after the slash, respectively. The total number of study sites for each fen type is given after the slash.

			Type of fen based on clitellate assemblage composition		
			Type A/19	Type B/16	Type C/20
Taxon name	Ind. value	*P*	Mean abundances from sites with species occurence		
***Trichodrilus strandi* Hrabě, 1936**	0.880	**0.001**	435.6/18	4/3	50.2/10
***Henlea* spp.**	0.638	**0.036**	8/16	4.4/14	4.2/6
Eiseniella tetraedra (Savigny, 1826)	0.583	0.731	23.4/18	28.5/15	20.1/18
Rhyacodrilus falciformis Bretscher, 1901	0.528	0.146	12.2/10	4.1/9	9.5/4
Enchytraeus spp.	0.510	0.787	7.5/11	2.4/12	1.5/13
***Stylodrilus heringianus* Claparède, 1862**	0.674	**0.004**	5.3/9	124.9/15	107.9/19
Cognettia sphagnetorum (Vejdovský, 1878)	0.622	0.080	17.3/9	117.8/14	75/18
Fridericia spp.	0.608	0.207	19.3/15	24/14	5.3/16
Mesenchytraeus armatus (Levinsen, 1884)	0.575	0.331	7.5/13	16.2/13	5.3/14
Henlea perpusilla Friend, 1911	0.410	0.390	2/5	8/5	3/4
Haplotaxis gordioides (Hartmann, 1821)	0.350	0.171	1.5/2	2/3	-
Trochaeta cylindrica Örley, 1886	0.338	0.292	1.5/2	3.3/3	2/1
Marionina argentea (Michaelsen, 1889)	0.182	0.746	1/1	1/1	-
***Erpobdella vilnensis* (Liskiewicz, 1925)**	0.849	**0.001**	1.8/5	25/2	48.1/17
***Limnodrilus hoffmeisteri* Claparède, 1862**	0.803	**0.001**	40.8/9	2.3/3	86.6/18
***Lumbriculus variegatus* (Müller, 1774)**	0.766	**0.001**	12/6	20.6/11	223.7/18
***Tubifex tubifex* (Müller, 1774)**	0.751	**0.001**	15.3/15	29.5/8	135.6/19
***Cognettia glandulosa* (Michaelsen, 1888)**	0.694	**0.002**	6/2	16.2/12	89.5/15
***Tubifex ignotus* (Štolc, 1886)**	0.655	**0.002**	1/1	-	15.7/9
***Nais communis* Piguet, 1906**	0.650	**0.019**	2/2	48/11	94.1/15
***Aulodrilus limnobius* Bretscher, 1899**	0.602	**0.003**	9/1	2/2	48.6/9
***Haemopis sanguisuga* (Linnaeus, 1758)**	0.519	**0.012**	1/1	-	2.5/6
Cernosvitoviella spp.	0.500	0.338	3.1/7	18.9/7	11.2/11
***Glossiphonia concolor* (Apáthy, 1888)**	0.500	**0.004**	-	-	6.2/5
Pristina bilobata (Bretscher, 1903)	0.496	0.102	2.7/3	13/4	9.2/9
Limnodrilus udekemianus Claparède, 1862	0.462	0.074	5/1	1.5/2	1.7/7
***Slavina appendiculata* (Udekem, 1855)**	0.447	**0.036**	-	-	24/4
***Glossiphonia nebulosa* Kalbe, 1964**	0.500	**0.004**	-	-	4.8/4
Pristina jenkinae (Stephenson, 1931)	0.496	0.102	14.5/4	-	10.6/7
Limnodrilus claparedeanus Ratzel, 1868	0.424	0.077	61/1	-	15.6/5
Rhyacodrilus coccineus (Vejdovský, 1875)	0.402	0.304	12/4	1/1	3/7
Marionina sp.	0.388	0.441	3.4/5	1/2	3.5/6
Pristina aequiseta Bourne, 1891	0.387	0.112	-	-	2.7/3
Spirosperma ferox Eisen, 1879	0.387	0.091	-	-	28.7/3
Nais variabilis Piguet, 1906	0.332	0.334	-	13/2	5/4
Aulodrilus japonicus Yamaguchi, 1953	0.316	0.319	-	-	130/2
Trichodrilus tatrensis Hrabě, 1937	0.316	0.342	-	-	7/2
Trichodrilus sp.	0.316	0.342	-	-	4.5/2
Mesenchytraeus spp.	0.246	0.786	-	1/2	3.5/2
Helobdella stagnalis (Linnaeus, 1758)	0.246	0.786	-	5/1	4/2
Pristina rosea (Piguet, 1906)	0.228	0.787	-	6/1	3.5/2
Nais bretscheri Michaelsen, 1899	0.217	0.756		4/1	1.5/2

Table 3

Summary results of the PCoA and dbRDA analyses. Regression coefficients for the multiple linear regressions for the explanatory variables and the PCoA site scores on the first two axes (PCoA 1 and 2), explained variation (R^2 %) and its significance (*P*) based on 9999 permutations. Adjusted percentage of explained variation ($AdjR^2$) in species data by each variable in separate dbRDA analyses (Gross effects) and in a single dbRDA model that includes all variables (Pure effects). The order of the variables entering the single dbRDA model was based on a forward selection procedure (FS). Significance (*P*) of explained variation was tested using a Monte Carlo test with 4999 runs. For abbreviations of environmental variables see Table 1.

	PCoA				dbRDA				
	Regression coefficients				Explained variability		Explained variability in FS		
Variable	PCoA 1	PCoA 2	R^2(%)	*P*	$AdjR^2$(%)	*P*	$AdjR^2$(%)	*P*	Order FS
TOC	1.000	-0.027	54.330	<0.001	18.09	0.005	16.46	0.001	1
Ca+Mg	-0.787	0.617	55.210	<0.001	16.62	0.005	5.46	0.001	2
Annual temp.	-0.764	0.646	36.610	<0.001	10.28	0.005	n.s.	n.s.	n.s.
O_2	-0.711	-0.703	34.890	<0.001	9.20	0.005	1.89	0.005	4
Water temp.	-0.014	1.000	42.230	<0.001	6.98	0.005	4.84	0.001	3
ORG	0.319	0.948	21.880	0.002	3.81	0.015	n.s.	n.s.	n.s.
NO_3	-0.930	-0.367	11.050	0.047	3.79	0.015	n.s.	n.s.	n.s.
INORG<500 µm	0.507	-0.862	11.610	0.040	2.26	0.045	n.s.	n.s.	n.s.

Table 4

Adjusted percentage of variance in the analysed species abundance explained by each predictor included in the final generalized linear models with Poisson distribution. The gross effect of the most important predictor for each taxa group (in bold) and the net effects of the other predictors are listed sequentially and summed up to give the Total. The effect of the linear term of each predictor and the quadratic term of the combined content of Ca+Mg and annual temperature are denoted by (+) and (-) for a positive and a negative effect respectively. Significance: ***, <0.001; **, <0.01; *, <0.05; <0.07 (marginally significant). For the explanation of predictor abbreviations see Table 1.

	Explained variance - pure effects										
	Total	Ca+Mg	Ca+Mg^2	Water temp.	O2	ORG	INORG	TOC	NO3	Annual	Annual temp.^2
Stylodrilus heringianus	75.8	**72.8 (-)*****					3.0 (-)*				
Lumbriculus variegatus	62.8	7.9 (-)**		15.3 (+)***				**39.7 (+)*****			
Trichodrilus strandi	50.2	19.5 (+)***			**30.7 (+)*****						
Nais communis	34.7	**24.4 (-)****					10.3 (-)**				
Cognettia sphagnetorum	47.1	**47.1 (-)*****									
Cognettia glandulosa	42.5						15.7 (+)**	**26.9 (+)****			
Pristina bilobata	36.8	8.9 (-)**		12.7 (-)*		10.6 (+)**			**14.3 (-)****		
Mesenchytraeus armatus	32.8			**22.9 (-)*****						<1.0 n.s.	9.9 (+)*
Tubifex tubifex	27.5			**16.6 (+)****		10.9 (+)*					
Rhyacodrilus falciformis	23.8		**13.2 (+)***			10.6 (-)*					
Erpobdella vilnensis	23.3				**23.3 (-)****						
Limnodrilus hoffmeisteri	17.9							**10.2 (+)***	7.7 (+).		

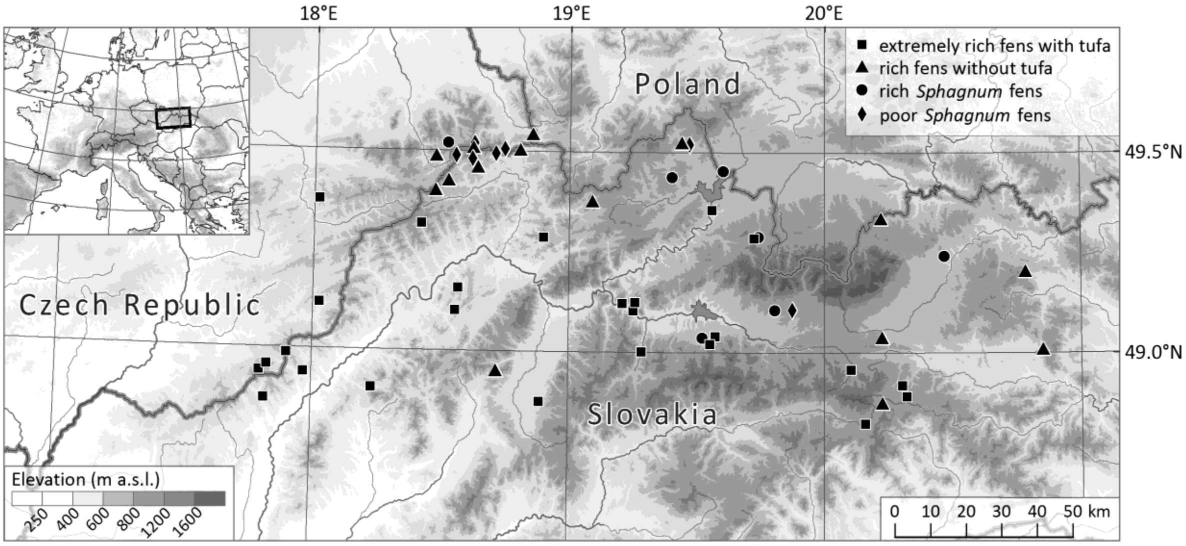

Fig. 1 Location of the study area and sites.

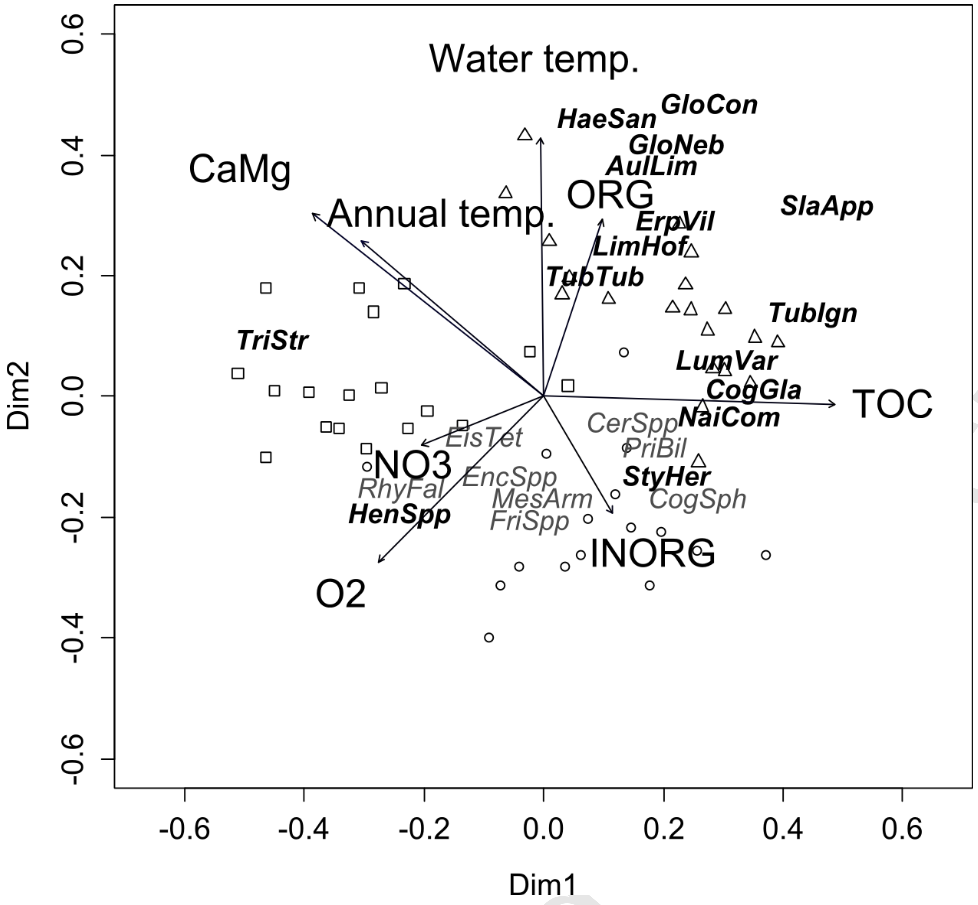

Fig. 2 PCoA ordination plot of the 54 clitellate assemblages with the position of 15 indicator species (given in bold) and the eight the frequent species (found at least at 15 sites). Eight environmental variables were fitted post-hoc into the ordination space of the first and second axes (all variables had significant fit, *P*<0.05). The classification of sites is based on the results of the cluster analyses (Fig. 3): rectangles, type A; circles, type B; triangles, type C. Abbreviations of taxa–**indicators of type A**: TriStr, *Trichodrilus strandi*; HenSpp, *Henlea* spp.; **indicator of type B**: StyHer, *Stylodrilus heringianus*; **indicators of type C**: ErpVil, *Erpobdella vilnensis*; LimHof, *Limnodrilus hoffmeisteri*; LumVar, *Lumbriculus variegatus*; TubTub, *Tubifex tubifex*; CogGla, *Cognettia glandulosa*; TubIgn, *Tubifex ignotus*; NaiCom, *Nais communis*; AulLim, *Aulodrilus limnobius*; HaeSan, *Haemopis sanguisuga*; GloCon, *Glossiphonia concolor*; SlaApp, *Slavina appendiculata*; GloNeb, *Glossiphonia nebulosa*; **frequent taxa**: CerSpp, *Cernosvitoviella* spp.; CogSph, *Cognettia sphagnetorum*; EisTet, *Eiseniella tetraedra*; EncSpp, *Enchytraeus* spp.; FriSpp, *Fridericia* spp.; MesArm, *Mesenchytraeus armatus*; PriBil, *Pristina bilobata*; RhyFal, *Rhyacodrilus falciformis*. For the abbreviations of the environmental variables see Table 1.

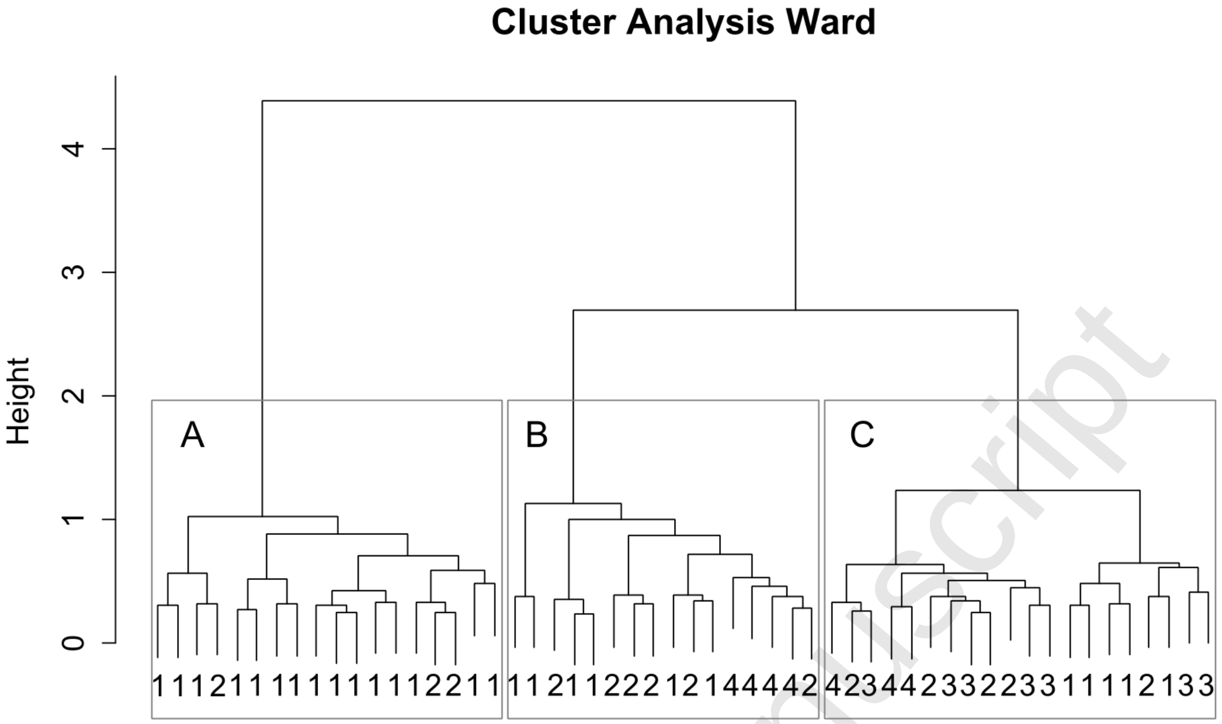

Fig. 3 Result of the cluster analysis (Bray-Curtis distance, Ward's method) of the 54 fen sites based on clitellate assemblages into the three types (A, B, C). Classification of the sites based on vegetation is shown by different numbers: 1, extremely rich fens with tufa; 2, rich fens without tufa; 3, mineral-rich *Sphagnum* fens and 4, mineral-poor *Sphagnum* fens.

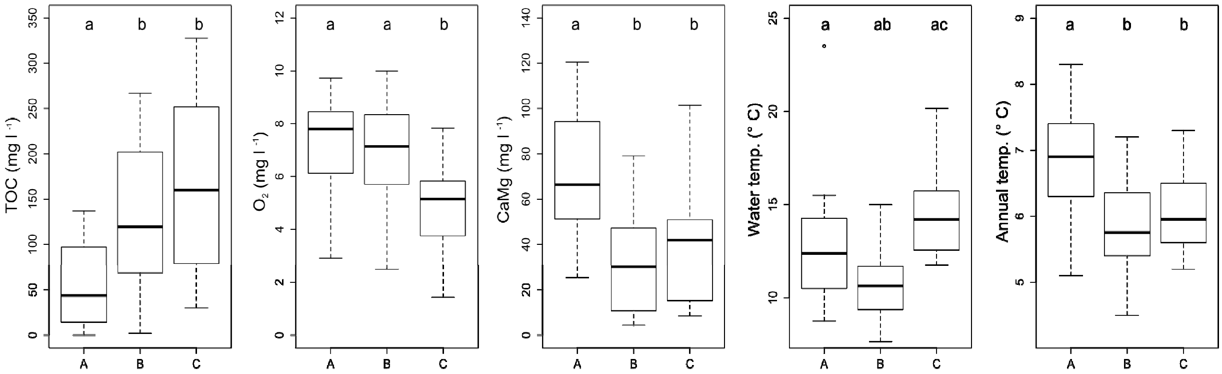

Fig. 4 Variation of five environmental factors among the three types of fens based on clitellate assemblage composition (see Fig. 3). The central line of each box refers to the mean value, box height indicates the interquartile range, whiskers to the non-outlier range (i.e. 1.5 times the interquartile range at each side), and a small cross for an outlier. Small letters at the top indicate statistically significant differences between the types (ANOVA and Tukey post-hoc test, *P*<0.05).

5.2 Druhová diverzita máloštětinatých opaskovců a její změny podél gradientů prostředí

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Strategies of Coexistence of Two Species: *Erpobdella octoculata* and *E. vilnensis* (Hirudinea: Erpobdellidae)

key words: seasonal dynamics, habitat preferences, diet

Abstract

Populations of *Erpobdella octoculata* and *E. vilnensis* were studied monthly over two years (May 1999–April 2001) at four sampling points on the river Rokytná (Czech Republic). The water depth and velocity were measured and the Froude number for each sample was calculated to follow flow conditions above the bottom. Temporal differences in biomass of the two species were studied by comparing their wet weights and evaluated by the time series analysis; differences in their diets were evaluated by gut content analyses. Significantly different hydrodynamic preferences for juvenile and adult stages of *E. octoculata* and *E. vilnensis* were recorded. In *E. vilnensis* only, an annual cycle was recorded with a one-month shift of mean weights compared to *E. octoculata*. This shift, together with the correlation of the leeches wet weights with their prey sizes and their distinct habitat preferences are suggested as mechanisms that explain their coexistence.

1. Introduction

The predatory species *Erpobdella octoculata* (Linnaeus, 1758) (Hirudinea: Erpobdellidae) seems to be one of the best-investigated organisms of the bottom fauna across different water bodies. Its life cycle in stagnant and running waters is completed principally in one (*e.g.*, Murphy and Learner, 1982; Dall, 1979, 1983) or, rarely, in two or three years (*e.g.*, Mann, 1953; Elliott, 1973). Certain authors recorded both one-year and two-year cycles but in different localities (Aston and Brown, 1975; Maltby and Callow, 1986). Many studies of the feeding habits of *E. octoculata* have been undertaken (*e.g.*, Young and Ironmonger, 1979, 1980; Dall, 1983; Toman and Dall, 1997; Kutschera, 2003), because the prey is swallowed as a whole and remains in the gut for some time almost undamaged. Only one study of *Erpobdella vilnensis* Liskiewicz, 1927 (former *E. monostriata* Gedroyć), has been published (Manoleli, 1976). This species probably has a similar life strategy to *E. octoculata* but is less common. In most populations of *E. vilnensis*, a one year cycle was recorded with only few breedings in their second year.

The goal of our study was to reveal the mechanisms enabling the coexistence of *E. octoculata* and *E. vilnensis* populations. These species are of similar size and weight, feed on similar prey and are commonly found together in Czech streams. Competition for space was assessed by the habitat preferences of individual juveniles and adults of the two species. From the environmental variables recorded, we selected those which regulate the reproduc-

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tion of leeches. The life cycles of *E. octoculata* and *E. vilnensis*, based on recording the leeches wet weights, were compared and their synchronisation estimated. The food niches were evaluated by the analysis of the leeches' gut contents and by comparing the composition, abundance and size of the prey.

2. Methods

2.1. Study Area

The study stream was the Rokytná (6th order) which has a length of 89.3 km and a catchment area of 585.4 km^2. It runs through an upland area in the southern part of the Czech Republic. In this area the climate is warm and dry with a moderate winter and a mean annual temperature of 8 °C. During the study period, the mean annual discharge varied from 1.0 to 1.3 m^3 s^{-1}, with winter and summer floods. The maximum discharge reached 24.3 m^3 s^{-1} and the minimum was 0.08 m^3 s^{-1}. The bottom substratum consisting of small pebbles, gravel, and sand, was rather unstable, especially in meandering reaches. In January and February, the river was frozen down to the bottom, in spring the substratum was colonized by periphytic algae and in summer the stream was completely shaded and the water was highly turbid because of organic pollution (ß-mesosaprobity). No macrophytes were observed on the river bottom during the course of study year.

2.2. Sampling and Laboratory Methods

Four habitats in the River Rokytná were chosen with different hydrological characteristics and substratum. These were situated on two cross-sections, A and B, 60 m distant (for details of sampling see SCHENKOVÁ and HELEŠIC, 2006). The first sampling point was on cross-section A in the straight part of the stream, here called A streamline (AS). In this part of the stream the substratum did not shift and did not form gravel bars. The three remaining sampling points were on cross-section B in the meandering part of the stream, here called B littoral (BL), B gravel bar (BG), B streamline (BS). At each sampling point, quantitative macro-invertebrate samples were taken monthly from May 1999 to April 2001, except for a two-month interval in winter. Samples were collected using a triangular sampling device (HELAN *et al.*, 1973) with an area of 0.1 m^2 and a mesh size 500 µm.

Due to the unstable substratum on cross-section B, we did not evaluate preference of leeches for particular sampling points but for particular hydrodynamic conditions. The values of Froude number (Fr) were computed for each sample from water velocity and depth using the formula $\mathrm{Fr} = \bar{U}/\sqrt{g}\ D$, where $\bar{U}$ is mean current velocity, g is acceleration due to gravity and D is total water depth (DAVIS and BARMUTA, 1989). The Froude number as a descriptor of the main channel flow represents the ratio of inertial forces to gravitational forces. The critical value of Fr is that at which the flow is changing from tranquil to broken and turbulent. Samples were divided into four categories according to their Fr values. Categories three and four were subsequently combined because of some anomalies with category four samples (Table 1 shows the respective categories 1–3). From the environmental variables recorded – water temperature, velocity, depth, discharge, pH, conductivity and biochemical oxygen demand (Table 2) – we aimed to select those that regulate the life cycles of leeches.

Macrozoobenthos samples were fixed in the field using 4% formaldehyde. Samples were sorted and leeches removed in the laboratory. These were stored in 70% ethanol and identified to species (Table 3). *E. octoculata* and *E. vilnensis* represented the eudominant Hirudinea species. Adults of both species can

Table 1. Categories of Froude number values.

Fr	0–0.3	0.31–0.6	0.61–0.9	0.91–1.23
Category	1	2	3	

Table 2. Environmental variables recorded 1999–2001.

Variable	Min.	Max.	Mean	Median
Water temperature [°C]	–0.2	19.4	11.0	13.0
Current velocity at 40% of depth [m s^{-1}]	0.00	1.46	0.67	0.68
Depth [m]	0.10	0.47	0.22	0.20
Mean daily discharge [m^3 s^{-1}]	0.13	3.95	1.36	1.08
pH	4.4	9.0	7.6	7.7
Conductivity [μS cm^{-1}]	453	725	615	615
BOD [mg l^{-1}]	1.9	15.8	5.4	4.5

be identified by the position of their male and female ducts but juveniles only by colour. As result of fixation with formaldehyde, the clitellum could not be observed (MURPHY and LEARNER, 1982). In *E. octoculata* dark spots cover the whole dorsal part, while *E. vilnensis* has a light median stripe without any spots. The smallest stages were colourless when fixed and their identification, unfortunately, was impossible. All leeches of the two species were weighed to the nearest 0.01 mg after 15 minutes of drying. According to their wet weights they were divided into six weight categories on a common logarithmic scale (Table 4).

Table 3. List of Hirudinea species recorded.

List of Hirudinea species	
Erpobdella octoculata juv.	315
Erpobdella octoculata ad.	49
Erpobdella vilnesis juv.	152
Erpobdella vilnesis ad.	191
Erpobdella spp. juv.	61
Glossiphonia complanata juv.	4
Glossiphonia concolor juv.	4
Glossiphonia nebulosa juv.	1
Piscicola geometra juv.	6
Helobdella stagnalis juv.	2
Hemiclepsis marginata juv.	1
Total	786

Table 4. *E. octoculata* and *E. vilnensis* wet weight categories, maximal, minimal and mean prey width. Category 1 prey widths were not analysed in this study.

Weight	Limits of wet	*Erpobdella octoculata*		*Erpobdella vilnensis*	
category	weight [g]	Mean width [μm]	Min. – Max. width [μm]	Mean width [μm]	Min.–Max. width [μm]
1	0.0001–0.0003	–	–	–	–
2	0.00031–0.001	172.0	129.5–220.4	190.3	132.5–248.0
3	0.00101–0.003	189.7	78.0–310.0	219.9	102.1–456.6
4	0.00301–0.01	210.8	76.2–616.2	245.4	103.4–676.2
5	0.01001–0.03	247.3	53.6–1021	321.9	104.2–673.0
6	0.03001–0.1	284.4	149.8–516.0	291.3	158.1–558.3

After being weighed, the gut contents of 48 individuals of *E. octoculata* and 51 of *E. vilnensis* were examined. We selected leeches from all sampling months within the year 2000. These included specimens from all weight categories except category one, which was too small for gut examination (Table 4). The gut contents were obtained by cutting their ventral parts and removing prey items under a stereomicroscope. Whole or fragments of invertebrates were identified to the most precise acheivable level in glycerine-water (1 : 5 solution) under an upright microscope. The head capsules widths of insects

Table 5. List of the prey taxa and their abundances from the analysed guts of *E. octoculata* and *E. vilnensis*.

Taxon	*E. octoculata*	*E. vilnensis*
Oligochaeta		
Bothrioneurum vejdovskyanum		2
Bythonomus lemani		3
Chaetogaster diastrophus	1	
Limnodrilus spp. juv.	26	17
Nais alpina	1	1
N. bretscheri	5	1
N. communis	3	1
N. elinguis	9	7
Potamothrix hammoniensis	1	
Psammoryctides barbatus	9	14
Rhyacodrilinae, with hair chetae		6
Rhyacodrilus coccineus		2
Stylodrilus spp. juv.	33	9
Tubifex tubifex		1
Tubificidae indet.	8	
Crustacea		
Gammarus roeselii	1	
Ephemeroptera		
Baetis rhodani	3	2
Trichoptera		
Hydroptila sp.	1	
Chironomidae		
Chironomini gen. sp.	1	
Eukiefferiella spp.	2	3
Micropsectra sp.	1	1
Microtendipes gr. *pedellus*		1
Nanocladius parvulus/rectinervis	1	
Orthocladiinae gen. sp.	10	10
Orthocladius sp.		2
Parametriocnemus stylatus	1	
Prodiamesa olivacea	1	
Rheocricotopus chalybeatus	4	2
Rheotanytarsus sp.	2	3
Tanypodinae gen. sp.		1
Tanytarsus brundini/curticornis	1	
Chironomidae indet.	2	1
unidentified taxa	7	5
empty gut	10	18
number of prey items	**118**	**84**
number of leeches analysed	**48**	**51**
mean number of prey items per ind.	**3.8**	**3.3**

and the mean body widths of other taxonomic groups (at least three measurements) were measured under the microscope and recorded. When more than one fragment of a taxon was recorded, especially for oligochaetes, we distinguished different individuals by their widths or, if this was not possible, by counting only fragments with prostomial parts.

2.3. Statistical Methods

Seasonal changes of Fr number values were tested by auto-correlations for all particular habitats (AS, BL, BG and BS) and no significant cyclic changes were found. The total trend of Fr number values was evaluated by Daniel's test (in fact Spearman correlation against their order) and no significant trend was found either for total data ($P = 0.718$) or for particular habitats AS: $P = 0.283$, BL: $P = 0.083$, BG: $P = 0.351$, BS: $P = 0.117$. Therefore samples from habitats were treated as independent and habitat preferences of adults and juveniles of *E. octoculata* and *E. vilnensis* in places with different hydrodynamic conditions were evaluated by chi square test. The null hypothesis, of equal distribution between habitats represented by categories of Fr number, was rejected at the 5% significance level.

The relationships between the mean abundances of leeches and mean environmental variables were computed by cross-correlation (BOX and JENKINS, 1970). The goal was to find correlations of values of environmental variables with abundances, shifted in time, and to compute the lag between changes of these values and the response in terms of the abundance of leeches.

Seasonal dynamics for both species were evaluated by time series analysis of the mean abundance of leeches, after first excluding the extremely abundant samples from May 1999. Moving average smoothing was applied for visualisation of the time series. The number of cycles per year and, thus, the length of the cycle were evaluated by spectrum (Furier) analysis of seasonality (SHUMWAY, 1988).

Food niches of both species and their overlap were tested by Bray-Curtis Similarity Index (BRAY and CURTIS, 1957), which was applied to invertebrate taxa found in leech guts (Table 5). The previous finding that, in *E. octoculata*, the size of prey is positively correlated with the leeche's wet weight (*i.e.* YOUNG and IRONMONGER, 1979; DALL, 1983) was tested for both *Erpobdella* species by the Spearman rank correlation coefficient (r_s). Statistica 7 for Windows (STATSOFT, 2004) was used for all analyses.

3. Results

3.1. Habitat Preferences

In total, 786 individuals of eight Hirudinea species belonging to the orders Pharyngobdellida and Rhynchobdellida were recorded in samples (Table 3). In this study, 315 juveniles and 49 adults of *E. octoculata* and 152 juveniles and 191 adults of *E. vilnensis* were recorded. As already mentioned, 61 individuals could not be identified to species and were, therefore, excluded from all analyses.

Significantly different habitat preferences were found by the chi square test: juveniles of *E. octoculata* preferred either the lowest flow (category 1, Fr 0–0.3) or the highest (category 3, Fr 0.6–1.23), juveniles of *E. vilnensis* preferred the middle flow (category 2, Fr 0.31–0.6). Densities of adults of *E. octoculata* increased significantly towards the lower flow. No significant preferences of *E. vilnensis* adults were found.

3.2. Seasonal Dynamics

The life cycle of *E. vilnensis* was regulated by physical parameters of the environment. The cross-correlations showed significant relationships with temperature, water depth and velocity with the three-month shift. Seasonal changes in densities of both species, after curve smoothing, are shown in Figure 1. While the densities of *E. vilnesis* followed significant, regular cyclic changes, the differences in densities of *E. octoculata* were not significant. The same results were obtained by periodograms of both species (Fig. 2). Furier's analysis revealed approximately a one year cycle for *E. vilnensis.* The cross-correlation analysis of temporal changes of mean wet weights revealed a one-month shift of *E. octoculata* weights against *E. vilnensis* (Fig. 1). Therefore the two species differed in weight at any time of the year. The mean monthly increase in wet weight was 0.0064 g for *E. octoculata* but only 0.0037 g for *E. vilnensis,* so that the difference in their size increased during their development.

The life cycle of each species was visualised by frequency histograms of the mean densities of juvenile and adult leeches in each of six weight classes. End of year classes were estimated and marked (Fig. 3). The end of each year generation enabled three distinct gen-

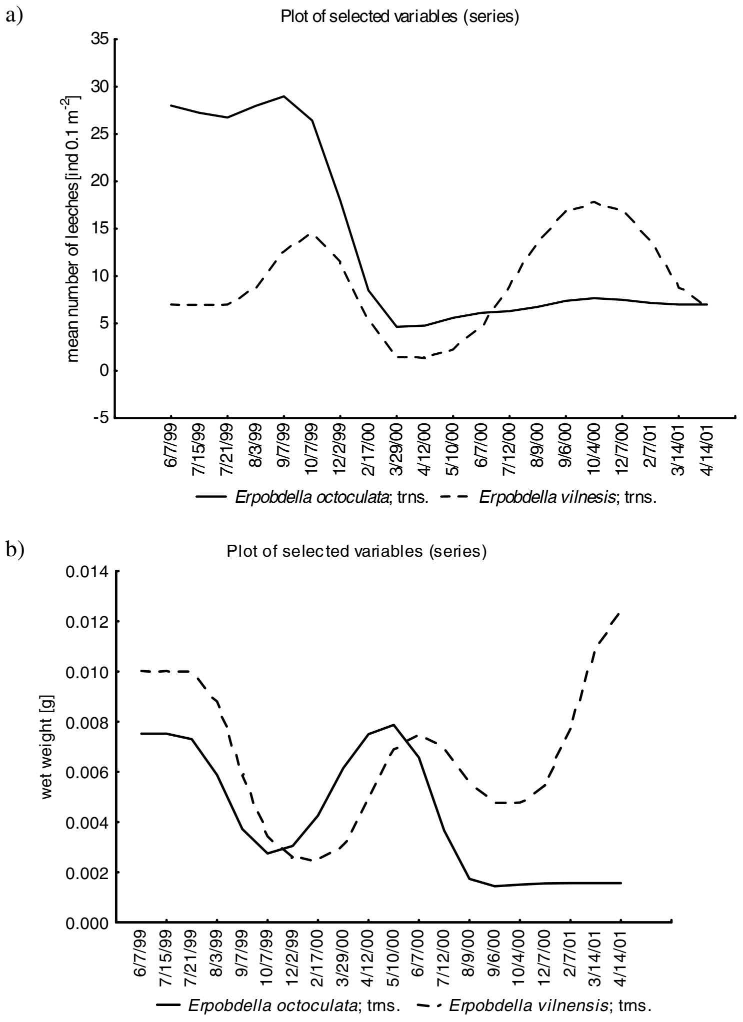

Figure 1. Seasonal changes in mean densities (a) and mean wet weights (b) of *E. vilnensis* and *E. octoculata.*

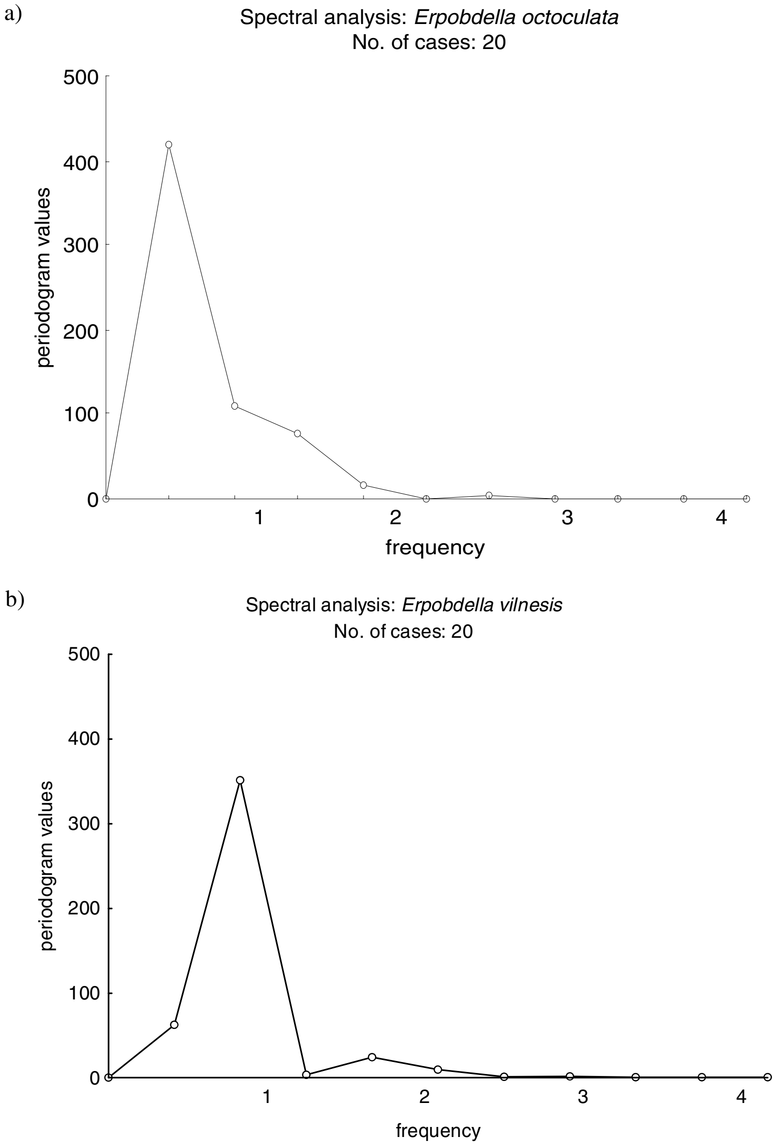

Figure 2. Periodograms of *E. octoculata* (a) and *E. vilnensis* (b).

erations to be distinguished: year class 1998 (individuals born in 1998 and dying in spring and summer 1999), year class 1999 and year class 2000. Mean wet weights of distinct year-classes are given for *E. octoculata* (Fig. 4) and *E. vilnensis* (Fig. 5). Though a one-year life cycle for the latter species was not confirmed by spectral analysis, all adults died after breeding and hence the reproduction was probably also annual.

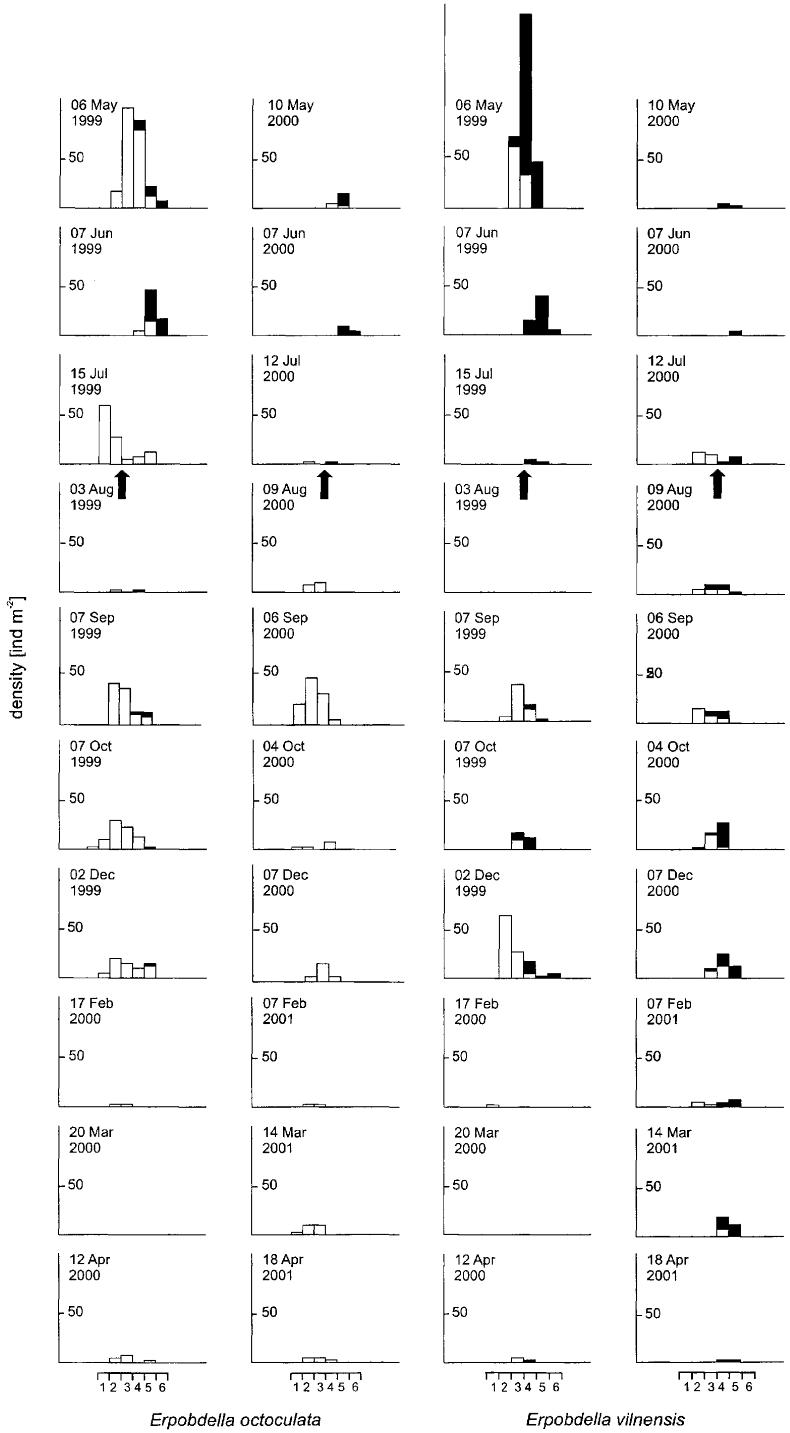

Figure 3. Frequency histograms of mean densities of *E. octoculata* and *E. vilnensis*. For ranges of weight categories see Table 4. Black arrows indicate the borders between year classes.

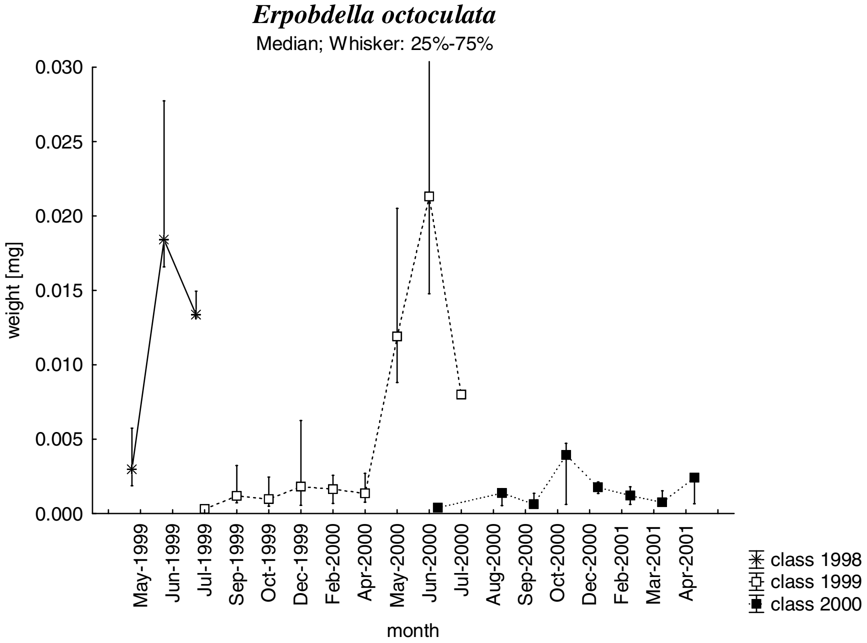

Figure 4. Mean densities (with 25 and 75% quartile) of the 1998, 1999 and 2000 year-classes of *E. octoculata* during the study period.

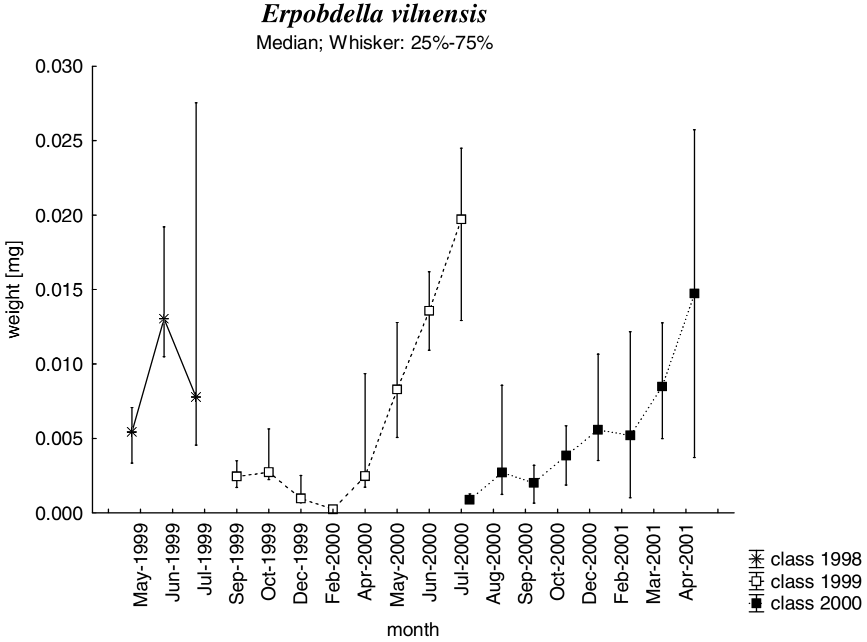

Figure 5. Mean densities (with 25 and 75% quartile) of the 1998, 1999 and 2000 year-classes of *E. vilnensis* during the study period.

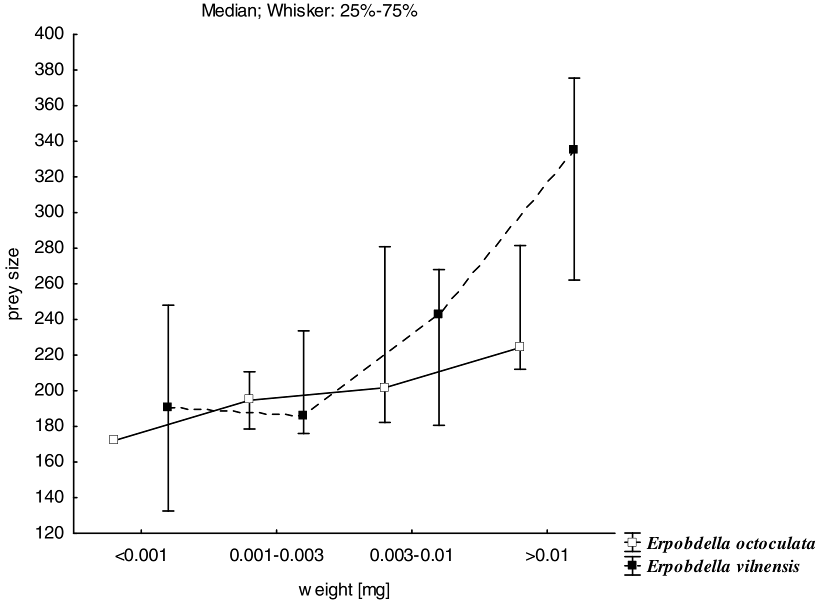

Figure 6. Mean prey size for individual weight categories of *E. octoculata* and *E. vilnensis*.

3.3. Gut Content Analysis

In the guts of 48 individuals of *E. octoculata* and 51 individuals of *E. vilnensis*, we identified 202 individual specimens of invertebrate of 15 taxa of Oligochaeta, 14 of Chironomidae and a single taxon each of Crustacea, Ephemeroptera and Trichoptera (Table 5). Oligochaeta and Chironomidae were the dominant components of the food. A Bray-Curtis similarity index was computed and the food composition of the two species had a high similarity of 83.7%. *E. octoculata* was adjudged to be more effective at hunting because the percentage of leeches with empty guts was higher for *E. vilnensis* (21.0%) than for *E. octoculata* (8.4%). However, the mean number of prey per leech was similar 3.8 for *E. octoculata* and 3.3 for *E. vilnensis* (leeches with empty guts not included). A significant Spearman correlation between mean wet weight leeches and their prey size was recorded for both *E. octoculata,* $r_s = 0.507$ and *E. vilnensis*, $r_s = 0.519$ at a significance level of 0.05. Figure 6 shows the increase in prey size with four leech weight categories; the fifth category (the biggest leeches) was omitted because of low sample size. The size of *E. vilnensis* prey seemed to increase more rapidly than that of *E. octoculata.*

4. Discussion

The present study of the coexistence of two sympatric populations of *E. octoculata* and *E. vilnensis* revealed a complex of mechanisms enabling them successfully utilise the same food niche. Based on time series analyses, no evidence of cyclic changes in densities were confirmed for *E. octoculata* and none of the environmental variables tested were shown to influence its life cycle. According to ASTON and BROWN (1975) breeding is regulated by a day-length. In our study, *E. octoculata* seemed to have an annual cycle, as previously recorded by Dall (1983). However, the duration of our study was too short to confirm this. On the other hand the annual life cycle of *E. vilnensis*, as previously recorded by Manoleli (1976), was confirmed, with reproduction significantly stimulated by changes of water temperature, depth and velocity. Increasing temperature in early spring, together with high discharges, trigged the development of the leeches clitella, followed by copulation and the deposition of cocoons. The hatching time was approximately the same in both species – juveniles occurred during July, but the mean wet weights of new-born leeches were slightly higher in *E. octoculata* and increasing more rapidly than for *E. vilnensis*. Juvenile *E. octoculata* were more successful in hunting the prey and they chose habitats with very low or very high flow, which probably increased the variability of the food on offer. This could have caused the one-month shift in the wet weights of the two species, which is another mechanism lowering food competition. Dall (1979) observed similar behaviour in sympatric populations of *E. octoculata* and *E. testacea* (Savigny, 1822) but he recorded that reproduction of *E. testacea* began a month earlier. We found that the size of the leeches' prey is weight-specific (Dall, 1983) in both species. The analysis of leech gut contents showed that their main food was comprised of Oligochaeta and Chironomidae, with an 83% similarity in the taxonspecies composition of their diets which means that they were not selective feeders. Two different life strategies of reproduction were observed. *E. octoculata* as a typical r-strategist produced a huge number of juveniles with high mortality. In all samples combined, juveniles represented 87% of individuals captured. For *E. octoculata,* high fecundity (juveniles per adult) was typical. More than half of the specimens of *E. vilnensis* found were adults and this would remain so even if all unidentified juveniles were of this species. In the conditions pertaining in our study, *E. vilnensis*, produced less juveniles than *E. octoculata*. Hence, *E. vilnensis* must have had a lower mortality resulting in low fecundity for this species. We also observed rapid sexual development of *E. vilnensis*. Specimens of *E. vilnensis* were also recorded in the winter months when individuals of *E. octoculata* were hidden in bottom substratum, suggesting that *E. vilnensis* is better adapted to running waters conditions.

In general, the life strategy of *E. octoculata* is based upon high cocoon production and high food source utilisation, but also on high mortality of juveniles. In contrast, *E. vilnensis* produces lower numbers of cocoons, its juveniles hunt for prey preferentially in mid-current conditions, but with lower success, and its all-year mortality is small. As a result, populations of the two species are able to co-exist to good effect.

5. Acknowledgements

We would like to thank our colleague V. Syrovátka for identifying representatives of the family Chironomidae and J. Gaisler and M. Furse for checking the language and other improvements. The project was supported by Long-term Research Plans from the Ministry of Education, Youth and Sports of the Czech Republic (MSM0021622416 and INCHEMBIOL MSM0021622412).

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Freshwater leeches (Annelida: Clitellata: Hirudinida) of the Czech Republic (Central Europe): check-list, new records, and remarks on species distributions

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Abstract

Freshwater leeches (Annelida: Clitellata: Hirudinida) of the Czech Republic were studied on the basis of recent literature, information in selected databases, and results of recent surveys conducted by the authors. The objectives of this study were to summarize recent taxonomic information, and to update the check-list of leeches using records collected during an eleven-year study (1998 through 2008). Altogether, twenty-four species representing 12 genera and five families are reported for the Czech Republic, including the first reports of *Piscicola* cf. *haranti* Jarry, 1960 and *Dina punctata* Johansson, 1927 for the country. A detailed description of the distributions of rare species and characterization of localities from which they are reported are presented. Plausible modes of dispersal and propagation of species in Central Europe are discussed.

According to recent records, leech species are divided into three groups: indigenous species with stable and strong populations (12 species), indigenous species with weak populations known from a limited number of localities (9 species), and species only recently recorded in the Czech Republic (3 species). For scarce or rare species, a category of threat in the Czech Republic according to the International Union for Conservation of Nature is recommended. Most of threatened species are inhabitants of lowland wetlands and lowland larger rivers—habitats that are negatively impacted, often catastrophically, by human activities throughout Central Europe. The protection of suitable habitats is the most effective way to protect extant populations of endangered leech species. Six species of leeches are recommended for permanent addition to the Red list of threatened species in the Czech Republic.

Key words: Annelida, Clitellata, Hirudinida, leeches, Glossiphoniidae, Piscicolidae, Haemopidae, Hirudinidae, Erpobdellidae, Czech Republic, IUCN, Red List, habitat, distribution, *Piscicola* cf. *haranti*, *Dina punctata*, first record

Introduction

A check-list of freshwater leeches (Hirudinida) of the Czech Republic has long been in need of revision. Available information on the distribution, taxonomy, and ecology of leeches in the Czech Republic has been scattered throughout various historical as well as recent papers, but none of these have focused on the country's fauna as a whole.

Based on molecular phylogeny, leeches (Hirudinida), Branchiobdellida, and Acanthobdellida form a monophyletic clade—with its common ancestor being an oligochaetous clitellate related to the family Lumbriculidae (Siddall *et al.* 2001, Erséus 2005). While most leeches in the Czech Republic are thermophilous freshwater species inhabiting stagnant as well as running waters, a few species are considered to be amphibious. Leeches are common in eutrophic waters and often serve as indicators of pollution (e.g., Sládeček & Košel 1984, Košel 1988, Grosser *et al.* 2001, Koperski 2005). Although some of them have been

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studied extensively, (e.g., the medicinal leech, *Hirudo medicinalis* Linnaeus, 1758), research focusing on the taxonomy and ecology of leeches occurring in the Czech Republic has been limited.

Several noteworthy papers presenting distributional information on leeches in the Czech Republic have been published over the last 55 years: Hrabě (1954) recorded eleven leech species in his key to fauna; Švec (1960) found only six species during a faunal study of southeastern Moravia, but one was a new record (*Alboglossiphonia hyalina* (O. F. Müller 1774)—considered as a "form" at the time of Švec's publication); Lucký & Dyk (1964) recorded four species of leeches during their study of fish parasites in rivers and ponds in Odra (Oder) and Dyje (Thaya) River catchment area, including a new record of *Piscicola fasciata* Kollar, 1842 (as *Cystobranchus fasciatus* Brumpt, 1900) for the Czech Republic; and Koubková & Vojtková (1973) reported eleven species in their publication on the fauna of the Czechoslovak Socialist Republic. More recently, Košel (1998 and 1999a) recorded nineteen and twenty species, respectively (Table 1). Unfortunately, the record of *P. fasciata* was not included in the check-list (Košel 1998). Although, most of these papers were published in the Czech or Slovak language, none provided a thorough review of the leech fauna known or thought likely to occur in the country.

The objectives of this study are to summarize historical, recently published, and unpublished records of leeches occurring in the Czech Republic, to present data resulting from our recently completed surveys for leeches throughout the country (1998–2008), and to provide a current check-list of Hirudinida occurring in the Czech Republic.

Methods and materials

The information and data summarized herein have been obtained from following sources: recent literature records (Košel 1999a, Sukop 2004, Košel & Beran 2006); records in PERLA, a database prepared for the assessment of running waters in the Czech Republic (Kokeš *et al.* 2006); records in a database of monitoring of running waters provided by the T. G. Masaryk Water Research Institute (monitoring of reference conditions, surveillance monitoring, Project Labe (Elbe) (Horecký *et al.* 2004), research projects and others); records obtained during the monitoring of *Hirudo medicinalis* carried out by the authors and organized by the Agency for Nature Conservation and Landscape Protection of the Czech Republic 2005–2008; records and information included in the diploma thesis of N. Kubová (Kubová 2008); and data resulting from recent collections by the authors, colleagues, and other personnel.

1. Košel (1999a), Sukop (2004), Košel & Beran (2006), Kubová (2008) and authors collected leeches with tweezers and with hand strainers (mesh size 1 mm) from solid submerged inorganic and organic substratum, e.g., gravel, stones, woody debris and vegetation. Leeches were then narcotized by graduating concentration of ethanol to final 70%, and then fixed in 4% formaldehyde.

2. PERLA database and monitoring of running waters (1998–2008). Leeches were collected with a hand net (mesh size 500 µm) during a 3-minute kick sampling period from running waters. All habitats (e.g., riffle, pool, macrophytes, and woody debris) were sampled proportionally. Leeches were then fixed in 4% formaldehyde.

3. Project Labe (1999–2005). Leeches were collected during a 3-min kick sampling period using a hand net (mesh size 500 µm), which was supplemented by qualitative sampling from submerged substratum and in-situ picking of individuals from additional material taken by hand net during extended sampling and sorting procedure. Samples were preserved in 80% ethanol.

All leech specimens collected by these methods were identified in a laboratory under a stereomicroscope according to the keys of Neubert & Nesemann (1999) and Košel (2001).

4. Monitoring of *Hirudo medicinalis* (2005–2008). Specimens were collected by hand, removing from both gumboots (waders) or from the bare legs of the collectors.

Maps noting the distributions of leeches in the Czech Republic (with Bohemia to the west, and Moravia to the east, see Figure 1) were prepared for most species discussed in this paper, with the exception of those commonly collected throughout the country.

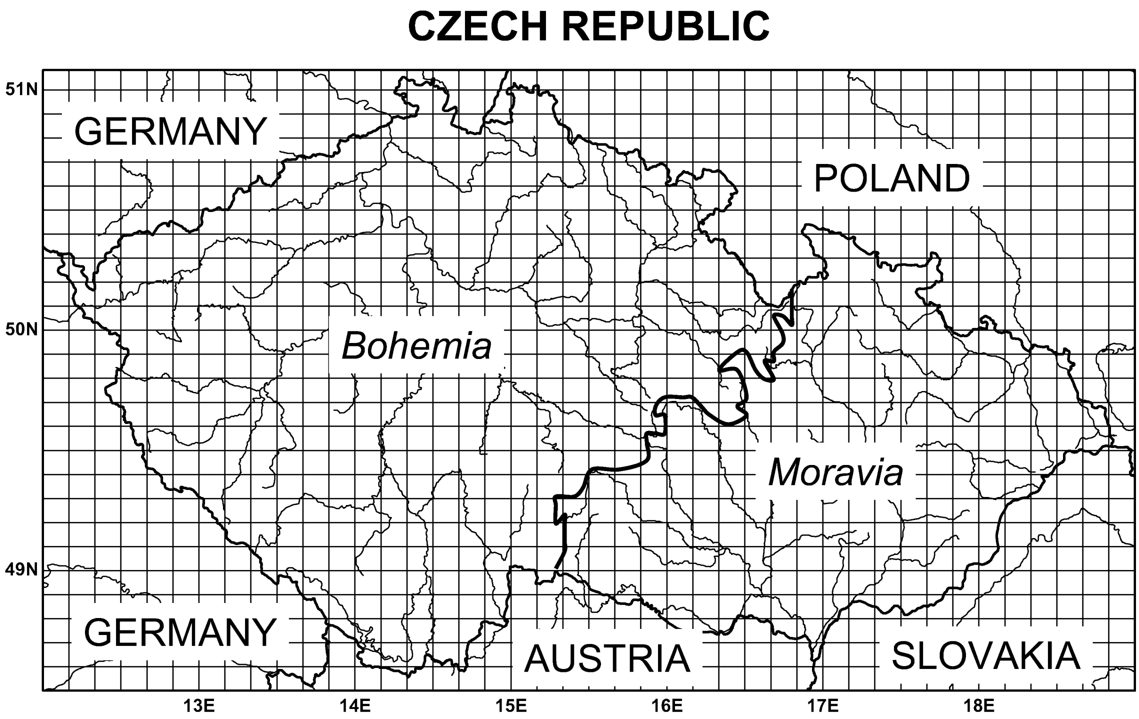


FIGURE 1. The geographical position of the Czech Republic in central Europe, including historical divisions within the country—Bohemia (Čechy) to the west, and Moravia (Morava) to the east.

Unpublished records of selected species are presented in the accounts, below, and (if available) include the following information associated with specimens: name of the water body, name of the settlement (town, village, city) closest to the site from which the specimen(s) was/were collected, index number [in brackets] of Central European map square (Pruner & Míka 1996) on which the collection site can be found, latitude and longitude coordinates for the collecting site, name(s) of the person(s) who collected and/or identified the species (collectors' names, abbreviated in the accounts below), and date of collection. Published records are presented in the same way, and include a valid citation. Typical habitats and species distribution in the Czech Republic are briefly described. For scarce or rare species, a category of threat in the Czech Republic according to the International Union for Conservation of Nature (IUCN) (1994) is recommended.

Abbreviations for the names of collectors associated with the species accounts presented below: A—anonymous, EP—Eva Podborská, FK—František Kubíček, GČ—Gašpar Čamlík, IS—Ivan Skála, JB—Jindřiška Bojková, JH—Jakub Horecký, JK—Jiří Kroča, JP—Jiří Procházka, JR—Jan Rucki, JSc—Jana Schenková, JSy—Jan Sychra, JŠ—Jan Špaček, KK—Kateřina Kolářová, MB—Milan Boukal, ML—Marek Liška, MS—Michal Straka, MŠ—Monika Štambergová, NK—Nela Kubová, NL—Natália Lapšanská, PK—Petr Komzák, PP—Petr Pařil, SV—Stanislav Větříček, VK—Vladimír Košel.

Results and species accounts

In total, twenty-four species of Hirudinida representing five families (Table 1) are now reported to occur in the Czech Republic based upon records in the literature and/or specimens collected during our eleven-year study (1998 through 2008). Detailed accounts for leeches occurring in the Czech Republic are presented below.

TABLE 1. Historical lists of leeches (Annelida, Clitellata, Hirudinida) and a new check-list of Hirudinida occurring in the Czech Republic, based upon published and unpublished records, and specimens from collections by the authors during the years 1998 through 2008.

	Hrabě (1954), Koubková & Vojtková (1973)	Švec (1960)	Lucký & Dyk (1964)	Košel (1998)	Košel (1999a)	this study (1998– 2008)
Family: Glossiphoniidae						
Alboglossiphonia heteroclita (Linnaeus, 1761)	+			+	+	+
Alboglossiphonia hyalina (O. F. Müller, 1774)		+			+	+
Alboglossiphonia striata (Apáthy, 1888)					+	+
Batracobdella paludosa (Carena, 1824)					misidentified	
Glossiphonia complanata (Linnaeus, 1758)	+			+	+	+
Glossiphonia concolor (Apáthy, 1888)				+	+	+
Glossiphonia nebulosa Kalbe, 1964				+	+	+
Glossiphonia slovaca (Košel, 1973)				+	+	+
Helobdella stagnalis (Linnaeus, 1758)	+	+		+	+	+
Hemiclepsis marginata (O. F. Müller, 1774)	+		+	+	+	+
Theromyzon tessulatum (O. F. Müller, 1774)	+			+	+	+
Family: Piscicolidae						
Caspiobdella fadejewi (Epshtein, 1961)				+	+	+
Piscicola geometra (Linnaeus, 1758)	+	+	+	+	+	+
Piscicola cf. *haranti* Jarry, 1960						+
Piscicola fasciata Kollar, 1842			+			
Piscicola respirans Troschel, 1850			+	+		+
Family: Haemopidae						
Haemopis sanguisuga (Linnaeus, 1758)	+	+		+	+	+
Family: Hirudinidae						
Hirudo medicinalis Linnaeus, 1758	+			+	+	+
Family: Erpobdellidae						
Dina lineata (O.F. Müller, 1774)	+	+		+	+	+
Dina punctata Johansson, 1927						+
Erpobdella nigricollis (Brandes, 1900)				+	+	+
Erpobdella octoculata (Linnaeus, 1758)	+	+		+	+	+
Erpobdella testacea (Savigny, 1822)	+			+	+	+
Erpobdella vilnensis (Liskiewicz, 1925)				+	+	+
Trocheta cylindrica Örley, 1886				+	+	+

Phylum: Annelida

Class: Clitellata

Order: Hirudinida

Family: Glossiphoniidae

Alboglossiphonia heteroclita (Linnaeus, 1761)

Habitats and status: The species prefers lowland vegetated pools or slow running waters with riparian vegetation. Because of both morphological and genetic differences, two new species—*A. hyalina* and *A. striata* —were recently separated from this taxon (Trontelj 1997, Neubert & Nesemann 1999). Unfortunately, only some of the older records are distinguished into forms 'heteroclita', 'hyalina' and 'striata', thus it is not possible to separate their habitat preferences at this time. *Alboglossiphonia heteroclita* seems to be the most common species occurring in the whole territory of the Czech Republic except at higher elevations (only rarely over 500 m a.s.l.).

Alboglossiphonia hyalina (O. F. Müller, 1774)

Records: Chabařovice artifical lake, Chabařovice [5349], *50°39'05''N / 13°57'11''E*, lgt. JŠ, det. JŠ (2005, 2006); concrete fire reserve, Jestřebice [5553], *50°27'49''N / 14°34'36''E* (Košel & Beran 2006); Harasov Pond, Janova Ves [5553] *50°24'43''N / 14°34'18''E*, (Košel & Beran 2006); irrigation ditch, Rakvice [7166], *48°49'45''N / 16°48'58''E*, (Košel 1999a); Kutnar Pond, Rakvice [7166], *48°50'11''N / 16°47'34''E*, lgt. NK, det. NK (2008); Milotický Pond, Milotice [7068], *48°57'52''N / 17°09'17''E*, lgt. JSy, det. JSy (2008); Štěpánek pond, Pozďatín [6762], *49°13'26''N / 16°02'19''E*, lgt. JSy (2006), det. JSy (2008); Tužínský Brook, Dřevěnice [5558], *50°26'33''N / 15°27'14''E*, lgt. JŠ, det. JŠ (2008) (Figure 2).

Habitats: The species was found in heterogeneous stagnant or slow-running waters, often of artificial origin, and all rich in organic material. The presence of submerged vegetation and aquatic snails also seems to be important.

Status: *Alboglossiphonia hyalina* is a rare species with only few localities from southern Moravia, Czech-Moravian Highland and northern Bohemia. These records suggest that this leech is probably more common in suitable habitats, most likely overlooked in previous collections due to its small size and translucent body.

Alboglossiphonia striata (Apáthy, 1888)

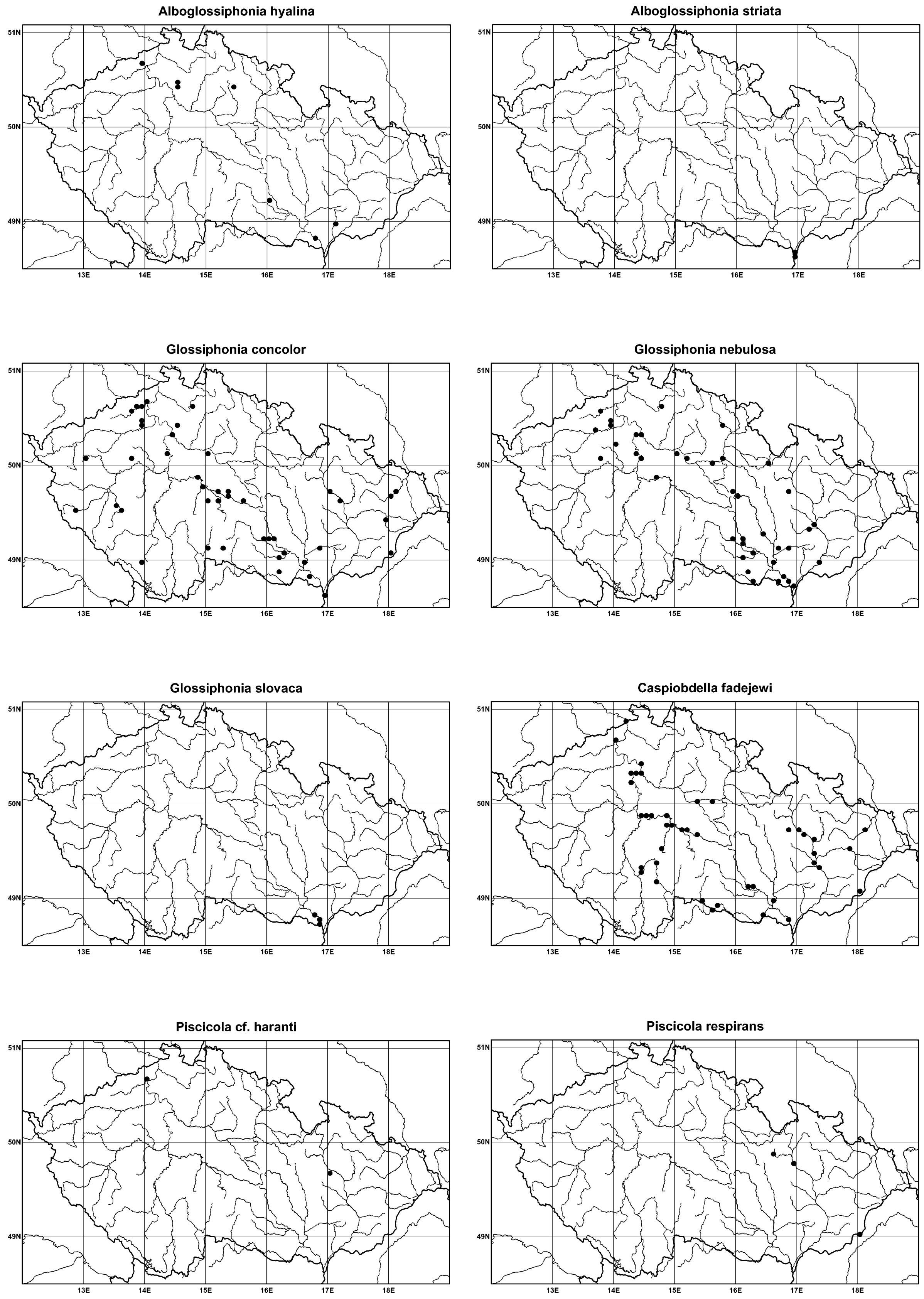
Records: Bažinka Pool, Lanžhot [7367], *48°38'05''N / 16°55'55''E*, lgt. JSc, det. JSc (2006); pool, Ranšpurk [7367], *48°40'27''N / 16°56'23''E*, (Košel 1999a) (Figure 2).

Habitats: The species was recorded in natural lowland pools with aquatic vegetation. Its ecology is still not well known.

Status: *Alboglossiphonia striata* is the rarest species in the genus known to occur in the Czech Republic, to date having been recorded only in southernmost Moravia. However, it could have been confused with or misidentified as *A. heteroclita* in previous studies.

Glossiphonia complanata (Linnaeus, 1758)

Habitats and status: *Glossiphonia complanata* represents a widely distributed species recorded from all kinds of water bodies, stagnant as well as running, with the exception of fast flowing mountain rivers and brooks. It is common throughout the Czech Republic. In past studies, this species was likely confused with or misidentified as one or more other *Glossiphonia* species because of its variable coloration.



Alboglossiphonia hyalina

Alboglossiphonia striata

Glossiphonia concolor

Glossiphonia nebulosa

Glossiphonia slovaca

Caspiobdella fadejewi

Piscicola cf. haranti

Piscicola respirans

FIGURE 2. The distribution of selected leech species (Annelida, Clitellata, Hirudinida: the families Glossiphoniidae and Piscicolidae) in the Czech Republic recorded during the years 1998 through 2008, based upon published and unpublished records, and specimens collected during this study.

Glossiphonia concolor (Apáthy, 1888)

Records: Bílina River, Bílina [5448], *50°33'17"N / 13°46'25"E*, lgt. JSy, det. JSy (2007); Bílina River, Kozlíky [5355], *50°36'41"N / 13°52'59"E*, lgt. JSy, det. JSy (2007); Bílina River, Lbín [5349], *50°36'20"N / 13°51'32"E*, lgt. JSy, det. JSy (2007); Bílina River, Stadice [5349], *50°37'08"N / 13°58'02"E*, lgt. JSy, det. JSy (2007); Bílina River, Ústí nad Labem [5350], *50°39'27"N / 14°02'28"E*, lgt. A, det. NK (2006); Blanice River, Nový Mlýn [6255], *49°46'40"N / 14°55'53"E*, lgt. A, det. FK (1998); Blažejovický Brook, Blažejovice [6357], *49°36'46"N / 15°12'19"E*, lgt. JH, NL, det. ML (2007); Bolíkovský Brook, Heřmaneč [6857], *49°07'07"N / 15°18'24"E*, lgt. A, det. JSc (2007); Břevnický Brook, Břevnice [6359], *49°38'01"N / 15°35'36"E*, lgt. A, det. FK (1998); Častava Brook, Chomoutov [7369], *49°37'54"N / 17°13'59"E*, lgt. JSy, det. JSy (2008); Čechtický Brook, Křivsoudov [6356], *49°38'26"N / 15°04'40"E*, lgt. JH, NL, det. JH (2007); Černý Brook, Meclov [6443], *49°30'36"N / 12°53'16"E*, lgt. A, det. FK (1999); Dlažkovice Pond, Dlažkovice [5549], *50°27'53"N / 13°58'02"E*, lgt. NK, det. NK (2007); Dolní Bartošovický Pond, Bartošovice [6374], *49°40'52"N / 18°01'45"E*, lgt. JSy, det. JSy (2005); Harasov Pond, Janova Ves [5553], (Košel & Beran 2006); Hranečník Pond, Častotice [6762], *49°14'03"N / 16°04'24"E*, lgt. JSy, NK, JSc, det. JSc (2008); Jihlava River, Vladislav [6761], *49°12'24"N / 15°59'49"E*, lgt. A, det. NK (2006); Káča Brook, Hořátev [5856], *50°08'33"N / 15°02'25"E*, lgt. A, det. JSy (2006); Kozárovický Brook, Zálezlice [5652], *50°18'35"N / 14°25'29"E*, lgt. A, det. JSy (2006); Křišťanovický Pond, Křišťanovice [7049], *48°58'05"N / 13°56'45"E*, lgt. JSy, det. JSy (2007); Lišanský Brook, Rakovník [5948], *50°05'35"N / 13°45'06"E*, lgt. JH, NL, det. JH (2007); Morava River, Nové Zámky [6268], *49°42'58"N / 17°01'28"E*, lgt. A, det. JSc (2007); Nový Častotický Pond, Častotice [6762], *49°14'09"N / 16°05'57"E*, lgt. JSy, det. PP (2002), lgt. NK, JSc, JSy, det. JSc (2008); Odra River, Petřvaldík [6274], *49°43'30"N / 18°07'53"E*, lgt. PK, SV, det. JSc (2007); Panenská Pool, Nové Mlýny [7166], *48°50'41"N / 16°43'40"E*, (Košel 1999a); Ploužnický Brook, Ploužnice pod Ralskem [5354], *50°38'10"N / 14°48'30"E*, lgt. A, det. JSy (2006); Podhrázský Pond, Lkáň [5549], *50°26'55"N / 13°56'57"E*, lgt. NK, det. NK (2007); Pstružný Brook, Františkodol [6358], *49°39'22"N / 15°22'58"E*, lgt. PK, SV, det. FK, JSc (1998, 2007); Rakovec Brook, Hrušky [6867], *49°07'44"N / 16°50'24"E*, lgt. PK, det. PK (2007); Rokytná River, Moravský Krumlov [6963], *49°03'45"N / 16°19'31"E*, (Schenková *et al.* 2007); Rokytná River, Vémyslice [6963], *49°02'06"N / 16°11'47"E*, lgt. PK, SV, det. JSc (2007); Řečička Brook, Jindřichův Hradec [6856], *49°07'22"N / 15°00'02"E*, lgt. JH, NL, det. JH (2007); Sázava River, Sázava [6155], *49°53'09"N / 14°52'26"E*, lgt. A, det. FK (1998); Sázava River, Soběšín [6255], *49°47'55"N / 14°57'19"E*, lgt. A, det. FK (1998); Sázava River, Vlastějovice [6257], *49°44'07"N / 15°10'45"E*, lgt. A, det. FK (1998); Sázavka River, Sázavka [6258], *49°44'11"N / 15°23'15"E*, lgt. A, det. FK (1998); Sekulská Morava (Morava River dead arm), Lanžhot [7367], *48°37'39"N / 16°57'12"E*, lgt. JSy, NK, JSc, det. JSy (2008); Skalička Brook, Práče [7163], *48°53'03"N / 16°12'56"E*, lgt. SV, PK, det. PK (2007); Střela River, Žlutice [5944], *50°05'45"N / 13°04'11"E*, lgt. A, det. FK (1999); Šatava River, Nosislav [7065], *48°59'33"N / 16°38'48"E*, lgt. A, det. JSy (2006); Únětický Brook, Únětice [5852], *50°08'56"N / 14°21'33"E*, lgt. JH, NL, det. JH (2007); Úslava River, Vilémov [6447], *49°32'40"N / 13°36'08"E*, lgt. A, det. FK (1999); Úslava River, Zdemyslice [6447], *49°35'58"N / 13°31'51"E*, lgt. A, det. FK (2000); Vlára River, Svatý Štěpán [6974], *49°03'18"N / 18°01'19"E*, lgt. PK, SV, det. JSc (2007); Vltava River, Praha [5952], *50°03'21"N / 14°24'60"E*, lgt. JH, NL, det. JH (2002, 2005), Vltava River, Zelčín [5652], *50°19'09"N / 14°23'56"E*, lgt. JH, NL, det. JH (2002); Vsetínská Bečva River, Bystřička [6573], *49°25'09"N / 17°57'10"E*, lgt. PK, SV, det. JSc (2007) (Figure 2).

Habitats: *Glossiphonia concolor* prefers especially stagnant waters or slowly running rivers or brooks. It occurs mainly in lowlands and moderate elevations (150–500 m, rarely up to 750 m a.s.l.).

Status: *Glossiphonia concolor* can be locally abundant. With the exception of the boundary mountain areas, *G. concolor* is distributed throughout the entire Czech Republic.

Glossiphonia nebulosa Kalbe, 1964

Records: Bačovka Brook, Velký Osek [5957], *50°05'43"N / 15°12'46"E*, lgt. A, det. JSy (2006); Bílina River, Bílina [5448], *50°33'17"N / 13°46'25"E*, lgt. JSy, det. JSy (2007); Brodečka River, Víceměřice [6669], *49°20'35"N /17°10'29" E*, lgt. SV, PK, det. PK (2007); Chomutovka Brook, Postoloprty [5648], *50°21'52"N / 13°42'10"E*, lgt. JSy, det. JSy (2008); ditch, Lanžhot [7267], *48°42'42"N / 16°56'27"E*, lgt. MS, det. JSy (2006); Dlažkovice Pond, Dlažkovice [5549], *50°27'53"N / 13°58'02"E*, lgt. NK, det. NK (2007); Dyje River, Břeclav [7166], *48°48'40"N / 16°48'59"E*, (Košel 1999a); Dyje River, Břeclav [7267], *48°46'22"N / 16°52'43"E*, (Košel 1999a); Jihlava River, Vladislav [6761], *49°12'24"N / 15°59'49"E*, lgt. A, det. NK (2006); Káča Brook, Hořátev [5856], *50°08'33"N / 15°02'25"E*, lgt. A, det. JSy (2006), Kozárovický Brook, Zálezlice [5652], *50°18'35"N / 14°25'29"E*, lgt. A, det. JSy (2006); Labe River, Němčice [5960], *50°05'38"N / 15°48'34"E*, lgt. JH, NL, det. JH (2005); Labe River, Valy [5959], *50°01'58"N / 15°37'05"E*, lgt. JH, NL, det. VK (2003); Labe River, Verdek [5560], *50°26'47"N / 15°47'05"E*, lgt. JH, NL, det. JH (2002, 2003); Lišanský Brook, Rakovník [5948], *50°05'35"N / 13°45'06"E*, lgt. JH, NL, det. JH (2007); Litava River, Vážany nad Litavou [6867], *49°07'47"N / 16°51'02"E*, lgt. SV, PK, det. PK (2007); Mlýnská strouha drain, Jaroslavice [7263], *48°45'44"N / 16°15'06"E*, lgt. SV, PK, det. PK (2007); Mnichovka Brook, Hrušov [6154], *49°53'36"N / 14°44'06"E*, lgt. JH, NL det. JH (2006); Návesník Pond, Vortová [6261], *49°42'46"N / 15°55'43"E*, lgt. JSy, det. JSy (2007); Nový Častotický Pond, Častotice [6762], *49°14'09"N / 16°05'57"E*, lgt. JSy, det JSy (2006); Nový Pond, Mikulov [7266], *48°46'55"N / 16°40'32"E*, (Košel 1999a); Okluky Brook, Uherský Ostroh [7070], *48°59'37"N / 17°24'10"E*, lgt. SV, PK, det. PK (2007); Oslava River, Náměšť nad Oslavou [6862], *49°11'55"N / 16°08'58"E*, lgt. A, det. EP (2007); Ploužnický Brook, Ploužnice pod Ralskem [5354], *50°38'10"N / 14°48'30"E*, lgt. A, det. JSy (2006); Podhrázský Pond, Lkáň [5549], *50°26'56"N / 13°56'57"E*, lgt. NK, det. NK (2007); Rakovec Brook, Hrušky [6867], *49°07'44"N / 16°50'24"E*, lgt. PK, det. PK (2007); Rokytná River, Moravský Krumlov [6963], *49°03'45"N / 16°19'31"E*, (Schenková *et al.* 2007); Rouchovanka Brook, Rešice [6962], *49°02'50"N / 16°08'47"E*, lgt. SV, PK, det. PK (2007); Říčka River, Sokolnice [6866], *49°06'32"N / 16°43'16"E*, lgt. SV, PK, det. PK (2007); Sendražický Brook, Velký Osek [5957], *50°05'26"N / 15°12'10"E*, lgt. A, det. JSy (2006); Skalička Brook, Práče [7163], *48°53'03"N / 16°12'56"E*, lgt. SV, PK, det. PK (2007); Svratka River, Křižánky [6362], *49°41'52"N / 16°03'39"E*, lgt. SV, PK, det. PK (2007); Svratka River, Veverská Bitýška [6764], *49°16'45"N / 16°26'15"E*, lgt. SV, PK, det. PK (2006); Šatava River, Nosislav [7065], *48°59'33"N / 16°38'48"E*, lgt. A, det. JSy (2006); Štemberský Brook, Slaný [5750], *50°13'29"N / 14°04'48"E*, lgt. JH, NL, det. JH (2008); Tichá Orlice River, Kunčice [5965], *50°00'17"N / 16°32'44"E*, lgt. A, det. JSc (2007); Třebůvka River, Loštice [6267], *49°43'21"N / 16°53'48"E*, lgt. PK, SV, det. JSc (2007); Únětický Brook, Únětice [5852], *50°08'56"N / 14°21'33"E*, lgt. JH, NL, det. JH (2007); Valová Brook, Polkovice [6669], *49°23'42"N / 17°15'51"E*, lgt. JSy, det. JSy (2008); Vltava River, Praha Podolí [5952], *50°03'21"N / 14°24'560"E*, lgt. JH, NL, det. JH (2002) VK (2003); Vltava River, Zelčín [5652], *50°19'09"N / 14°23'56"E*, lgt. JH, NL, det. JH (2002, 2005) (Figure 2).

Habitats: *Glossiphonia nebulosa* is considered typical of running waters, but some of the records are from ponds at middle and higher elevations (about 600 m a.s.l.). Its ecological valences are probably broader than have been documented to date.

Status: Prior to this study, *Glossiphonia nebulosa* had been recorded only from two localities in the southernmost part of the country (Košel 1999a); more recently, this species has been reported as a common species from additional localities in both Bohemia and Moravia. Most of localities are situated in southern Moravia (Dyje (Thaya), Svratka, and Jihlava River basins) and middle Bohemia (Labe (Elbe), Vltava, and Ohře River basins); no records have yet been reported from the southwestern part of the country. The species was probably overlooked in previous studies, and perhaps may have been confused with or misidentified as *G. complanata* in past studies.

Glossiphonia slovaca (Košel, 1973)

Synonym: *Batracobdella slovaca* Košel, 1973

Records: Dyje River, Břeclav [7166], *48°48'40"N / 16°48'59"E*, (Košel 1999a); Dyje River, Břeclav [7267], *48°46'22"N / 16°52'43"E*, (Košel 1999a); Dyje River, Břeclav [7267], *48˚43'25"N / 16˚53'07"E*, lgt. PK (2006), det. PK (2008) (Figure 2).

Habitats: Limited information suggests that *Glossiphonia slovaca* occurs in larger lowland rivers.

Status: Both records of this species in the Czech Republic were collected from Dyje River in the southernmost part of the country, which limit its northern range margin (Grosser & Nesemann 2004). Because of its scarceness, *G. slovaca* was classified as vulnerable in the Red list of threatened species in the Czech Republic (Schenková & Košel 2005).

Helobdella stagnalis (Linnaeus, 1758)

Habitats and status: *Helobdella stagnalis* is a very common species throughout the Czech Republic, especially in stagnant and slowly running waters. Most of the records are from eutrophic water bodies; it is absent only in brooks at the highest elevations in the country (over 800 m a.s.l.).

Hemiclepsis marginata (O. F. Müller, 1774)

Habitats and status: *Hemiclepsis marginata* is relatively abundant in stagnant water bodies, and usually those with aquatic vegetation occurring in lowlands and mid-elevations (150–450 m a.s.l.). It is less frequent in slowly running waters. Its distribution is scattered throughout the Czech Republic.

Theromyzon tessulatum (O. F. Müller, 1774)

Habitats and status: *Theromyzon tessulatum* is a locally abundant leech occurring in stagnant water bodies with aquatic vegetation or in larger rivers and slowly running brooks. Its distribution is scattered throughout the Czech Republic.

Family: Piscicolidae

Caspiobdella fadejewi (Epshtein, 1961)

Synonym: *Piscicola fadejewi* Epshtein, 1961

Records: Bakovský Stream, Vepřek [5651], *50°18'18"N / 14°18'58"E*, lgt. JH, NL, det. JH (2005); Bečva River, Choryně [6473], *49°30'12"N / 17°53'43"E*, lgt. PK, SV, det. JSc (2007); Bílina River, Ústí nad Labem [5350], *50°39'28"N / 14°02'31"E*, lgt. A (2007), det. JSy (2008); Blanice River, Mladá Vožice [6454], *49°32'52"N / 14°49'02"E*, lgt. JH, NL, det. NL (2007); Blanice River, Radonice [6155], *49°47'20"N / 14°55'58"E*, lgt. JH, NL, det. JH, NL (2004, 2005, 2007); Doubrava River, Záboří nad Labem [5958], *50°01'34"N / 15°20'40"E*, lgt. JSy, det. JSy (2008); Dyje River, Břeclav [7267], *48°46'22"N / 16°52'43"E* (Košel 1999a); Dyje River, Jevišovka [7164], *48°49'41"N / 16°28'24"E*, lgt. A (2007), det. JSy (2008); Dyje River, Podhradí [7159], *48°53'29"N / 15°38'30"E*, lgt. JŠ, det. JŠ (2008); Chotýšanka Brook, Libež [6255], *49°45'32"N / 14°54'53"E*, lgt. JH, NL, det. NL (2006); Dyje River, Podhradí [7159], *48°53'29"N /*

15°38'30''E, lgt. PK, SV, det. JSc (2007); Dyje River, Podhradí [7060], *48°54'12''N / 15°41'21''E*, lgt. A (2007), det. JSy (2008); Haná River, Bezměrov [6670], *49°19'53''N / 17°20'18''E*, lgt. JSy, det. JSy (2008); Jevanský Brook, Stříbrná Skalice [6155] *49°53'22''N / 14°51'26''E*, lgt. JH, NL, det. JH (2006); Kozský Brook, Sezimovo Ústí [6654], *49°22'57''N / 14°41'38''E*, lgt. JH, NL, det. JH (2006); Labe River, Hřensko [5151], *50°52'28''N / 14°14'08''E*, lgt. JSy, det. JSy (2008); Labe River, Liběchov [5552], *50°24'08''N / 14°26'53''E*, lgt. A (2007), det. JSy (2008); Labe River, Valy [5959], *50°01'59''N / 15°37'00''E*, lgt. A (2007), det. JSy (2008); Lužnice River, Bechyně [6752], *49°17'21''N / 14°28'11''E*, lgt. PP, det. JSc, JSy (2007, 2008); Lužnice River, Dobronice [6652], *49°19'07''N / 14°29'36''E*, lgt. A, det. JSc (2007); Lužnice River, Veselí nad Lužnicí [6854], *49°10'41''N / 14°41'51''E*, lgt. A (2007), det. JSy (2008); Morava River, Černovír [6369], *49°36'44''N / 17°15'14''E*, lgt. A (2007), det. JSy (2008); Morava River, Lhota [6368], *49°40'59''N / 17°08'59''E*, lgt. A, det. JSc (2007); Morava River, Lobodice [6669], *49°23'48''N / 17°18'25''E*, let. PK, SV, det JSc (2007); Morava River, Nové Zámky [6268], *49°42'57''N / 17°01'27''E*, lgt. A, det. JSc (2007); Morava River, Větrovany [6569], *49°27'55''N / 17°18'19''E*, lgt. JSy, det. JSy (2008); Moravská Dyje River, Písečné u Slavonic [7058], *48°57'48''N / 15°27'50''E*, lgt. A (2007), det. JSy (2008); Nežárka River, Veselí nad Lužnicí [6854], *49°10'53''N / 14°42'33''E*, lgt. A (2007), det. JSy (2008); Nučický Brook, Stříbrná Skalice [6155], *49°52'58''N / 14°51'33''E*, lgt. JH, NL, det. JH (2007); Odra River, Petřvaldík [6274], *49°43'30''N / 18°07'53''E*, lgt. PK, SV, det. JSc (2007); Oslava River, Nová Ves [6863], *49°07'21''N / 16°18'47''E*, lgt. PK, SV, det. JSc (2007); Oslava River, Skřípina [6863], *49°08'27''N / 16°12'30''E*, lgt. PK, SV, det. JSc (2007); Ostrovský Brook, Zruč nad Sázavou [6256], *49°44'31''N / 15°06'14''E*, lgt. JH, NL, det. JH (2006); Sázava River, Kamenný Přívoz [6153], *49°51'31''N / 14°31'49''E*, lgt. A, det. JSc (2007); Sázava River, Nespeky [6153], *49°51'27''N / 14°39'25''E*, lgt. JH, NL, det. JH NL (2004, 2006, 2007); Sázava River, Pikovice [6152], *49°52'38''N / 14°26'11''E*, lgt. A, det. JSc (2007); Sázava River, Sázava [6155], *49°52'51''N / 14°52'57''E*, lgt. PK, SV, det. JSc (2007); Sázava River, Smrčná [6358], *49°39'37''N / 15°20'39''E*, lgt. PK, SV, det. JSc (2007); Sázava River, Soběšín [6255], *49°47'35''N / 14°57'03''E*, lgt. PK, SV, det. JSc (2007); Sázava River, Vlastějovice [6257], *49°43'41''N / 15°10'31''E*, lgt. PK, SV, det. JSc (2007); Sázava River, Zruč nad Sázavou [6256], *49°44'32''N / 15°06'04''E*, lgt. JH, NL, det. JH NL (2004, 2007), lgt. A (2007), det. JSy (2008); Svratka River, Vranovice [7065], *48°57'02''N / 16°37'07''E*, lgt. A (2007), det. JSy (2008); Třebůvka River, Loštice [6267], *49°43'21''N / 16°53'48''E*, lgt. PK, SV, det. JSc (2007); Valová Brook, Polkovice [6669], *49°23'42''N / 17°15'51''E*, lgt. JSy, det. JSy (2008); Vlára River, Svatý Štěpán [6974], *49°03'18''N / 18°01'19''E*, lgt. PK, SV, det. JSc (2007); Vltava River, Zelčín [5652], *50°19'09''N / 14°23'56''E*, lgt. JH, NL, JSy det. JH, JSy (2005, 2006, 2008); Zákolanský Brook, Kralupy nad Vltavou [5751], *50°14'22''N / 14°18'14''E*, lgt. JH, NL, det. JH (2004) (Figure 2).

Habitats: *Caspiobdella fadejewi* occurs in large or medium size rivers with slow moving reaches. This species is often found on substratum – in contrast to *Piscicola geometra*, which is more often collected directly from its fish host. Based upon the distributional records of these two species, the higher number of current records of *C. fadejewi* than of *P. geometra* probably reflects the differences in collecting methodologies, rather than some other ecological factors.

Status: Prior to this study, *Caspiobdella fadejewi* was known only from southern Moravia. However, it has been recently recorded at many additional localities in the Labe (Elbe), Odra (Oder), and Morava (March) River basins. The main areas of its distribution in the Czech Republic are associated with the Vltava and Morava River basins. Although it is likely that *C. fadejewi* has been confused occasionally with *P. geometra*, an overall trend of rapid spreading of *C. fadejewi* along the rivers with suitable habitats has been observed during our eleven-year study.

Piscicola fasciata Kollar, 1842

Synonym: *Cystobranchus fasciatus* Brumpt, 1900

Habitats and status: *Piscicola fasciata* is a rare ectoparasitic species living most often on the catfish (*Silurus glanis* Linnaeus, 1758) (Neubert & Nesemann 1999). In the Czech Republic it was reported only once from the lower Dyje (Thaya) River (Lucký & Dyk 1964). During their survey, *P. fasciata* was found frequently on catfish in spring period. This species is hence supposed to live in the Czech Republic, although by the sampling method used it was not collected recently (1998 through 2008).

Piscicola geometra (Linnaeus, 1758)

Habitats and status: *Piscicola geometra* is the most common species of the family Piscicolidae; some specimens found in Czech rivers and streams are quite variable in color. While this species inhabits stagnant as well as running waters, it prefers slower flow conditions and the presence of various fish hosts.

Piscicola cf. *haranti* Jarry, 1960

Records: Bílina River, Ústí nad Labem [5350], *50°39'28"N / 14°02'31"E*, lgt. A (2007), det. JSy (2008); Mlýnský Brook, Litovel [6368], *49°41'58"N / 17°04'08"E*, lgt. JSy, det. JSy (2008) (Figure 2).

Habitats: The species was recorded in slow running waters located in the lowlands of the country.

Status: *Piscicola* cf. *haranti* is reported in this paper to be a new record for the Czech Republic. The first two records of this species in the country were collected from the Morava (March) and Labe (Elbe) River basins, and indicate its possible wider distribution in the country. Over the last 20 years this species has likely been slowly expanding its range throughout central Europe.

Piscicola respirans Troschel, 1850

Synonym: *Cystobranchus respirans* Brumpt, 1900

Records: Lukovský Brook, Luková [6165], *49˚52'10"N / 16˚37'39"E*, lgt. SV (2007), det. PK (2008); Morava River, Moravičany [6267], *49˚45'19"N / 16˚58'32"E*, lgt. SV (2006), det. PK (2008); Vlára River, Svatý Štěpán [6974], *49˚02'05"N / 18˚03'03"E*, lgt. PK (2007), det. PK (2008) (Figure 2).

Habitats: *Piscicola respirans* has been collected from small to medium-sized rivers and brooks, located in low or mid-elevations (250–400 m a.s.l.) of the country. Similar to *P. geometra*, *P. respirans* is most commonly collected from its fish host; it is rarely present in general macrobenthic samples.

Status: Recent records for this species suggest that *P. respirans* is a rare species with a scattered distribution in the Czech Republic. All recent records for *P. respirans* are from the Morava River basin.

Family: Haemopidae

Haemopis sanguisuga (Linnaeus, 1758)

Habitats and status: *Haemopis sanguisuga* has been found in small and medium sized running waters, small shallow ponds and pools, and often on wet soil next to these water bodies. Preference for habitats in higher elevations in Central Europe was reported by Košel (1982). Although infrequently collected in the Czech Republic, the current distribution of this species likely has been under-estimated due to its semi-aquatic life style and insufficient survey effort in its preferred habitats.

Family: Hirudinidae

Hirudo medicinalis Linnaeus, 1758

Records: Černá jezera – Dyje River dead arm, Břeclav [7267], *48°43'09"N / 16°54'22"E*, lgt. JP, det. JSy (2008); Kačení Louka Pool, Moravičany [6267], lgt. MB, det. MB (1998, 2003), lgt. MŠ, det. MŠ (2008); Kutnar Pond, Rakvice [7166], *48°50'11"N / 16°47'34"E*, lgt. JSy, det. JSy (2006); Milotický Pond, Milotice [7068], *48°57'47"N / 17°09'15"E*, lgt. JSc, JSy, GČ, det. JSc (2008); Pastvisko Pool, Lednice [7166], *48°48'30"N / 16°47'53"E*, (Sukop 2004), lgt. JSc, NK, det. JSc (2005), lgt. JSc, MŠ, NK, JSy, det. JSc (2006), lgt. JSc, JSy, MS, det. JSc (2007), lgt. JSc, JSy, MS, det. JSc (2008); pond, Ostrovec [6550], lgt. MŠ, det. MŠ (2008); pond, Varvažov [6550], lgt. MŠ, det. MŠ (2008); Sekulská Morava (Morava River dead arm), Lanžhot [7367], *48°37'39"N / 16°57'12"E*, lgt. JSc, JSy, NK, det. JSc (2008); Zámecký Pond, Lednice [7166], *48°48'46"N / 16°48'32"E*, lgt. JSy, det. JSy (2006), lgt. JSc, JSy, MS, det. JSc (2008) (Figure 3).

Habitats: *Hirudo medicinalis* was found in shallow stagnant lowland water bodies with abundant vegetation, stable water levels, and presence of amphibian and mammal hosts for juveniles and adults, respectively. All localities were in rather temperate climatic conditions.

Status: *Hirudo medicinalis* is a critically endangered species in the Czech Republic (Schenková & Košel 2005) due to intensive agri- and aquaculture in the country, which has resulted in extensive destruction of natural wetlands by drainage and flood-control management. Further loss of critical habitat for this species has resulted from intensive fishery and recreation in the few remaining unaltered wetlands, ponds, and small pools in the country. Based on authors' monitoring experiences, the intensive mud removal by heavy machinery in the Czech Republic has had destructive impact on *H. medicinalis* populations. Isolated pools that historically supported populations of medicinal leeches have failed to yield specimens after mud removal operations have been conducted. Recent distributional records for this species in the Czech Republic have been limited to only a few localities in southern and middle Moravia and southern Bohemia.

Family: Erpobdellidae

Dina lineata (O.F. Müller, 1774)

Records: Dyje River dead arm, Břeclav [7267], *48°43'09"N / 16°54'22"E*, lgt. JSy, det. JSy (2008); Myšník Pond, Častotice [6762], *49°14'12"N / 16°06'13"E*, lgt. JS (2006), det. JS (2008); Ohře River, Terezín [5450], *50°30'06"N / 14°09'39"E*, lgt. IS, det. IS (2005); Opatovický Brook, Ústí [6472], *49°31'19"N / 17°45'48"E*, lgt. PK, SV, det. PK (2008); Panenská Pool, Nové Mlýny [7166], *48°50'41"N / 16°43'40"E*, (Košel 1999a); Radičeveská strouha Brook, Trnovany [5647], *50°19'00"N / 13°35'54"E*, lgt. IS, det. IS (2006); Reslava River, Pomezí nad Ohří [5939], *50°05'31"N / 12°15'07"E*, lgt. IS, det. IS (2005); Soutěska Spring, Pavlovské vrchy [7165], *48°51'39"N / 16°38'46"E*, (Košel 1999a); Skluzavka Pool, Plošiny [7267], *48°44'17"N / 16°53'19"E*, (Košel 1999a); Sviní Brook, Kozlíky [5349], *50°36'43"N / 13°53'11"E*, lgt. IS, det. IS (2005) (Figure 3).

Habitats: *Dina lineata* occurs in smaller or medium-sized stagnant and running water bodies or in springs in lowlands (150–250 m a.s.l.); rarely also in pond or river littorals in mid-elevations (about 450 m a.s.l.).

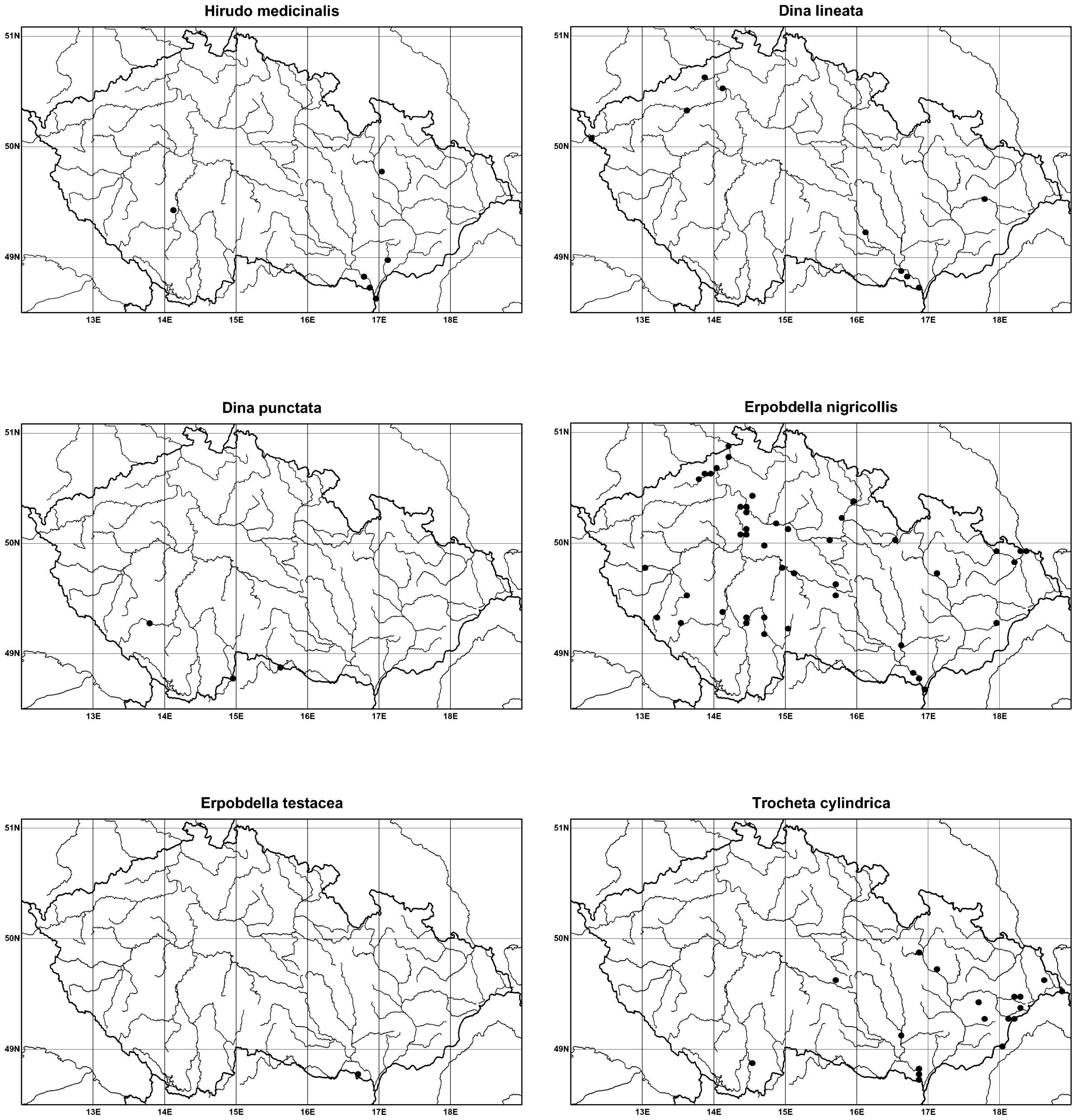
Status: *Dina lineata* is a rare species in natural habitats and commonly collected only in some lowland areas of the Czech Republic.

Dina punctata **Johansson, 1927**

Records: Dyje River, Podhradí [7159], *48°53'29"N / 15°38'30"E*, lgt. PK, SV, det. JSc (2007); Lužnice River, České Velenice [7255], *48°45'53"N / 14°58'03"E*, lgt. JR, det. JR (2007); Otava River, Katovice [6748], *49°16'22"N / 13°49'22"E*, lgt. KK, det KK (2007) (Figure 3).

Habitats: *Dina punctata* lives in littoral zones of larger rivers and brooks.

Status: *Dina punctata* is reported in this paper to be a new species record for the Czech Republic. This species likely has spread into the country from the Dunaj (Danube) River basin, where it is more common. This is the case of the occurrence in the Dyje (Thaya) River. Its occurrences in southern Bohemia (in the Vltava River basin) need additional documentation. Other new records can be expected, especially in the southern part of the country.


Hirudo medicinalis

Dina lineata

Dina punctata

Erpobdella nigricollis

Erpobdella testacea

Trocheta cylindrica

FIGURE 3. The distribution of selected leech species (Annelida, Clitellata, Hirudinida: the families Hirudinidae and Erpobdellidae) in the Czech Republic recorded during the years 1998 through 2008, based upon published and unpublished records, and specimens collected during this study.

Erpobdella nigricollis (Brandes, 1900)

Records: Bílina River, Brozánky [5349], *50°36'35" / 13°56'25"*, lgt. JSy, det. JSy (2007); Bílina River, Hostomice [5448], *50°35'23"N / 13°49'36"E*, lgt. JSy, det. JSy (2007); Bílina River, Kozlíky [5355], *50°36'41"N / 13°52'59"E*, lgt. JSy, det. JSy (2007); Bílina River, Lbín [5349], *50°36'20"N / 13°51'32"E*, lgt. JSy, det. JSy (2007); Bílina River, Stadice [5349], *50°37'08"N / 13°58'02"E*, lgt. JSy, det. JSy (2007); Bílina River, Ústí nad Labem [5350], *50°39'27"N / 14°02'28"E*, lgt. A (2007), det. JSy (2008), lgt. EP, det. EP (2008); Dyje River, Břeclav [7166], *48°48'40"N / 16°48'59"E*, (Košel 1999a); Dyje River, Břeclav [7267], *48°46'22"N / 16°52'43"E*, (Košel 1999a); Holínková dead arm, Rakvice [7166], *48°50'08"N / 16°48'06"E*, (Košel 1999a); Káča Brook, Hořátev [5856], *50°08'33"N / 15°02'25"E*, lgt. A, det. JSy (2006); Kamenice River, Nekrasín [7053], *49°13'20"N / 15°04'29"E*, lgt. A, det. FK (1998); Kyjovka River, Lanžhot [7367], *48°41'26"N / 16°57'07"E*, lgt. A, det. NK (2006); Labe River, Děčín [5251], *50°46'09"N / 14°12'45"E*, lgt. JH, NL, det. VK (1999, 2003); Labe River, Hradec Králové [5760], *50°12'20"N / 15°49'29"E*, lgt. A, det. EP (2007); Labe River, Hřensko [5151], *50°52'24"N / 14°14'07"E*, lgt. JH, NL, det. JH (1999); Labe River, Lysá [5855], *50°10'49"N / 14°50'08"E*, lgt. JH, NL, det. JH (2002); Labe River, Obříství [5652], *50°18'12"N / 14°29'08"E*, lgt. JH, NL, det. JH (1999); Labe River, Obříství [5752], *50°17'54"N / 14°28'55"E*, lgt. A, det. EP (2007); Labe River, Valy [5959], *50°01'58"N / 15°37'05"E*, lgt. JH, NL, det. JH (1999, 2002, 2005); Lužnice River, Bechyně [6752], *49°17'29"N / 14°28'18"E*, lgt. A, det. FK (1998); Lužnice River, Roudná [6854], *49°18'59"N / 14°43'21"E*, lgt. A, det. FK (1998); Lužnice River, Vlkov [6756], *49°09'41"N / 14°43'18"E*, lgt. A, det. FK (1998); Moravice River, Opava [6073], *49°55'38"N / 17°56'44"E*, lgt. A, det. NK (2006); Mže River, Stříbro [6244], *49°45'14"N / 13°00'36"E*, lgt. A, det. EP (2007); Odra River, Bohumín [6075], *49°55'04"N / 18°19'27"E*, lgt. A, det. EP (2007), lgt. A, det. EP (2008); Odra River, Ostrava [6175], *49°49'13"N / 18°13'03"E*, lgt. A, det. EP (2008); Olše River, Kopytov [6076], *49°56'49"N / 18°20'07"E*, lgt. A, det. NK (2006); Opava River, Třebovice [6175], *49°49'57"N / 18°13'10"E*, lgt. A, det. EP (2007); Oskava River, Pňovice [6268], *49°43'11"N / 17°09'37"E*, lgt. A, det. EP (2007); Ostružná River, Sušice [6747], *49°15'18"N / 13°33'01"E*, lgt. A, det. FK (1998); Otava River, Topělec [6650], *49°21'00"N / 14°08'40"E*, lgt. A, det. NK (2006); Ploučnice River, Děčín [5251], *50°46'32"N / 14°12'30"E*, lgt. A, det JSy (2006); Pšovka River, Kokořín [5553], *50°26'47"N / 14°34'41"E*, (Košel & Beran 2006); Rokytka River, Strašín [6054], *49°59'59"N / 14°41'12"E*, lgt. A, det. JSy (2006); Sázava River, Soběšín [6255], *49°47'55"N / 14°57'19"E*, lgt. A, det. FK (1998); Sázava River, Utín [6360], *49°36'09"N / 15°40'01"E*, lgt. A, det. FK (1998); Sázava River, Zruč nad Sázavou [6256], *49°44'32"N / 15°06'06"E*,lgt. A, det. NK (2006); Senice River, Leskovec [6773], *49°16'52"N / 17°59'16"E*, lgt. A, det. FK (2000); Smutná River, Bechyně [6654], *49°18'33"N / 14°27'38"E*, lgt. A, det. FK (1998); Svratka River, Rajhrad [6965], *49°05'35"N / 16°37'10"E*, lgt. A, det. EP (2007); Šlapanka Brook, Věžnice [6460], *49°30'56"N / 15°41'06"E*, lgt. JH, NL, det. JH (2006); Tichá Orlice River, Kunčice [5965], *50°00'33"N / 16°32'15"E*, lgt. A, det. FK (2000); Úhlava River, Dubová Lhota [6645], *49°20'10"N / 13°11'24"E*, lgt. A, det. FK (1999); Úpa River, Jaroměř [5661], *50°21'24"N / 15°55'53"E*, lgt. A, det. EP (2007); Úslava River, Vilémov [6447], *49°32'40"N / 13°36'08"E*, lgt. A, det. FK (1999); Vltava River, Praha Podolí [5952], *50°03'21"N / 14°24'60"E*, lgt. JH, NL, det. VK (2003) JH (1999, 2002, 2005); Vltava River, Podolí [5952], *50°03'01"N / 14°24'47"E*, lgt. A, det. EP (2007); Vltava River, Praha Trója [5951], *50°04'06"N / 14°15'00"E*, lgt. JH, NL, det. JH (2006, 2007); Vltava River, Zelčín [5652], *50°19'09"N / 14°23'56"E*, lgt. JH, NL, det. VK (2003) JH (1999, 2002, 2005); Zákolanský Brook, Kováry [5851], *50°11'27"N / 14°15'52"E*, lgt. JH, NL, det. JH (2006, 2007) (Figure 3).

Habitats: *Erpobdella nigricollis* is an inhabitant of large- or medium-sized running waters, where it occurs frequently with (although less abundantly than) *Erpobdella octoculata*.

Status: The distribution of *E. nigricollis* in the Czech Republic is scattered throughout the entire country, especially in lowland habitats; however, it seems to be absent from habitats located at higher elevations (over 500 m a.s.l.).

Erpobdella octoculata (Linnaeus, 1758)

Habitats and status: *Erpobdella octoculata* is a very common leech species in all types of both running and stagnant waters throughout the Czech Republic. It is less abundant in running waters at higher elevations, where *Erpobdella vilnensis* is more commonly collected.

Erpobdella testacea (Savigny, 1822)

Records: Nesyt Pond, Lednice [7266], *48°46'48''N / 16°43'25''E*, (Košel 1999a) (Figure 3).

Habitats: The occurrence of *Erpobdella testacea* is restricted to lowland stagnant waters; to date, this species had been recorded from only one pond located in the south-eastern part of Czech Republic.

Status: Although *Erpobdella testacea* is one of several species that are difficult to identify, it is nonetheless considered to be very rare in the Czech Republic; thus, we recommend a certain degree of protection for this species until further studies can establish its true status in the country.

Erpobdella vilnensis (Liskiewicz, 1925)

Habitats and status: *Erpobdella vilnensis* is common in the Czech Republic, especially in smaller brooks and springs or in the littoral areas of ponds occurring in the mid and higher elevations (usually over 350 m a.s.l.). It is distributed throughout the country and can be locally very abundant.

Trocheta cylindrica Örley, 1886

Synonym: *Trocheta bykowskii* Gedroyč 1913

Records: Blazický Brook, Loukov [6572], *49°24'23"N / 17°43'05"E*, lgt. A, det. JSy (2006); Brook, Halenkov [6775], *49˚16'33''N / 18˚10'43''E*, lgt. JK (2006), det. JSy (2008); Brook, Trojanovice [6575], *49˚29'58''N / 18˚12'25''E*, lgt. JK (2007), det. JSy (2008); Malše River, Straňany [7153], *48°53'14''N / 14°30'42''E*, lgt. A, det. FK (1998) Bystrý Brook, Řeka [6377], *49˚37'16''N / 18˚36'09''E*, lgt. JK, det. JSy (2008); Javorový Brook, Rovné [6377], *49˚37'38''N / 18˚36'57''E*, lgt. JK (2007), det. JSy (2008); Malá Ráztoka Brook, Trojanovice [6575], *49˚29'57''N / 18˚15'56''E*, lgt. JK, det. JSy (2008); Malá Tisová Brook, Halenkov [6774], *49˚16'29''N / 18˚08'38''E*, lgt. JK, det. JSy (2008); Moravská Sázava River, Rájec [6167], *49°51'43"N / 16°54'46"E*, lgt. A, det. NK (2006); Oskava River, Pňovice [6268], *49°43'11''N / 17°09'38"E*, lgt. A (2007), det JSy (2008); Sázava River, Štukheilský Mlýn [6360], *49°36'09''N / 15°40'02"E*, lgt. A, det. FK (1998); Skluzavka Pool, Plošiny [7267], *48°44'17''N / 16°53'19''E*, (Košel 1999a); springbrook, Bukovec [6479], *49°32'51''N / 18°51'24''E*, lgt. JB, det. JSy, (2005, 2006); Svitava River, Brno [6865], *49°08'35''N / 16°37'44"E*, lgt. A., det. JSy (2008); Trnávka River, Hrobice [6772], *49°16'14''N / 17°49'30"E*, lgt. A, det. FK (2000); Vlára River, Brumov [6974], *49°02'16"N / 18°03'04"E*, lgt. A, det. NK (2006); Vsetínská Bečva River, Velké Karlovice [6675], *49°21'45"N / 18°18'09"E*, lgt. A (2008), det. JSc (2009); Zaplavená Pool, Lubeš [7167], *48°48'12''N / 16°50'14''E*, (Košel 1999a); Žábronožková Pool, Lanžhot [7267], *48°46'05''N / 16°52'08''E*, (Košel 1999a); Živkovský Brook, Břeclav [7267], *48°47'30"N/16°52'32"E*, lgt. A, det. JSy (2006) (Figure 3).

Habitats: *Trocheta cylindrica* has commonly been collected in the Czech Republic from springs, and from small brooks at higher elevations (up to 900 m a.s.l.). Other records of this species were reported from lowland pools and wetlands, and (although less frequently) from larger rivers.

Status: *Trocheta cylindrica* has a limited distribution in the Czech Republic; most of the records to date are from localities in eastern Moravia. Although infrequently collected in the Czech Republic, the current distribution of this species likely has been under-estimated due to its semi-aquatic life style and insufficient survey effort in its preferred habitats.

Discussion

Geographically, the Czech Republic is situated in the centre of Europe (Figure 1), on the watersheds of the Labe (Elbe), Dunaj (Danube), and Odra (Oder) Rivers; each of these major watersheds serve as ‘avenues’ for leech distribution. Elevations throughout the country range from 120 to 1600 m, with a mean elevation of about 430 m.

Czech leeches (Hirudinida) can be classified into three groups according to population strength and increasing or decreasing number of localities:

1) indigenous species with stable and strong populations – those with records from many sites throughout the country;
2) indigenous species with weak populations – those known from only a limited number of localities in the country; and
3) species recently recorded in the Czech Republic – those which have passively spread or have been introduced (in one or more ways) into the Czech Republic.

Group 1. Various kinds of water bodies in the Czech Republic provide optimal conditions for most species of leeches known to occur in the country—indigenous species representing the European, Palaearctic fauna with historical records and stable populations. *Alboglossiphonia heteroclita*, *Glossiphonia complanata*, *Helobdella stagnalis*, *Hemiclepsis marginata*, *Theromyzon tessulatum*, *Piscicola geometra*, *Haemopis sanguisuga*, *Erpobdella octoculata* and *E. vilnensis* belong to this first group. Three other species are now included in this group: *Glossiphonia concolor*, *G. nebulosa*, and *Erpobdella nigricollis*. Previously considered to be rare in this country, these three species are now considered common due to the accurate identification of specimens and presence in the diversity of localities that have been surveyed for the presence of leeches.

Group 2. The second group includes species considered historically extant in the Czech territory (mostly those considered to be restricted to several finite areas or habitats based upon historical records), but now considered to be a declining or more restricted species in the country based upon a limited number of records and/or specimens collected during this and other recent studies. The most important region for thermophilous leeches is the southernmost part of the Czech Republic, particularly in southern Moravia near the confluence of the Morava (March) and Dyje (Thaya) Rivers; this area lies biogeographically within the Pannonian Basin. In the cases of *Alboglossiphonia hyalina* and *Alboglossiphonia striata*, we have recorded an increase in reports of these two species in the Czech Republic—likely attributable to their recent separation, taxonomically, from *Alboglossiphonia heteroclita* (Trontelj 1997, Neubert & Nesemann 1999). In fact, *A. hyalina* and *A. striata* are probably indigenous to Central Europe. Very little is yet known about their ecology, but neither ever occurs in large populations. Because records of *A. hyalina* are limited in the Czech Republic, we suggest that this species be considered for listing as vulnerable. The distribution records for *A. striata* in this country are quite limited, and we believe this species to be threatened. Because we are aware of only two recent records of *A. striata* from the southeastern part of the country, we believe this species should be considered for listing as endangered.

Of the species restricted to the Southern Moravia, only *Glossiphonia slovaca* is considered vulnerable (Schenková & Košel 2005). This species occurs mainly in running waters (all of our records come from the lower reaches of the Dyje River), although its occurrence is also likely in river branches and ponds. We believe this species to now be extirpated from the Morava River above its confluence with the Dyje River (Košel 1995, 1999b). Southern Moravia represents the northernmost limit of the current known distribution of *G. slovaca* (Grosser & Nesemann 2004).

Dina lineata is another rare species with a limited distribution in the Czech Republic; recent records come mainly from southern Moravia and western Bohemia. This species has only been recorded in smaller brooks, in stagnant, often temporal bodies, and in springs. To date, the known distribution of *Dina lineata* in the Czech Republic is based upon only a few specimens; most of these were recorded from unique and sensitive habitats. The limited known distribution and restricted habitats in which this rare species seems to occur provides plausible support for the listing of *Dina lineata* as vulnerable in the country.

Another amphibious species, *Trocheta cylindrica*, is known from only a few scattered localities, primarily springs and small brooks, and (less frequently) from pools and slow running rivers. Although large populations of *T. cylindrica* have been recorded in its preferred habitat, its apparent restriction to unique and finite localities in the Czech Republic supports assignment of this species to vulnerable status.

Hirudo medicinalis represents a unique case. It is a critically endangered species (Schenková & Košel 2005), yet has a historical distribution covering larger areas in the Morava and Labe River basins —with records of its broad distribution documented in several publications in the early and mid-20th century (Zavřel 1923, Hrabě 1936, Blažka 1958, 1960). Intensive collection pressure on natural populations of *Hirudo medicinalis* for use in a variety of medical applications has resulted in reductions—sometimes even decimation—of previously strong, naturally occurring populations of this species in the Czech Republic and elsewhere in Europe. The dramatic decrease in abundance of this species can also be attributed to the significant increase in intensive agri- and aquaculture activities in the Czech Republic (especially widespread and uncontrolled drainage of land) leading to the destruction of preferred habitats. This decrease in populations of *H. medicinalis* in the Czech Republic is quite obvious when compared to populations of this species in some other European countries, e.g., Poland (Buczyńsky 2003). During this present survey, *H. medicinalis* was recorded at only nine localities in the Czech Republic, most of them restricted to the Morava and Dyje River basins. Nevertheless, regular monitoring of *H. medicinalis* over the last five years—using specific, quantifiable methods for surveying and capture of specimens—has resulted in additional distributional information and new habitat preferences for this species. Our study of this critically endangered species in the Czech Republic is continuing.

A closely related thermophilous species, *Hirudo verbana* Carena 1820, occurs in Austria and Hungary (Neubert & Nesemann 1999), which are located (respectively) south and east of the Czech Republic. This species, however, has not yet been recorded—either historically or during this and other recent studies—within the Czech Republic. Several records of *H. verbana*, especially those from Western Europe, are likely specimens that were obtained (or had escaped from) commercial cultures. *Hirudo medicinalis* and *H. verbana* can easily be distinguished from one another by comparison of their ventral color patterns: dark spots are present on *H. medicinalis*, but absent from *H. verbana.*

Another uncommon species, *Piscicola respirans*, was recorded sporadically from several medium-sized rivers and brooks, within the Morava River basin only. Its preference for running water habitats at higher elevations (Neubert & Nesemann 1999, Košel 2001) was not confirmed during this study. Because of its scarceness, we believe this species should be considered vulnerable. The collection of *P. respirans* from fish hosts would most likely provide additional information about its distribution.

Piscicola fasciata, a very uncommon species, to date, it has only been recorded in the Czech Republic by Lucký & Dyk (1964). This species is probably more common in the country than presently known from verifiable records, because it lives permanently attached to its catfish host, and thus has not been collected via the methodologies used for most free-living leeches during this eleven-year study. We expect to document the presence of *P. faciata* in the Czech Republic in the near future.

Erpobdella testacea represents a very rare species, based upon a single known record from the southeastern part of the country. This species used to be reported from more stagnant water bodies in this area (Vojtek *et al.* 1967, Koubková & Vojtková 1973); the reason for its recent low occurrence, however, is still unknown. For this reason we suggest assignment of endangered status for *Erpobdella testacea*.

Group 3. The third group includes leeches only recently recorded in the Czech Republic, with an increasing number of localities. For these species, there are no historical records from the Czech Republic.

One species—*Caspiobdella fadejewi*—had been reported only once from the country prior to this study. Two species are reported for the first time in the country—*Piscicola* cf. *haranti* and *Dina punctata*.

Caspiobdella fadejewi, non-indigenous species of Ponto-Caspian origin, is quickly spreading throughout central Europe up the Dunaj River. It is a parasitic species that uses its hosts (several fish species) for transport. Since the 1980s, this species has been recorded in Poland, Austria, Germany and the Netherlands (Tittizer *et al.* 2000, Bij de Vaate *et al.* 2002, van Haaren *et al.* 2004). Today, *C. fadejewi* is the dominant piscicolid species in the Austrian part of the Dunaj River, and it has also been reported from many localities in the Dunaj and Rhein (Rhine) River basins in Germany. In the Czech Republic, it was initially recorded in the 1990s in the vicinity of the Morava-Dyje confluence (Košel 1998, 1999a) and it has recently spread into various slow flowing larger rivers in the Morava, Odra, and Labe River basins. *Caspiobdella fadejewi* is often more frequent than *Piscicola geometra* in macrozoobenthos samples, because it spends more time apart its fish host. In the same habitats, these two species are probably mutually exclusive (Jueg *et al.* 2004).

Piscicola cf. *haranti*, reported here as a new record for the Czech Republic, is also thought to be spreading throughout central Europe based upon recent records. The origin and mode of distribution of this species, however, is still unclear. Recent records of this species are from Romania, Hungary, Austria, and Germany (from the Dunaj River), and more recently from the Rhein River basin (Neubert & Nesemann 1999, Tittizer *et al.* 2000, Jueg *et al.* 2004). Unfortunately, the original description of *P. haranti* by Jarry in 1960 was based only on the external morphology of specimens collected from a single locality—a spring in southern France. Given the dissimilarity of 1) the habitats in which populations of *P. haranti* (as originally described by Jarry in 1960) and *P.* cf. *haranti* (as reported from our surveys in the Czech Republic and from other records included herein), and 2) the distance of these two populations from one another, it is possible that these two taxa could in fact be different species. In consideration of its probable Dunaj basin origin, our record of this taxon from the Labe River basin is remarkable. This species appears to be more widespread than previously thought. Furthermore, there is the possibility of confusion with its congener, *P. geometra*.

Dina punctata, recently reported as a new species for the country, can be considered a non-indigenous species that has been spreading into the Czech Republic from the west—likely from the Rhein River basin (Germany) through the Main River and floating channel to the Dunaj River. From the Dunaj, *D. punctata* has moved upstream into its tributaries, initially into the Slovak part of the Morava River (Košel 1999b), then more recently into the Dyje River in Vranov. During the eight years since it was first recorded in Slovakia, the species has moved about one hundred km upstream into the Dyje River. Two other Czech localities for *D. punctata* were found in southern Bohemia— both in upper parts of the Vltava River basin. The occurrence of this species in this region is still somewhat of a surprise. Because of its possible semi-aquatic life-style, we can speculate about its terrestrial spread from near situated brooks in the Dunaj River basin, where this species was previously recorded (Nesemann 1997). One possible explanation is that young *D. punctata* specimens are transported by waterfowl. Additional specimens of *D. punctata* collected from the lower parts of the Vltava or the Labe River basins may provide us with insight into this question. Its current northern distribution probably extends into, but not north of, the Czech Republic. According to the observations of this species in Slovakia, *D. punctata* seems to be an invasive and aggressive species, which can negatively influence the indigenous leech community (personal observation), and thus we expect more specimens to be collected from areas in the Czech Republic in the future.

In general, the most diverse leech fauna in the Czech Republic has been recorded in southern Moravia, mainly in the vicinity of the Morava-Dyje confluence—an area into which some species (e.g., *Glossiphonia slovaca*) are known to have spread from south-eastern Europe. Other species such as *Batracobdelloides moogi* Nesemann & Csányi 1995 and *Glossiphonia verrucata* (Fr. Müller 1844) were found not far from the Czech border, and likely will be collected in the country in the near future. We also expect a recently confirmed species, *Haemopis elegans* Moquin-Tandon, 1846, to eventually spread into the Czech Republic; to date, *H. elegans* has been reported from Austria, Hungary, Slovenia and Germany (Grosser 2004a, Grosser & Neseman 2004).

Most of the records for leeches discussed in this paper were collected during recent monitoring programs of running waters in the Czech Republic, conducted over the last 11 years (1998 through 2008). Additional research focusing specifically on wetlands and habitats associated with smaller stagnant water bodies is necessary to increase our knowledge of leech distributions in the Czech Republic and elsewhere in Europe.

As is true for other invertebrates, the conservation and legal protection of critical habitats used by threatened species is the most effective protection for leeches. Most of the threatened species of leeches are inhabitants of lowland wetlands and larger lowland rivers, usually those with aquatic macrophytes. These habitats are often heavily impacted by human activities throughout central Europe, usually with negative and often irreversible results. Leeches are usually not very sensitive to moderate changes and fluctuations in water quality; however, their populations have been declining steadily, often drastically, because of the destruction of their preferred habitats, or because of other unsuitable or incompatible influences there. Therefore, habitats with extensive and perennial populations of vulnerable, threatened, and/or rare leech species should be strictly protected; only carefully designed, well-managed, and regularly monitored construction projects should be permitted.

Conclusions

The check-list of leeches (Hirudinida) of the Czech Republic discussed in this paper and presented in Table 1 includes twenty-four species, reflecting historical and recent records published in the literature, unpublished records, and specimens from collections by the authors during this eleven-year study (1998 through 2008). Two of these—*Piscicola* cf. *haranti* and *Dina punctata*—are reported here as new records for the Czech Republic. Two species—*Hirudo medicinalis* and *Glossiphonia slovaca*—are already considered to be threatened species (Schenková & Košel 2005). The results of our research presented in this paper support our recommendation that six additional species be added permanently to the Red list of threatened species in the Czech Republic: four of these – *Alboglossiphonia hyalina*, *Dina lineata*, *Piscicola respirans*, and *Trocheta cylindrica*—should be listed as “vulnerable”, and two—*Alboglossiphonia striata* and *Erpobdella testacea* – should be listed as “endangered” according to IUCN Red List Categories and Criteria (IUCN 1994).

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Environmental determinants of leech assemblage patterns in lotic and lenitic habitats.

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Environmental determinants of leech assemblage patterns in lotic and lenitic habitats

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Leeches (Clitellata: Hirudinida) are abundant predators or ecto-parasites inhabiting various freshwater habitats; however many biotic and abiotic drivers of their assemblage patterns have been deduced rather than directly tested. To study species richness and composition changes in leech assemblages, 109 sites of running and stagnant water bodies were sampled in three regions of the Czech Republic in 2007–2010, together with several explanatory variables that are known or expected to be important predictors of leech distribution. In total, 17 species of leeches were recorded, varying between 0–7 and 0–9 species in lotic and lenitic sites, respectively. These differences in species richness of lotic and lenitic sites were highly significant, contrary to the abundances, which varied between 0–283 and 0–295 individuals. The main change in species composition was controlled by water temperature and morphological characteristics (e.g. substrate and cover of macrophytes), mostly reflecting the differences between lotic and lenitic habitats. We found the density of benthos (i.e. prey availability) to be the best predictor of species composition in both lotic and lenitic sites, together with the percentage of canopy cover. However, the other significant predictors (i.e. the substrate and water conductivity found to be significant in lotic sites, and the mean annual temperature and PO_4^{3-} in lenitic sites), differed between these habitats. Other than mean annual temperature and water temperature, which had different effects on species richness in lotic and lenitic sites, there were no other differences between lotic and lenitic sites in terms of how species richness and abundance responded to all other analyzed predictors. Our results stress the importance of prey availability and canopy for leech distribution patterns. Differences in the significant predictors of leech assemblage patterns between lotic and lenitic sites raise fundamental questions about the underlying mechanisms and ecological constraints to leech distribution in these main types of aquatic systems.

Introduction

The life strategies of leeches (Clitellata: Hirudinida), blood-sucking ecto-parasites of vertebrates or predators of invertebrates, determine their distribution patterns due to a tight relationship between food availability and leech occurrence (Sawyer, 1974). The importance of prey availability has however only been deduced from other habitat characteristics, such as the chemistry and trophic status of the water, without quantification and/or a detailed statistical evaluation (Pawłowski, 1936; Sandner, 1951; Mann, 1955; Elliott and Mann, 1979). Specific data exist only for some species of which the diet was explored in detail, with the majority of studies being related to *Erpobdella octoculata* (e.g. Young and Ironmonger, 1979; Dall, 1983; Toman and Dall, 1997; Schenková et al., 2007). Substrate, water depth, type of aquatic habitat and chemical parameters of the waterbody have also been determined to be the other significant predictors of leech distribution (Sawyer, 1974; Koperski, 2006). As leeches have adapted to most aquatic environments, covering a rich variety of trophic levels from dystrophic to eutrophic aquatic systems (Mann, 1955; Sládeček and Košel, 1984), testing other environmental factors can reveal some important determinants of their distribution patterns. However, other biotic parameters of waterbodies, such as the presence of a canopy or macrophytes, have only exceptionally been considered in ecological studies to date. We are aware only of the work of Koperski (2010) evaluating the effect of riparian vegetation. Several hypotheses that are also unexplored relate to the effect of temperature, which is expected to have a great importance for leech distribution (Sawyer, 1974, 1986; Elliott and Mann, 1979). In the conditions found in central European, leeches are generally described as thermophilous organisms, but without specifying whether higher importance should be given to seasonal variations in water temperature within the habitat (which differ between running and standing waters) or the variation of temperature between sampling sites (mostly correlating with altitude).

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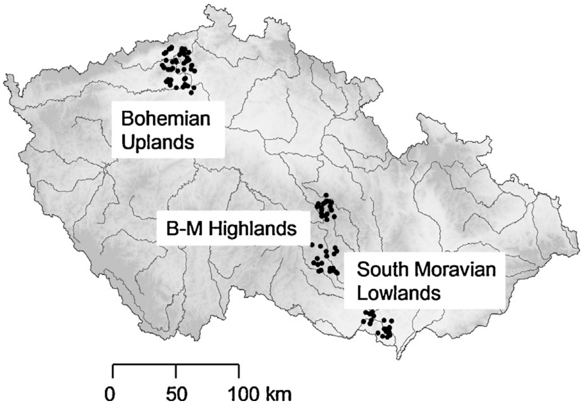

Fig. 1. Map of the Czech Republic showing the position of study areas with all 109 sampling sites. Note that the Bohemian–Moravian Highlands (B–M Highlands) include two distinct local sampling areas.

Previous ecological studies on leeches were focused either solely on stagnant (e.g. Young and Ironmonger, 1981; Spelling and Young, 1987) or running waters (e.g. Grosser et al., 2001; Koperski, 2005, 2010). Existing studies summarizing the response of the whole assemblage in various types of water habitats are always focused only on a part of all the potential environmental variables. Descriptive studies summarizing leech data related to a certain geographical area (e.g. Bennike, 1943; Elliott and Mann, 1979; Košel, 1988, 1989; van Haaren, 2004; Schenková et al., 2009) cover mostly habitat characteristics, pollution status and distribution of particular leech species. Other studies emphasize the importance of water chemistry (e.g. Mann, 1955; Grosser et al., 2001) or solely the trophic status of the water (Sládeček and Košel, 1984). Particular attention was given to various water types and their features by Koperski (2006) in his meta-analysis, but unfortunately without any evaluation of water chemistry. Some ecological studies refer to a different geographical range, with only a small overlap with European species (Herrmann, 1970; Moser et al., 2006).

In this study, we used a geographically extended data set, covering both lotic and lenitic habitats, accompanied by the sampling of macrozoobenthos as potential prey, in order to segregate the influence of diet availability from the other environmental drivers. Specifically we attempted to: (i) determine the main predictors of species richness and composition of leech assemblages; and (ii) compare the observed assemblage patterns between lotic and lenitic habitats.

Methods

Study area

Sampling sites were located in three different areas (i.e. Bohemian Uplands, the Bohemian–Moravian Highlands and the South Moravia Lowlands), covering the main types of waterbodies in the Czech Republic (Fig. 1). The majority of the waterbodies occurring within these areas (total extent 4320 km^2) were sampled. In each area we tried to cover as much as possible of the broad variety of waterbodies in respect to their size (stream width in running waters, total area in stagnant waters), depth, substratum composition, relative cover and composition of the riparian/littoral vegetation.

The Bohemian Uplands Protected Landscape Area is characterized by a patchy landscape, composed of fields, pastures and deciduous forests, with the exception of the northern part, which has been affected by brown coal mining. It represents a relatively warm and medium-dry region of a broader range of elevation (Table 1). The Žďárské vrchy Hills (in the northern part of the Bohemian–Moravian Highlands) are covered by spruce forests and meadows, which are irrigated by numerous brooks and small rivers. This region is rather cold and humid and has the highest elevation of all the study sites. The Náměšť nad Oslavou territory (the southern part of the Bohemian–Moravian Highlands) is characterized by an undulating landscape, covered by fields and pine forests. Although brooks and rivers are often present there, the most frequent waterbodies are ponds. It is warmer than the northern part of the region, but drier and of lower elevation. The third region, the South Moravia Lowlands, is an agricultural territory, dominated by fields and arable lands; most of the waterbodies studied were to a certain extent organically polluted. This region has the highest mean annual temperature, the lowest elevation and is semi-dry.

Field sampling and laboratory work

The sampling was carried out from the end of April to the beginning of June in 2007–2010. In total, 109 sites of lotic (51) and lenitic (58) waters were explored. Leeches were collected for 30 min by hand along 10 m of shoreline, including areas of bare shore and riparian/littoral vegetation at each study site. The method applied of collecting by tweezers or with bare hands (Elliott and Mann, 1979; Košel, 1988) was used mainly because leeches were usually tightly attached to the substratum. Leeches were identified in the laboratory, after standardized fixation procedures (Košel, 1988), according to Neubert and Nesemann (1999) and Grosser and Nesemann (2004).

Several explanatory variables, which were known or expected to be important predictors of leech distribution, were established for each site: (i) water temperature, dissolved oxygen, water pH and conductivity were measured in the field by portable instruments (WTW Multi 340i/SET) two times in one week, and the mean value was used in the analyses to reduce the effect of short-term variation; (ii) the concentration of nitrates, ammonia and orthophosphates, total carbon and total nitrogen were determined in the laboratory from water samples; (iii) the GPS position was directly measured in the field and used to determine the altitude and several climatic conditions (i.e. mean annual temperature, mean January and July temperatures, and the annual precipitation), obtained by using overlays of plot locations with a digital elevation model and climatic maps, based on Tolasz (2007), in the ArcGIS 8.3 program (ESRI, 2003); and (iv) water depth was measured in the field, the percentage of canopy and the percentage of the shoreline with riparian/littoral vegetation (macrophytes) were estimated and recorded in the field, and the substrate composition was estimated as a percentage of particle size categories in the field and converted using the phi scale (Hynes, 1970; Gordon et al., 1992).

Samples of the macrozoobenthos were collected by three minute kick-sampling using a hand net with 250-μm mesh size. All mesohabitats at each site were sampled proportionally, i.e. the sampling time given to each mesohabitat corresponded to their proportional representation at the site (Kokeš et al., 2006; Kubová and Schenková, 2012). The numbers of dominant taxa of macrozoobenthos (i.e. “Oligochaeta”, Mollusca, Ephemeroptera, Plecoptera, Trichoptera and Chironomidae) were counted and entered into the analyses; other taxa with a very low abundance were excluded from further evaluation. Invertebrate densities were converted on a scale from 1 to 5 (1, up to 150 individuals; 2, 150–300 individuals; 3, 300–500 individuals; 4, 500–1000 individuals; 5, more than 1000 individuals).

Data analyses

The preference of each species for either lotic or lenitic sites was tested based on a comparison of recorded individuals between the studied lotic and lenitic sites using a Mann–Whitney *U* test.

Table 1
Climatic characteristics of the studied areas.

Area	Altitude (m a.s.l.)	Mean annual temperature (°C)	Mean January temperature (°C)	Mean July temperature (°C)	Precipitation (mm year^{-1})
Bohemian Uplands	135–667	6.1–9.1	−3.8 to −0.8	15.7–18.8	488–713
Bohemian–Moravian Highlands					
Žďárské vrchy Hills	553–719	6.0–6.8	−4.1 to −3.3	15.1–16.1	684–768
Náměšť nad Oslavou territory	541–630	6.7–7.9	−3.5 to −2.8	16.5–17.8	355–586
South Moravia Lowlands	161–207	9.0–9.4	−1.9 to −1.5	19.0–19.4	480–504

Differences among number of species, total abundance and all explanatory variables were compared between lotic and lenitic sites using a *t*-test, as all these variables had a normal distribution (Kolmogorov–Smirnov test, $p < 0.05$). As all the climatic variables (altitude, temperature and precipitation) were strongly correlated (Spearman's rank correlations $r_s > 0.795$, $p < 0.001$), only the mean annual temperature was used in the analysis.

The species-by-site matrix was analyzed using Detrended Correspondence Analysis (DCA; detrended by segments) to expose the major compositional gradients in the data. The numbers of individuals were transformed as $\log(x+1)$ to exclude the effect of dominant species; rare species were downweighted according to the recommendations given by Lepš and Šmilauer (2003). The length of the main gradient (3.31 SD) in the species data was adequate for use in unimodal modelling (ter Braak and Prentice, 1988). To explain the gradients in the species data, the correlations with climate and environmental variables were examined using Spearman correlations (r_s), with site scores on the first four DCA axes; a Mann–Whitney *U* test was used for nominal variables. Sequential Bonferroni corrections of the significance level were used for multiple comparisons of continuous environmental variables (Holm, 1979). Canonical Correspondence Analysis (CCA) and Redundancy Analysis (RDA) with a forward selection procedure and a Monte-Carlo test with 999 permutations were used to reveal the environmental variables that were able to explain a significant portion of variability in the leech data.

Generalized Linear Models (GLM; McCullagh and Nelder, 1989), with Poisson family corrected for overdispersion (i.e. quasipoisson) in the data, were used to determine the explanatory variables that were significant for leech species richness and abundance. All variables were entered into the analysis in linear and quadratic shape; the interactions between variables were not included. The accuracy of the final models was checked using distribution of residuals and Cook's distances. In the case of the existence of two good models for one species, Akaike's information criterion (AIC) and the number of parameters were used as the deciding parameters (Guisan and Zimmermann, 2000). In order to test dietary preferences, the total abundance of macrozoobenthos and the numbers of "Oligochaeta", Mollusca, Ephemeroptera, Plecoptera, Trichoptera and Chironomidae were entered stepwise into the analysis.

All analyses, except for the DCA, were computed separately for lotic and lenitic sites. Species and environmental data were analyzed using the statistical programs CANOCO (ter Braak and Šmilauer, 2002), Statistica (StatSoft Inc., 2001) and R (version 2.10.1; R Development Core Team, 2010).

Results

In total, 4547 specimens of 17 leech species were collected (Table 2). Table 3 shows the environmental differences between the lotic and lenitic sites studied based on all the explanatory variables. The total abundance per site varied from 0 to 283 and from 0 to 295 individuals at lotic and lenitic sites, respectively. There was no difference between lotic and lenitic sites in terms of total abundance ($p = 0.176$); however, the abundance of several species differed significantly. *Erpobdella vilnensis* ($p = 0.018$) and *Glossiphonia complanata* ($p = 0.042$) expressed a significantly higher population size at lotic sites, contrary to *Hemiclepsis marginata* ($p < 0.001$) and *Theromyzon tessulatum* ($p < 0.001$), which had a higher number recorded at lenitic sites. In contrast to abundance, species numbers differed significantly between lotic and lenitic sites ($p < 0.001$), varying from 0 to 7 and from 0 to 9 species, respectively (Table 3).

The DCA analysis of leech assemblages significantly separated lotic and lenitic sites along the first axis (Mann–Whitney *U* test, $p = 0.038$) (Fig. 2). The sample scores on this axis were strongly correlated with water temperature and several morphological variables: phi, macrophytes (positively) and canopy (negatively) (Table 4). The sample scores on the second axis were significantly correlated with the nitrate content (negatively), which represented the water chemistry gradient. The other correlations with the first four DCA axes were weaker and not significant after Bonferroni corrections (Table 4). The CCA analysis revealed significant relationships between the variance in leech compositional data and benthos densities (prey availability), water conductivity (water chemistry) and phi (substratum characteristics) (Table 5). Single RDA analyses, which were run separately for both habitat types, showed that the largest part of the variability in species data was also explained by benthos density: 24% in lotic and 15% in lenitic sites (Table 5). However, other than the percentage of canopy the other significant predictors differed between habitats, with phi and water conductivity found to be significant at lotic sites, and the mean annual temperature and PO_4^{3-} at lenitic sites (Table 5).

The type of habitat, along with mean annual temperature, water temperature, density of benthos, substratum composition and canopy significantly affected the species richness of leeches in the whole dataset ($p < 0.001$). The numbers of species in lotic sites

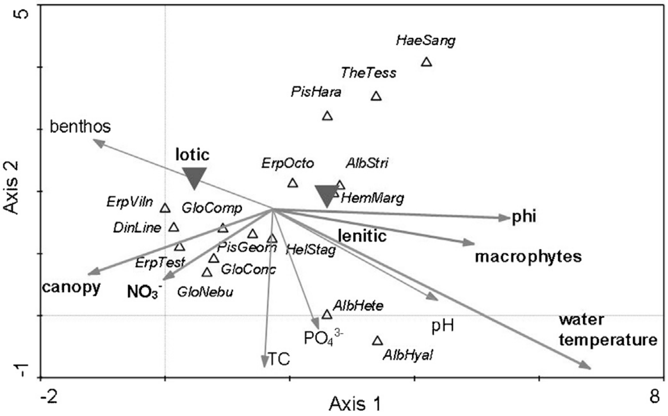

Fig. 2. DCA ordination diagram of study sites positioned on the first two ordination axes, based on leech assemblages of 109 study sites. All species except *Hirudo medicinalis*, which was an outlier in the ordination space, were plotted. Variables with a significant correlation ($p < 0.05$) with at least one of the first four DCA axes are shown, and those significant after Bonferroni corrections ($p < 0.0026$) are in bold. Nominal variables (i.e. lotic or lenitic sites) are displayed as dark triangles. The first four DCA axes explained 16.9, 10.9, 7.0, and 4.4% of the species data variance, respectively. The sum of all eigenvalues was 3.31.

Table 2
List of recorded leech species with total abundances, number of their records and relative abundance (%) in all sampling sites, with lotic and lenitic sites shown separately.

Species	Both habitats ($n = 109$)			Lotic habitats ($n = 51$)			Lenitic habitats ($n = 58$)		
	No. of individ.	No. of sites	Relative abund. (%)	No. of individ.	No. of sites	Relative abund. (%)	No. of individ.	No. of sites	Relative abund. (%)
Alboglossiphonia heteroclita (Linnaeus, 1761)	226	18	4.97	35	5	1.98	191	13	6.98
Alboglossiphonia hyalina (O.F. Müller, 1774)	6	3	0.13	4	2	0.22	2	1	0.07
Alboglossiphonia striata (Apáthy, 1888)	8	4	0.17	3	1	0.17	5	3	0.18
Glossiphonia complanata (Linnaeus, 1758)	188	34	4.13	136	21	7.65	52	13	1.87
Glossiphonia concolor (Apáthy, 1888)	31	10	0.68	13	4	0.73	18	6	0.64
Glossiphonia nebulosa Kalbe, 1964	33	6	0.72	30	4	1.68	3	2	0.10
Helobdella stagnalis (Linnaeus, 1758)	774	58	17.02	187	23	10.52	587	35	21.19
Hemiclepsis marginata (O.F. Müller, 1774)	99	29	2.17	5	5	0.28	94	24	3.39
Theromyzon tessulatum (O.F. Müller, 1774)	43	22	0.94	7	3	0.39	36	19	1.23
Piscicola geometra (Linnaeus, 1758)	22	8	0.48	2	1	0.11	20	7	0.72
Piscicola haranti Jarry, 1960	1	1	0.02	1	1	0.05	0	0	–
Haemopis sanguisuga (Linnaeus, 1758)	30	6	0.66	1	1	0.05	29	5	1.04
Hirudo medicinalis Linnaeus, 1758	12	1	0.26	0	0	–	12	1	0.43
Dina lineata (O.F. Müller, 1774)	1	1	0.02	1	1	0.05	0	0	–
Erpobdella octoculata (Linnaeus, 1758)	2552	78	56.12	873	27	49.12	1679	51	60.61
Erpobdella testacea (Savigny, 1822)	1	1	0.02	1	1	0.05	0	0	–
Erpobdella vilnensis (Liskiewicz, 1925)	520	41	11.43	478	25	26.89	42	16	1.51

Table 3
Descriptive statistics of environmental and response variables, recorded in lotic and lenitic study sites; differences between habitat types were tested by a *t*-test.

Variable	*p*	Lotic habitats			Lenitic habitats		
		Min	Median	Max	Min	Median	Max
Explanatory variables							
Altitude (m a.s.l.)	n.s.	136	318	719	135	263	708
Mean annual temperature (°C)	n.s.	6.0	7.7	9.4	6.0	8.0	9.4
Mean January temperature (°C)	n.s.	−4.1	−1.9	−0.8	−4.1	−1.8	−0.9
Mean July temperature (°C)	n.s.	15.1	17.6	19.4	15.1	17.7	19.4
Annual precipitation (mm)	n.s.	485	552	768	480	554	759
Water temperature (°C)	0.001	10.5	14.3	22.9	13.3	18.4	26.7
Water pH	0.035	5.4	7.9	9.4	5.6	8.1	10.4
Water conductivity (μS/cm)	n.s.	50	424	2890	45	540	2014
Dissolved oxygen (mg/l)	n.s.	3.4	9.8	15.7	2.3	8.9	21.8
Total carbon (mg/l)	n.s.	0.5	35.0	290.4	0.7	41.0	125.1
Total nitrogen (mg/l)	0.001	0.7	7.0	20.0	0.1	2.2	18.0
Nitrates (mg/l)	0.001	0.0	13.5	38.4	0.0	1.8	28.1
Ammonium ions (mg/l)	0.046	0.0	0.6	28.5	0.0	0.7	9.4
Phosphates (mg/l)	n.s.	0.0	0.2	1.9	0.0	0.1	5.9
Phi	0.028	−5.9	−1.3	8.0	−6.0	2.3	8.0
Water depth (cm)	0.004	15	28	60	21	45	85
Percentage of canopy (%)	0.001	0	30	95	0	10	80
Percentage of macrophytes (%)	0.001	0	0	80	0	20	100
Categorical density of benthos	n.s.	1	3	5	1	3	5
Response variables							
Total abundance	n.s.	0	20	183	0	23	295
Species richness	0.001	0	2	7	0	3	9

Table 4
Eigenvalues of the first four axes of the DCA, Spearman's rank correlations (r_s) and the Mann–Whitney *U* test (M–W) between environmental factors and site scores on the DCA axes.

	DCA			
	AX1 r_s	AX2 r_s	AX3 r_s	AX4 r_s
Eigenvalues	0.984	0.863	0.779	0.686
Morphological variables				
Phi	**0.38**[***]	0.21[*]	n.s.	n.s.
Macrophytes	**0.44**[***]	n.s.	n.s.	−0.27[*]
Canopy	**−0.42**[***]	−0.22[*]	n.s.	n.s.
Density of benthos	n.s.	n.s.	−0.25[*]	n.s.
Water temperature	**0.45**[***]	n.s.	n.s.	−0.21[*]
Chemical variables				
pH	0.25[*]	n.s.	n.s.	n.s.
Nitrates	−0.26[*]	**−0.34**[***]	n.s.	n.s.
Total carbon	n.s.	−0.23[*]	0.22[*]	n.s.
Phosphates	−0.22[*]	n.s.	0.24[*]	n.s.

Only those factors that significantly correlated with at least one DCA axis are shown.
Significant correlations after Bonferroni corrections are in bold (the cut level was $p = 0.0026$); non-significant (n.s.) correlations are not shown.

[*] Significance level: $p < 0.05$.

[***] Significance level: $p < 0.001$.

Table 5
Percentage variance in leech species data explained by each variable in separate canonical correspondence analyses (CCA, all sites) or redundancy analyses (RDAs, lotic or lenitic sites), and the percentage of independent variation explained in a single CCA/RDA for all variables together using the forward selection procedure (FS).

Variable	CCA – all sites			RDA – lotic sites			RDA – lenitic sites		
	Expl. var. %	Order in FS	Expl. var. in FS %	Expl. var. %	Order in FS	Expl. var. in FS %	Expl. var. %	Order in FS	Expl. var. in FS %
Density of benthos	13.28*	1	13.28*	24.12**	1	24.12**	15.53**	1	15.53**
Conductivity	11.11*	2	11.38*	11.47*	4	11.76*	14.77**	7	n.s.
Phi	10.26*	3	10.57*	16.76**	2	11.47*	7.95	6	n.s.
Nitrates	10.02*	4	n.s.	1.76*	12	n.s.	6.43	5	n.s.
Water temperature	8.67	5	n.s.	11.17	5	n.s.	8.71	9	n.s.
pH	8.67	6	n.s.	7.06	8	n.s.	3.03	12	n.s.
Mean annual temperature	7.05	7	n.s.	10.0	6	n.s.	14.85**	2	14.77*
Canopy	7.32	8	n.s.	11.76*	3	17.9**	14.39**	3	10.22*
Phosphates	7.59	9	n.s.	5.59	10	n.s.	12.12*	4	9.85*
Total carbon	7.86	10	n.s.	11.47	7	n.s.	7.57	11	n.s.
Ammonium ions	7.05	11	n.s.	9.71	9	n.s.	7.19	8	n.s.
Total nitrogen	7.32	12	n.s.	5.0	11	n.s.	7.19	10	n.s.

The significance (*p*) of explained variation (expl. var.) was tested using the Monte Carlo test with 999 runs. The order of the variables during forward selection is shown.
* Significance level: $p < 0.05$.
** Significance level: $p < 0.01$.

were controlled by mean annual temperature, water conductivity and canopy, contrary to lenitic sites, with species richness significantly driven by benthos densities, water temperature, canopy and the quantity of ammonium (Table 6). We found that the total abundances of leeches in the whole dataset responded to the same variables as species richness. However, the abundances of leeches at the lotic sites were affected by water temperature, mean annual temperature, substratum composition, concentration of nitrates and canopy (Table 6). At the lenitic sites, the abundances were also associated with mean annual temperature and substratum composition; however, benthos density and the amount of total carbon were revealed as additional significant variables (Table 6).

Using response curve modelling, we mostly found consistency in the responses of species richness and abundances to those environmental factors that had a significant effect on the analyzed data (Fig. 3). Other than for mean annual temperature and water temperature, which had different effects on species richness in lotic and lenitic sites, there were no other differences between lotic and lenitic sites in terms of the way in which species richness and abundance responded to all the other analyzed predictors.

Discussion

Assemblage patterns in the whole dataset

Using the whole dataset, we documented significant differences in leech species composition between lotic and lenitic sites,

Table 6
Results of the GLM modelling for species richness and total abundance recorded in all sites and in lotic and lenitic sites analyzed separately.

Habitat	Variable categorical	Linear		Quadratic		*p* Value	Var. expl. %
Species richness		Positive/negative response		Positive/negative response			
All sites	Habitat type					0.001	9.0
		+	Mean ann. temp.	–	Mean ann. temp.	0.013	8.7
		+	Water temp.	–	Water temp.	0.001	6.8
		+	Dens. of benthos		–	0.002	4.1
		–	Phi		–	0.033	3.4
		–	Canopy		–	0.046	2.9
Lotic sites		+	Mean ann. temp.	–	Mean ann. temp.	0.018	10.3
		+	Conductivity	–	Conductivity	0.031	8.2
		+	Canopy		–	0.011	3.6
Lenitic sites		+	Dens. of benthos	–	Dens. of benthos	0.001	12.1
		+	Water temp.	–	Water temp.	0.012	9.0
		–	Canopy		–	0.041	6.5
		–	Ammonium		–	0.047	3.1
Abundance							
All sites	Habitat type					0.024	8.5
		+	Water temp.	–	Water temp.	0.001	7.8
		+	Dens. of benthos		–	0.002	5.5
		+	Canopy	–	Canopy	0.021	4.3
		+	Mean ann. temp.	–	Mean ann. temp.	0.025	4.2
		–	Phi		–	0.034	3.1
Lotic sites		+	Water temp.	–	Water temp.	0.006	9.1
		+	Mean ann. temp.	–	Mean ann. temp.	0.017	7.1
		–	Phi		–	0.005	6.3
		+	Nitrates	–	Nitrates	0.029	4.6
		+	Canopy		–	0.006	2.8
Lenitic sites		+	Mean ann. temp.	–	Mean ann. temp.	0.008	7.7
		+	Dens. of benthos	–	Dens. of benthos	0.001	6.8
		+	Phi	–	Phi	0.023	4.2
		+	Total carbon		–	0.036	2.8

Mean ann. temp., mean annual temperature; water temp., water temperature; dens. of benthos, density of benthos; var. expl., explained variation. In the cases of both linear and quadratic term significant at $p = 0.05$ based on *F* test, a lower value of significance is shown.

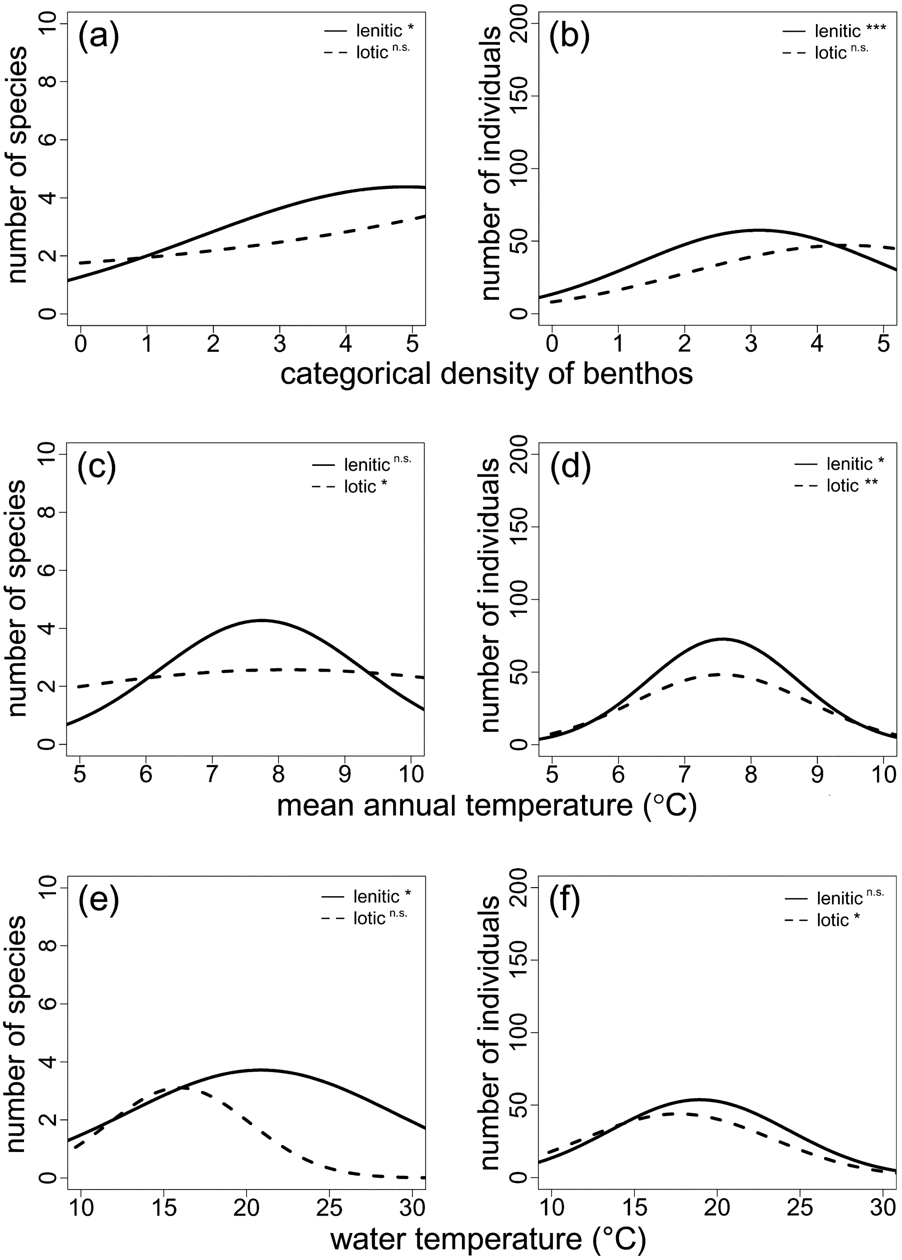

Fig. 3. Response curves of species richness and abundance in lotic and lenitic sites against selected significant environmental factors. Significance of curves based on *F* test: $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; n.s., not significant.

formerly also reported by Mann (1953) and Koperski (2006). Contrary to Koperski (2006, 2010), who distinguished further habitat types within lotic and lenitic waters, we only established these two basic categories in order to obtain gradients of physical–chemical and morphological characteristics that were as wide as possible.

Habitat type and several environmental factors, i.e. water temperature, phi (substratum composition), presence of macrophytes, percentage of canopy and nitrates, were found to be important in driving the main compositional changes in leech assemblages (Fig. 2). Variables connected with site morphology – phi value and the presence of macrophytes – increased along the first DCA axis towards lenitic sites, while the canopy variable increased towards lotic sites. It has already been pointed out that the coarser substratum in lotic habitats is generally preferred by leeches (Koperski, 2010). However, in lenitic habitats the fine substratum was accompanied by the presence of macrophytes, which could substitute for the lack of a coarse substratum (Sawyer, 1986). The direct ordination analysis (CCA) confirmed the importance of substratum composition and nitrates; significant effects of water conductivity and prey availability were also determined. However, as most of these important variables significantly differed between the two habitat types (Table 3), it was not possible to segregate the effect of habitat type and these variables based on the whole dataset. It can be concluded that these variables rather serve to define ecological differences between lotic and lenitic waters, which are also important predictors of leech distribution. As leech species composition was controlled by a combination of morphological, climatic, chemical and biological variables, it was not possible to conclude which of them was of higher importance. We therefore analyzed lotic and lenitic sites separately to segregate the effects of these confounding predictors.

Comparison between lotic and lenitic habitats

We found several important differences in variables that significantly shaped changes in the patterns of leech species composition, as well as species richness and abundance in lotic and lenitic sites. The results from lotic sites were more similar to those based on the whole dataset, suggesting the overall patterns of species composition to be mostly constrained by the lotic assemblages (Table 5). In all three datasets the density of benthos (i.e. prey availability) appeared consistently to be the best explanatory variable of leech species composition. To test the causality of these relationships, we ran separate CCA and RDA models, with all the significant environmental variables (see Table 5) included in each respective model, and tested the significance of benthos density for the residual variation in the leech data. The effect of benthos density was significant in all models ($p < 0.05$, data not shown).

Although this factor also possessed the highest predictive power for changes in species richness at lenitic sites, no significant relationship was found for lotic sites (Table 6). Similar results were also found for the total abundances of leeches. Despite the great importance of prey availability for structuring leech assemblages, we did not determine any indicators of the specific food preferences of leeches as a whole, as has been reported earlier for individual genera, for example *Erpobdella* (e.g. Dall, 1983; Toman and Dall, 1997) or *Helobdella* (Davies et al., 1979). Moreover, leech abundance at lenitic sites expressed a clear unimodal response, with a significant decrease of leech abundance with increasing benthos densities (Fig. 3b). This can probably be explained by a compounding effect of organic pollution and high temperatures in lenitic habitats. This was further indicated by a higher percentage of Chironomidae larvae and "Oligochaeta", suggesting a notable decrease in water quality to levels at which even some of the most tolerant species, *Helobdella stagnalis* and *E. octoculata*, were not able to increase their densities. Rather low and non-significant changes in both number of species and of individuals in lotic sites together with changes in prey density (Fig. 3a and b) suggested a presumably higher importance of abiotic variables in lotic habitats.

These observations on prey availability are in good concordance with those previously published; however, they either give broader support to these earlier findings or have revealed some new underlying mechanisms. For example, Koperski (2005) found a high influence of phosphates, but only for lotic habitats and on the basis of only eleven sites in three relatively unpolluted rivers. It has previously also been reported that several chemical variables, closely connected with the trophic status of the water (e.g. alkalinity, conductivity, concentration of phosphates, nitrates and oxygen, and various direct indices of trophic status), are significant for leech distribution (e.g. Bennike, 1943; Mann 1955; Herrmann, 1970; Koperski, 2005, 2010). However, it is rather unlikely that these variables would exert a direct effect on the leeches as all of them are more or less linked with prey density and availability. Similar patterns were also obtained in our data, with water conductivity having a significant influence on species composition changes in all three datasets (Table 5). Phosphate content also explained a significant and notable amount of the variations in leech species composition, but only for lenitic sites. However, as mentioned above, it would appear that prey availability is a more important predictor of leech assemblage patterns in lenitic than in lotic systems.

We found the surrounding terrestrial vegetation (percentage of canopy) to have an important and previously unreported role for leech species distribution. It explained a high portion of the variations in the species composition data for both lotic and lenitic sites (Table 5). The surrounding terrestrial vegetation cover was also a significant predictor for species richness, but the effects observed in lotic and lenitic sites were opposite to each other. It is likely that the higher canopy at lotic sites, on one hand, probably prevented thermal fluctuations and offered patches of cold water as refuges for running water species (Aguiar et al., 2002; Ebersole et al., 2003), which are less tolerant of higher temperatures. On the other hand, a low canopy can offer suitable warm-water conditions for thermophilous/temperature-tolerant species in lenitic habitats. In contrast to the canopy, the influence of macrophytes was of lower importance, being significant only in the context of the whole dataset (Fig. 2), and probably reflecting the difference between lotic and lenitic habitats. In agreement with this, Koperski (2010) observed no significant effect of riparian vegetation on leech assemblages in running water. As most of the benthic taxa observed in our study were found on the coarse substratum at the bottom rather than on macrophytes, several explanations for this behaviour can be proposed. The underside of stones can be a suitable shelter, reducing the risk of fish predation and/or of being washed away during high flow events (Young and Spelling, 1986). We observed that these microhabitats can also provide rich sources of food, as they supported the highest densities of prey recorded on the river bottom during our sampling.

We found a compounding effect of mean annual temperature and water temperature on leech assemblages, with importance differences of these two variables for each habitat type as a result of the different habitat features of lotic and lenitic sites. In lenitic sites, the mean annual (air) temperature explained a significant proportion of the variance in the leech composition data, and was more important than the directly measured water temperature (Table 5). In lotic sites, in contrast, both water and mean annual temperature had almost the same importance, as water temperature was probably more associated with stream morphology and substrate character than it was in lenitic sites. The character of the substrate was one of the most important predictors of compositional changes, but only in lotic sites. When considering species richness, however, opposite results were found for lotic and lenitic sites. There was a significant, but rather flat, response to mean annual temperature at lotic sites (Fig. 3c). In contrast, water temperature had a significant effect on lenitic species richness, presenting a unimodal response (Fig. 3e). Interestingly, we observed a rather narrow range of higher species richness in lotic sites along water temperature, with a maximum of 16 °C (based on water temperature range during the sampling), though the response curve was not significant (Fig. 3e). This can only suggest there are possible ecological limits to having a higher number of species towards both ends of the temperature gradient, as some leech species have a thermal threshold for starting reproduction (Bennike, 1943). In warmer sites there was an even sharper drop in species richness, starting from around 20 °C, probably caused by a lack of thermophilous/high temperature-tolerant species, which were represented only by *E. octoculata*, *G. complanata* and *H. stagnalis* in our data (Madsen, 1963). Thus, species that prefer simultaneously a higher water current and temperature would appear to be very rare.

There is good ecological support for a unimodal response of species richness in lenitic sites along the mean annual temperature (Fig. 3c). Low temperatures are not favourable to most of the species: a negative correlation with altitude in stagnant waters has been reported for some leech species in British lakes (Young and Ironmonger, 1981). In contrast, higher mean annual temperatures, which were reached in the South Moravian sampling sites, are associated with the negative influence of organic pollution (caused by agricultural land-use in the surroundings). Similar limits can also shape the response of leech density along mean annual temperature, which expressed a significant unimodal pattern in both types of waterbodies (Fig. 3d).

As reported previously by Koperski (2006), intensity of fish predation is another important controller of leech assemblages; this was not examined in detail in our study. We designed the present study to achieve a rather large and representative data set of various freshwater bodies, which presented difficulties to obtain exact data on fish stock for all the lotic and lenitic sites. Thus, we were only able to estimate fish densities in the field (on a six-grade scale from 0, no fish, to 5, high abundance of fish). Using these rather coarse-scale estimates we were not able to determine any significant relationships between leech assemblages and fish densities.

Habitat requirements and preferences of study species

Most of the central European leech species are often reported as being rather generalist (Sawyer, 1974; Košel, 1988, 1989; Neubert and Nesemann, 1999). Despite the common occurrence of most of the study species in both types of habitats, there were several statistical preferences of some species for one habitat or the other. We observed that four species, *E. vilnensis*, *G. complanata*, *H. stagnalis* and *T. tessulatum*, significantly preferred only one type of site, although they are able to inhabit either (Elliott and Mann, 1979; Neubert and Nesemann, 1999). These preferences have already been reported by Koperski (2006) for the former two species (which prefer lotic habitats) and by Bennike (1943) for the latter two species (which prefer lenitic habitats), but without an exact statistical evaluation. The rare species *Dina lineata* and *Erpobdella testacea* were found only in lotic habitats, though they are reported elsewhere predominantly from lenitic habitats (Elliott and Mann, 1979). This was probably linked to the presence of suitable mesohabitats in the study streams (e.g. muddy bottoms or shallow banks) and favourable water chemistry. The choice of study sites could be another possible explanation of this deviance from its known habitat requirements. However, this seems of low importance, as the observed habitat preferences of frequent species are in good agreement with those previously reported. For example, the common species *E. octoculata* and *H. stagnalis* were dominant in both lotic and lenitic habitats, as is generally known. These two species are the most dominant and frequent leech species in the Czech Republic (Košel, 1988) and Europe (Elliott and Mann, 1979; Neubert and Nesemann, 1999).

We did not find any significant differences in total abundance between lotic and lenitic sites, but significantly higher numbers of species were recorded in lotic habitats. In contrast to this result, Koperski (2006) recorded a higher diversity in lakes and a lower diversity in rivers and ponds. As there are no natural lakes present in our sampling area, it is difficult to compare these results. It is likely that large and natural lakes can offer a combination of suitable microhabitats and conditions characteristic of both lotic and lenitic habitats. They also possess a unique combination of ecological parameters: they are able to provide high prey availability, but without having a high trophic status. Thus, in order to achieve a better understanding of the processes driving local species richness, a perspective for further research would be to study a broader variety of water types, from springs and marches to large natural lakes.

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Fundam. Appl. Limnol. Vol. 185/2 (2014), 167–180 **Article**

Tolerance, optimum ranges and ecological requirements of free-living leech species (Clitellata: Hirudinida)

Nela Kubová[1, *] and **Jana Schenková**[1]

With 4 figures and 2 tables

Abstract: Deep knowledge of the ecology of aquatic invertebrate species has always been essential background for water quality assessment and leeches (Clitellata: Hirudinida), which are at the top of the benthic invertebrate food chain, are hence a group of principal importance. To study the biological and habitat requirements of free-living leeches, 109 sites of running and stagnant water were sampled in the Czech Republic, between 2007 and 2010. The morphological characteristics of the sampling site, chemical parameters of the water and climatic data were available for each site. Leeches were collected by hand, counted and identified. In total, seventeen species of genera *Alboglossiphonia*, *Glossiphonia*, *Helobdella, Hemiclepsis*, *Theromyzon*, *Piscicola*, *Haemopis*, *Hirudo*, *Dina* and *Erpobdella* were recorded. Environmental and species data were used in the analyses. The optima of twelve environmental variables for nine common species were compared using boxplots. The structure of the leech data set was recognised by cluster analysis, where eurytopic species formed a distinct group, well separated from all other species, which was further subdivided according to their ecology. Predictive models designed for seven of the leech species, revealed significant variables, which determined species occurrence and the extent of the effect of these variables. The most frequent significant variables were the type of habitat (lotic or lenitic) and the mean annual temperature.

Key words: Hirudinida, cluster analysis, species optima, predictive models.

Introduction

Most of the Central European leech species are adapted to lower water quality, and above all to higher trophy, from beta-mesosaprobic to alpha-mesosaprobic (water saprobity by Kolkwitz & Marsson 1909). In such waters, hundreds of individuals of common species, e.g., *Erpobdella octoculata* or *Helobdella stagnalis*, inhabit one sampling site (Schönborn 1985, Kutschera 2003). Hirudinida can therefore serve as good indicators of organic pollution (Sládeček & Košel 1984, Koperski 2005). Despite broad and systematic research into this group, the ecology of some of the rare species is poorly known. The life strategy and life cycles of the common species are the most-studied areas of leech biology (Mann 1957a, Mann 1957b, Elliott 1973, Learner & Potter 1974, Davies & Reynoldson 1976, Maltby & Calow 1986a, Maltby & Calow 1986b, Mason & Sayers 2005). Co-existence or competition of related species in one family (Calow & Riley 1982, Milne & Calow 1990) or in one genus (Dall 1983, Schenková et al. 2007) are further well-known research topics. In such studies, not only rare, but also less abundant common species are seldom covered and therefore their ecology on a large scale is only partly known.

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There are only a few ecological studies based in the field, which summarise the response of Hirudinida species to various environmental variables on a larger scale (Bennike 1943, Mann 1955, Elliott & Mann 1979, Sládeček & Košel 1984, Košel 1988, Košel 1989, Koperski 2006, Moser et al. 2006); unfortunately, these mostly refer to relatively small geographical areas. Focusing solely on stagnant (Young & Ironmonger 1981, Spelling & Young 1987) or running water (Grosser et al. 2001, Koperski 2005, Koperski 2010) is a further limitation. Predictors of species richness and composition of the leech assemblages were revealed within a large-scale study on freshwater leeches (Kubová et al. 2013), however, there was no inclusion of species ecology.

To fill the gap in knowledge concerning the ecological demands of leech species, we focused on leech assemblages across the Czech Republic (Central Europe). Twenty-four species from five families have been reported in this territory: ten in the family Glossiphoniidae, four in the Piscicolidae, seven in the Erpobdellidae, one in the Haemopidae, and one in the Hirudinidae (Schenková et al. 2009). The main object of our study was therefore to evaluate the responses of leeches to various physical, chemical and morphological variables covering the lotic and lenitic habitats and mutually compare their responses between lotic and lenitic environments. To include the whole range of selected variables, we extended our study sites beyond the commonly sampled pool, to include those where leeches do not usually occur, such as brooks at a higher altitude. Appropriate mesohabitats were chosen to cover the whole spectrum of preferences of free-living species. Using such a data set, we were able to evaluate the response of single species to the gradient of environmental variables.

Our main targets were (1) to evaluate ecological valences in various environmental parameters for selected species and (2) to test the response of species in lotic and in lenitic habitats separately.

Material and methods

Study area

Leeches were sampled from April to June in 2007–2010 in 109 sites from running (51) and stagnant (58) water; each site was sampled once. With respect to leech occurrence, four areas were selected: the Bohemian Uplands Protected Landscape Area, the Žďárské vrchy Hills (the Bohemian-Moravian Highlands), the Náměšť nad Oslavou territory (the Bohemian–Moravian Highlands) and the South Moravian Lowlands (Fig. 1). These represented the main types of landscapes in the Czech Republic that are rich in water bodies and covered the main environmental gradients, as well as the gradients of latitude and longitude.

Field sampling and laboratory procedure

Within each area, sites were selected to sample the whole gradient of altitude, stream width in running water, the total area in stagnant water, depth, substratum composition, and the composition and amount of the littoral/riparian vegetation. The majority of water bodies in the study area (960–1,440 km^2) were sampled. At first, the type of habitat was determined at each sampling site as categorical variable: lotic, i.e. water with at least slow current (brooks, streams), and lenitic, i.e. water with absence of current (ponds, reservoirs). At each site, 10 m of shoreline was chosen for sampling. This sampling line included the different mesohabitats available at the site (Kubová & Schenková 2012). The amount of littoral/riparian vegetation was estimated as a percentage of shoreline covered by macrophytes within the selected sampling line. Leeches were collected manually according to the hand-collecting method (Elliott & Mann 1979, Košel 1988). The coarse substratum, macrophytes, wood and different submerged objects were checked. We collected leeches for 30 min at each site, to standardise sampling time (Kubová & Schenková 2012). Live leeches were transported from the field to the laboratory, where they were narcotised by a gradually increasing concentration of ethanol to a final level of 70 % and, after their death, the mucus was removed from their bodies. Leeches were then transferred onto paper saturated with 4 % formaldehyde to strengthen their bodies (Košel 1988) and stored in 4 % formaldehyde. They were identified according to Neubert & Nesemann (1999) and Grosser & Nesemann (2004). The abundances of adult leeches only were used for all statistic analyses.

The GPS position was directly measured in the field and used to obtain the altitude and the climatic conditions (i.e., mean annual temperature, mean January and July temperatures and the annual precipitation) based on Tolasz (2007), in the ArcGIS 8.3 program (ESRI 2003). The pH, conductivity, dissolved oxygen and water temperature were measured in situ by portable instruments (WTW Multi 340 i/SET) twice-weekly at each site, and the mean values were used in analyses, to reduce the effect of short-term excesses. The concentration of nitrates, ammonium and orthophosphates, total carbon and total nitrogen were evaluated in the laboratory from water samples. The percentage of the canopy above the whole water body (lenitic habitats) or above the sampling river stretch (lotic habitats) was estimated in the field. The percentage of shoreline with littoral/riparian vegetation was also estimated in the field.

Substrate composition was estimated as a percentage of particle size categories in the field and converted on the phi scale (Hynes 1970, Gordon et al. 1992); phi values were then used for the respective statistic analyses.

The quantity of all benthic macroinvertebrates was recorded, to obtain information concerning food availability. Samples of the benthos were collected by three-minute kick-sampling using a hand net with 250 µm mesh size. All habitats were sampled proportionally. The number of individuals from the total macrozoobenthos, Oligochaeta (sensu oligochaetous Clitellata; Erséus 2005), Mollusca, Ephemeroptera, Plecoptera, Trichoptera and Chironomidae were counted and entered the analyses; the other very low abundant taxa were excluded from further evaluation.

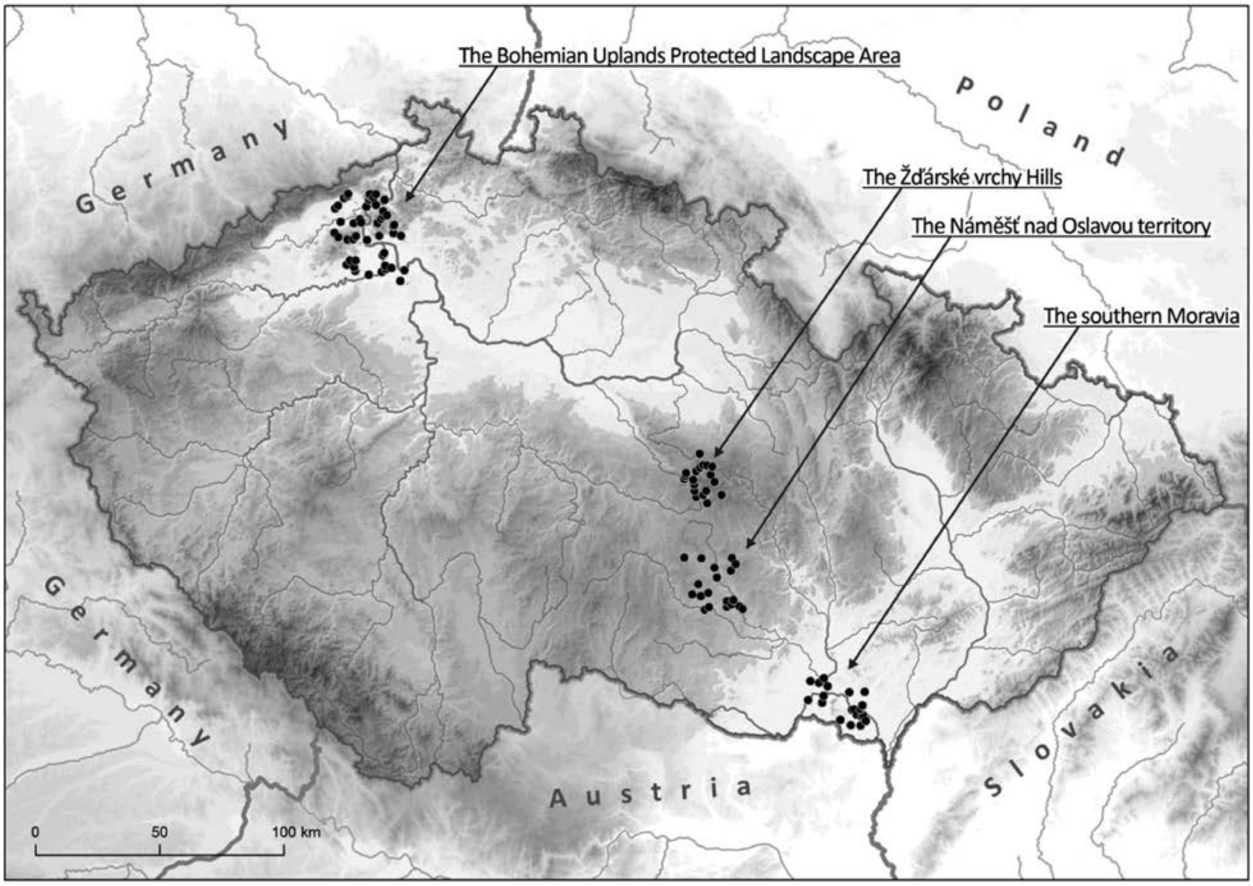

Fig. 1. Map of the Czech Republic, showing all sampled sites.

Data analyses

Cluster analysis was used to arrange leech species into groups of similar ecological requirements. After data (abundances of all species) standardisation, Ward's method with Manhattan distances as the linking method was used to get the most objective results.

Ecological preferences for leech species recorded at eight and more sites were visualised using box plots. Box plots for eight leech species and 12 selected environmental variables were constructed, based on the originally measured values, weighted by the abundance of each species at particular sites. The median, minimum and maximum values and the upper and lower quartile were depicted as box plots. The original values were shown on the x-axis.

Predictive generalised linear models (GLM) based on an assumed relationship between the mean response variable and the linear combination of the explanatory variables was used to determine the explanatory variables that were significant for species with eight and more records. All variables were entered into the analysis in linear and quadratic shape, the interactions between variables were not included into the model formulae. As all climatic variables (temperatures and precipitation) were strongly correlated (Spearman's rank correlations $rs > 0.795$, $p < 0.001$), only mean annual temperature was used in the analysis. For testing diet preferences, the total abundance of macrozoobenthos and numbers of Oligochaeta, Mollusca, Ephemeroptera, Plecoptera, Trichoptera and Chironomidae entered the analysis stepwise. Two models were computed for each species: one model based on the Poisson (quasipoisson in the case of over-dispersion in the data) distribution, recommended for the abundance data, and another model with logarithmic distributions. Poisson models were tested by the Chi-squared test at the $p = 0.05$ significance level, quasipoisson models by the *F*-test and logarithmic models by the *t*-test at the same significance level. The accuracy of final models was checked using the distribution of residuals and Cook's distances. In the case when both models for one species complied with both criteria, Akaike's information criterion (AIC) and the number of parameters were the deciding factors (Guisan & Zimmermann 2000). Seven final models complied with all the criteria.

Based on the predictive models, graphs with selected leech species and mean annual temperature were constructed. The original discontinuous data were used to predict continuous sequences within the observed range in the models, which were depicted separately for lotic and lenitic habitats in the respective graphs.

Species and environmental data were analysed using the statistics program Statistica (StatSoft Inc. 2001) and program R, version 2.10.1 (R development core team 2010).

Results

Species composition

Overall, 4,547 individuals of 17 leech species were found (Table 1). The maximum number of leeches (295) was found at the stagnant water site in the Bohemian Uplands, and the maximum abundance of one species (208 specimens) was recorded for *Helobdella stagnalis*. We counted the total abundance and frequency of each species in lotic and lenitic habitats, both separately and together (Table 1).

The structure of the leech data set was revealed using cluster analysis, which divided the whole assemblage into two main clusters (Fig. 2). The first cluster (A) was composed of eight more or less common species, which were recorded at ten or more sites. *Erpobdella octoculata*, which was found at 71.6 % of the observed sites (Table 1) was the last species to join the cluster (Fig. 2). The remaining species of cluster A formed two subgroups. The first group included three species (*E. vilnensis*, *Glossiphonia complanata* and *G. concolor*), which inhabited both lotic and lenitic habitats, but preferred habitats where the mean annual temperature did not exceed the mean values of the observed range. The second subgroup included four common stagnant water species. Two of these, *Theromyzon tessulatum* and *Hemiclepsis marginata*, were almost absent in lotic habitats and preferred less organically polluted sites, whereas two other rather eurytopic species, *Alboglossiphonia heteroclita* and *H. stagnalis*, inhabited similar, but more polluted sites in both lotic and lenitic habitats. The second cluster (B) included nine species, formed again from the two smaller subgroups. The first of these included free-living species of genus *Alboglossiphonia*: *A. striata* and *A. hyalina*, and the parasitic *Piscicola geometra*. Both species of the genus *Alboglossiphonia* are quite rare in the Czech Republic and prefer similar lenitic mesohabitats with macrophytes. *Piscicola geometra* was not rare, but with the sampling method used, their numbers should

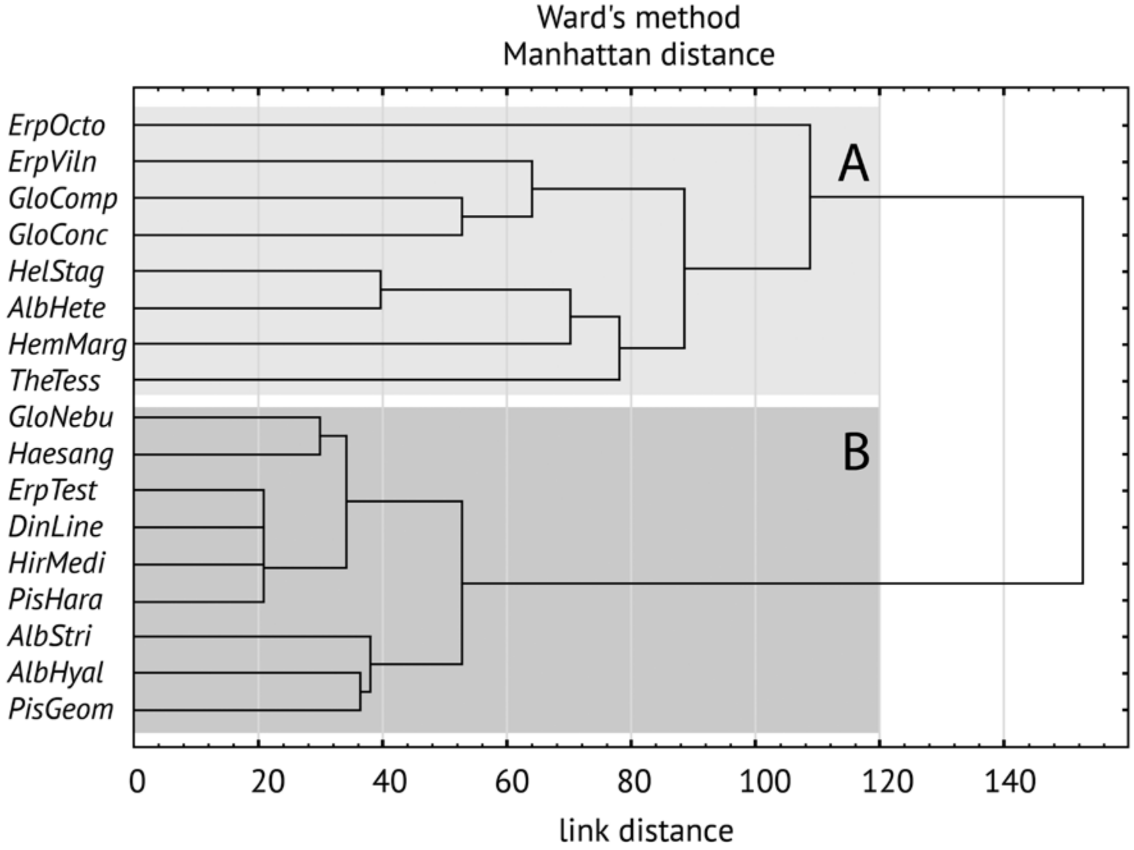

Fig. 2. Cluster analysis diagram for all leech species with displayed link distances. Species abbreviations: AlbHete – *Alboglossiphonia heteroclita*, AlbHyal – *Alboglossiphonia hyalina*, AlbStri – *Alboglossiphonia striata*, DinLine – *Dina lineata*, ErpOcto – *Erpobdella octoculata*, ErpTest – *Erpobdella testacea*, ErpViln – *Erpobdella vilnensis*, GloComp – *Glossiphonia complanata*, GloConc – *Glossiphonia concolor*, GloNebu – *Glossiphonia nebulosa*, HaeSang – *Haemopis sanguisuga*, HelStag – *Helobdella stagnalis*, HemMarg – *Hemiclepsis marginata*, PisGeom – *Piscicola geometra*, PisHara – *Piscicola haranti*, TheTess – *Theromyzon tessulatum*.

Table 1. Total abundances and frequencies of species at all sampling sites: abundances and frequencies (displayed as the percentage of sites with occurrence of each species) of species in lotic and lenitic habitats were recorded separately.

Species	Total		Lotic habitats				Lenitic habitats			
	n individuals	**frequency of occurrence (% of sites)**	**min**	**max**	**median**	**frequency of occurrence (% of lotic sites)**	**min**	**max**	**median**	**frequency of occurrence (% of lenitic sites)**
Alboglossiphonia heteroclita (Linnaeus, 1761)	226	16.5	1	18	2	9.8	1	62	5	22.4
Alboglossiphonia hyalina (O. F. Müller, 1774)	6	2.7	2	2	2	3.9	2	2	2	1.7
Alboglossiphonia striata (Apáthy, 1888)	8	3.7	3	3	3	1.9	1	3	1	5.2
Glossiphonia complanata (Linnaeus, 1758)	188	31.2	1	33	4	41.2	1	12	3	22.4
Glossiphonia concolor (Apáthy, 1888)	31	9.2	1	5	3.5	7.8	1	7	2.5	10.3
Glossiphonia nebulosa Kalbe, 1964	33	5.5	1	25	2	7.8	1	2	1.5	3.4
Helobdella stagnalis (Linnaeus, 1758)	774	53.2	1	52	5	45.1	1	208	7	60.3
Hemiclepsis marginata (O. F. Müller, 1774)	99	26.6	1	1	1	9.8	1	12	2	41.4
Theromyzon tessulatum (O. F. Müller, 1774)	43	20.2	1	3	3	5.9	1	6	1	32.8
Piscicola geometra (Linnaeus, 1758)	22	7.3	2	2	2	1.9	1	12	1	12.1
Piscicola haranti Jarry, 1960	1	0.9	1	1	1	1.9	0	0	0	0
Haemopis sanguisuga (Linnaeus, 1758)	30	5.5	1	1	1	1.9	1	20	1	8.6
Hirudo medicinalis Linnaeus, 1758	12	0.9	0	0	0	0	12	12	12	1.7
Dina lineata (O. F. Müller, 1774)	1	0.9	1	1	1	1.9	0	0	0	0
Erpobdella octoculata (Linnaeus, 1758)	2552	71.6	1	135	19	52.9	1	152	26	87.9
Erpobdella testacea (Savigny, 1822)	1	0.9	1	1	1	1.9	0	0	0	0
Erpobdella vilnensis (Liskiewicz, 1925)	520	37.6	1	107	9	49	1	9	1.5	27.6

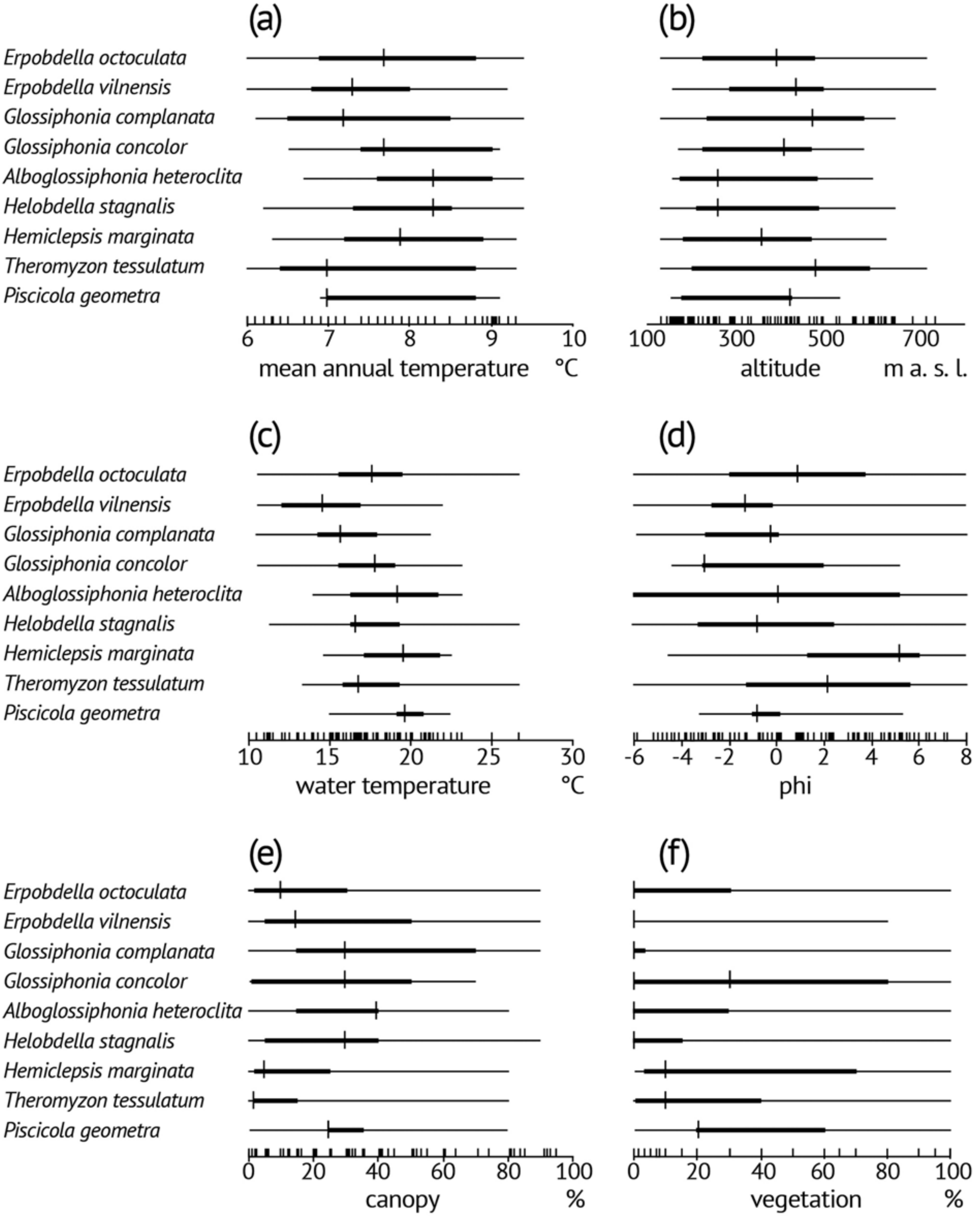

Fig. 3. Environmental preferences for species with more than eight records and selected variables. Displayed values are weighted by the abundance of a particular species. —— min, max (except the outlying values), — 25 %–75 %, | median. Tick marks pointing upwards indicate the distribution of measured data.

have been underestimated when taking into consideration its parasitic life. The second subgroup included one rare species, *G. nebulosa*, and the species *Haemopis sanguisuga*, both of which prefer colder habitats, and four other species that are very rare in the Czech Republic and were found only once during sampling.

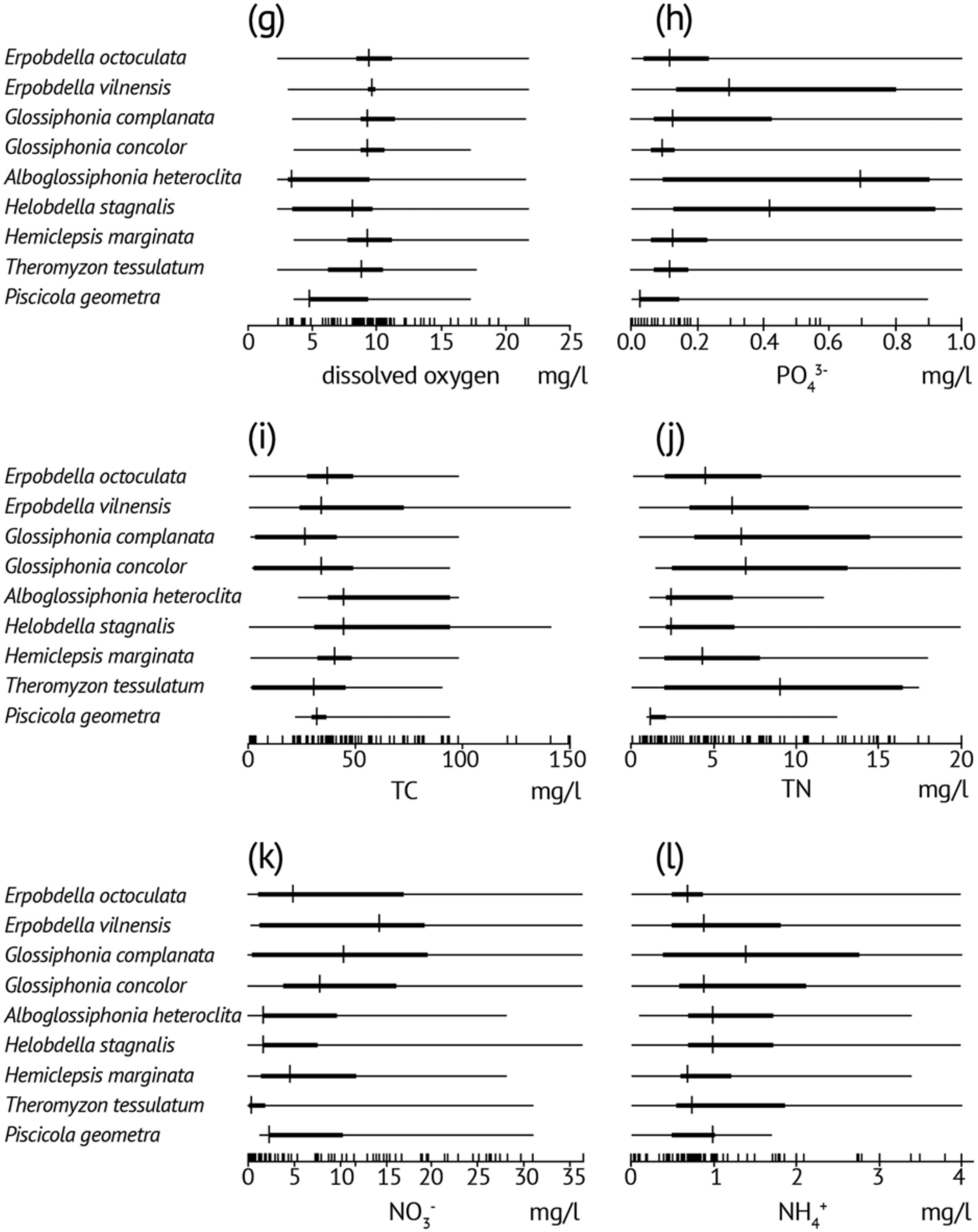

Fig. 3. Continued.

Species environmental optima

For the visualisation of the differences between the environmental optima of particular species, box plots were used (Fig. 3). These were prepared for all available variables, but only those where species responses were different from each other were presented.

The mean annual temperature and altitude (Fig. 3a, b) represent the climatic variables. The thermophilous species (*A. heteroclita* and *H. stagnalis*) preferred

Table 2. Results of the GLM models tested by the *F*-test (Poisson model) or Chi-squared test (quasipoisson model) for seven species in both habitats analysed together and separately. For the quasipoisson models, the dispersion parameter is noted. From two habitats (lotic/lenitic) only this with higher predicted abundance for each species is displayed. Abbreviations: P – Poisson model; Q – quasipoisson model; mean ann. temp. – mean annual temperature; var. exp. – variability explained.

Species	Model	Variable					% var. exp. per model
		Categorical: habitat type	Linear positive/negative response		Quadratic positive/negative response		
Alboglossiphonia heteroclita	Q: 9.16	lenitic	–	phi	+	phi	47.9
			+	PO_4^{3-}	–	PO_4^{3-}	
Glossiphonia complanata	Q: 5.13	lotic	–	mean ann. temp.			20.3
			–	phi			
			–	NH_4^+			
Helobdella stagnalis	Q: 33.26	lenitic	–	phi			21.1
			+	TC			
Hemiclepsis marginata	P	lenitic	+	mean ann. temp.	–	mean ann. temp.	35.6
			+	phi			
			+	NO_3^-			
Theromyzon tessulatum	P	lenitic	+	mean ann. temp.			26.4
			–	TN	+	TN	
Erpobdella octoculata	Q: 1.18	lenitic	+	mean ann. temp.	–	mean ann. temp.	23.4
			–	TC			
			–	canopy			
Erpobdella vilnensis	P	lotic	+	mean ann. temp.	–	mean ann. temp.	27.4
			+	NH_4^+			

warmer sites at lower altitudes, whereas *G. complanata* and *Theromyzon tessulatum* had an optimum in colder sites at higher altitudes. Other species are rather eurytopic and the climatic variables were not limiting for these.

Water temperature, substratum, canopy and vegetation (Fig. 3c–f) are variables that characterise the type of habitat. Water temperature was the most fluctuating variable. The optimum of single species is connected to their affinity to the habitat type (lotic, lenitic) and partly to altitude. The substratum composition represented by the phi value was also related to the habitat type. Most of the leech species preferred a coarse substratum; only the parasitic *Hemiclepsis marginata* and *T. tessulatum* preferred sites with a fine substratum. The canopy and vegetation represented the macrophytes above and inside the water body, respectively. The canopy had an influence on the stability of the water temperature and was usually higher in lotic habitats in contrast to water vegetation (macrophytes), which was more abundant in lenitic habitats. The parasitic species preferred sites with a higher percentage of vegetation.

Among the chemical variables, dissolved oxygen (Fig. 3g) is a variable that changes considerably in its dependence on time, microhabitat, or water flow. All the species, with the exception of *A. heteroclita* and *P. geometra* had an optimum of dissolved oxygen of about 10 mg l^{-1}.

The amount of phosphates (Fig. 3h) represented the trophy of observed habitats and most of the species preferred the mean concentrations. *Erpobdella vilnensis* and two small species, *H. stagnalis* and mainly *A.heteroclita*, were very tolerant and had optima at a high concentration of phosphates. For this chemical variable, we noted the highest range of species optima. The species optima did not strongly differ according to total carbon and ammonium ion concentrations, contrary to total nitrogen and nitrates, higher values of which were limiting for *A. heteroclita*, *H. stagnalis* and *P. geometra* (Fig. 3i–l). *Theromyzon tessulatum* only preferred very low concentration of nitrates (Fig. 3k).

Predictive models

The most parsimonious predictive models for seven selected leech species (*A. heteroclita*, *G. complanata*, *H. stagnalis*, *Hemiclepsis marginata*, *T. tessulatum*, *E. octoculata* and *E. vilnensis*) were computed (Table 2). Final models were computed from the Poisson or quasipoisson distributions, and in quasipoisson, the dispersion parameter was noted. Variables significant for species distribution ($p < 0.05$) were selected from all variables and the type of species response was recorded (Table 2). The categorical variable, type of habitat (lotic or lenitic), was significant for all selected species and those habitat types for which the abundance of each species was predicted to be higher is shown in Table 2. Only *G. complanata* and *E. vilnensis* preferred lotic habitats. For both of these species, the mean annual temperature and ammonium ions were the most decisive variables, together with the substratum composition for *G. complanata*. All other species were more abundant in lenitic habitats and the significant variables were more diversified. In addition to the mean annual temperature, we noted the importance of substratum composition and water chemical parameters.

Species response curves to mean annual temperature

Mean annual temperature was significant in the majority of predictive models; hence, the response curves of three common species (*E. octoculata*, *E. vilnensis*, *G. complanata*) to this variable were constructed (Fig. 4). The significant effect of mean annual temperature was expressed for lotic and lenitic habitats separately, to allow better comparison both within and between species.

For two out of three dominant species (*E. octoculata* and *E. vilnensis*), mean annual temperature was significant for both linear and quadratic shapes in both, lotic and lenitic habitats. These models were used for creating species response curves and their quadratic shapes were visualized (Fig. 4). *Erpobdella octoculata* was more frequently recorded in lenitic habitats and it had a lower temperature optimum in lenitic then in lotic habitats. The temperature optimum of *E. vilnensis* in both habitats was similar, although this species preferred lotic habitats. In contrast to two previous species, quadratic shape of mean annual temperature for *Glossiphonia complanata* was significant only in lotic habitats (Fig. 4). *G. complanata* was less abundant than two previous species and preferred colder sites in lotic habitats.

Response curves were not produced for the other species, because of their relatively low abundances and/or small numbers of records, which were further reduced by division between the two habitat types. The input data for creating predicted sequences were weak and curves had a low predictive effect.

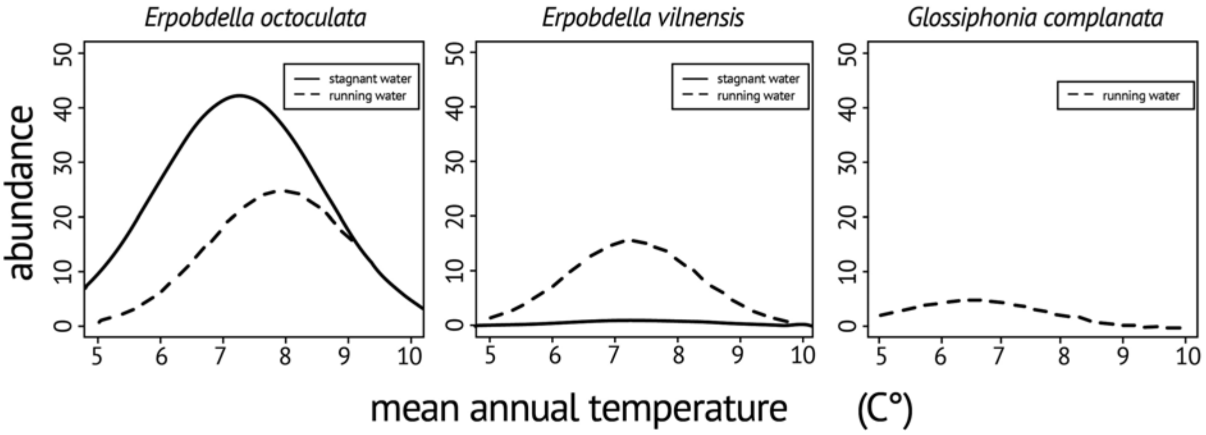

Fig. 4. Significant curves ($p < 0.05$) of the common species response to the mean annual temperature, based on the quadratic predictive models. — lenitic habitats, – – – lotic habitats.

Discussion

Factors determining leech assemblage composition

In this study, 17 out of 24 species reported from the Czech Republic (Schenková et al. 2009) were found. The type of habitat (lotic/lenitic) was an important determinant of leech occurrence (Table 2); generally, we recorded higher abundances (of common species) and lower total species diversity in lenitic than in lotic habitats. Conversely, out of the total of seventeen species found, sixteen were present in lotic habitats, with only *Hirudo medicinalis* being absent, and only fourteen were present in lenitic habitats.

The cluster analysis identified two main clusters with eight common species in cluster A and nine less common and rather rare species in cluster B (Fig. 2). Such a division suggests species grouping according to two main leech life strategies: broad ecological valences for one part of leech species, where habitat preferences of a particular species are rather a quantitative function (Sawyer 1974), and alternatively, more specific ecological requirements for another group of leech species with special habitat preferences that enable them to inhabit only a few suitable sites with a certain combination of environmental characteristics. The habitat requirement of seven out of eight species from cluster A with the exception of *G. concolor*, were described using predictor modelling (Table 2).

Habitat preferences of common leech species (cluster A)

Erpobdella octoculata is the most common Central European leech, although its occurrence was restricted in hard water (Mann 1955). We found it at various site types from clean brooks to eutrophic ponds. This species is tolerant to the complete range of water quality and chemical conditions (Elliott 1973, Elliott & Mann 1979, Grosser et al. 2001) and has even been considered a low water-quality indicator (Sládeček & Košel 1984, Koperski 2005). We recorded the highest abundances in water with sufficient, but not an overabundance, of organic matter, which provided enough available diet (indirectly via benthic invertebrates feeding on it and later serving as a prey). A suitable altitude with an optimum of 300–500 m a.s.l. in predictive models, substituted by a mean annual temperature with an optimum of about 7 °C in stagnant and 8 °C in running water, respectively, was another crucial condition for the occurrence of *E. octoculata*. A negative correlation with the canopy in this model showed that it preferred unshadowed waters, which created slightly higher temperatures.

The most abundant and frequent running water species, *E. vilnensis*, is common in Central European running water (Košel 1989, Neubert & Nessemann 1999, Koperski 2006). At the intermediate altitudes, *E. vilnensis* was syntopic with *E. octoculata*, however, *E. vilnensis* was almost absent from the southern Moravian territory (lower altitudes), where both lotic and lenitic habitats had higher temperatures and were more productive. On the contrary, *E. vilnensis* was more frequent and abundant than *E. octoculata* at higher altitudes. A surprising result was the tolerance of *E. vilnensis* to high concentrations of all analysed chemical variables. Considering its preference for higher altitudes and lotic habitats, which are usually less affected by chemical pollution, these results were not expected. Van Haaren (2004) only indicated a sporadic occurrence of *E. vilnesis* in lowland brooks and ditches, but the high tolerance of *E. vilnensis* to chemical pollution has not been so far discovered. *Erpobdella octoculata* and *E. vilnensis* niche portioning is probably controlled by temperature, which rather than chemical conditions, regulates differences in their life cycles (Schenková et al. 2007).

Glossiphonia complanata is an evenly distributed species in all types of water bodies: lakes, ponds, marshes and running water (Bennike 1943). We recorded *G. complanata* in both habitat types, with a preference for lotic habitats. This species was syntopic with *G. concolor* and *G. complanata*, which, however, did not reach the same abundance. We found that *G. complanata* preferably inhabited water at higher altitudes and water with a sufficient amount of phosphates. These preferences, together with tolerance to a higher amount of nitrates and ammonium ions, also reported by Grosser et al. (2001) and Koperski (2005), enabled this species to colonise habitats with a high supply of both oxygen and organic matter. Organic matter was also essential for the occurrence of molluscs, the main component of its diet (Elliott & Mann 1979, Neubert & Nesseman 1999).

Infrequent records of *G. concolor* confirmed its previously reported preference for stagnant water or slowly running water from lowlands to moderate elevations (Košel 1989, Neubert & Nessemann 1999, Koperski 2006, Schenková et al. 2009). Unfortunately, its ecological requirements have not yet been statistically evaluated, also based on insufficient frequencies in previous studies. New information concerning its ecological valences was the unusual combination of preference for habitats with a coarse substratum, typical for the genus *Glossiphonia*, and the high percentage of canopy and littoral/riparian vegetation. Its preference for shore covered with vegetation, separated its optimum habitat from the other congeners, *G. complanata* and *G. nebulosa*.

Theromyzon tessulatum was almost absent from lotic habitats. Its preferred sites were ponds with abundant water birds. Its chemical preferences (e.g., the amount of oxygen, phosphates or total nitrogen) and saprobe status did not considerably differ from those reported from the Czech Republic (Košel 1989) and Germany (Grosser et al. 2001). Similar to Mann (1955), we recorded distinct behaviour in different size categories: small individuals were almost exclusively attached to the vegetation, whereas large, adult specimens were also found on the underside of stones. Such a strategy enables young leeches to more easily attach to birds for feeding and consequently grow to mature size. Adult leeches that do not feed but are breeding (Mann 1955), are safer under the stones (Sawyer 1986).

Hemiclepsis marginata is a species that strongly prefers lenitic habitats, often weedy ponds (Mann 1955, van Haaren 2004). We usually found *H. marginata* on submerged vegetation, most often in eutrophic ponds with a muddy bottom, higher water temperature and sufficient fish stock. We recorded the importance of fine substratum and nitrates in the predictive model. In contrast to species with similar habitat preferences, such as *H. stagnalis* or *A. heteroclita*, *Hemiclepsis marginata* did not prefer high concentrations of chemical variables, especially phosphates. An unexpected finding was that this species was not abundant at the southernmost sites (with the highest mean annual temperatures) and at sites at the lowest altitudes with the richest fish stocks. Higher values of chemical parameters were probably limiting at these habitats. Such ecological valence might be the cause of its recent classification among high quality indicator species (Koperski 2005).

The most common species of the family Glossiphoniidae was *H. stagnalis*. The ecological status of this species is still rather ambivalent. It occurs in heavily organically polluted waters and is considered as an indirect indicator of such conditions (Mann 1955, Sládeček & Košel 1984, van Haaren 2004), whereas in Poland, it belongs to a group of species that indicates high quality water (Koperski 2005). The explanation might be due to its tolerance of high content of phosphates on the one hand, and to limiting total nitrogen and nitrates on the other (this study, Grosser et al. 2001). The latter limitation might cause an undesirable increase in nutrients without any positive effect on habitat conditions or the amount of available diet, which is one of the most important parameters for the occurrence of *H. stagnalis* (Elliott & Mann 1979, Sawyer 1986).

Alboglossiphonia heteroclita is a eurytopic species that often inhabits eutrophic sites with a high amount of phosphates, a muddy bottom and a low amount of oxygen (Mann 1955, Schenková et al. 2009), i.e., habitats which provide sufficient food, particularly oligochaetes, chironomids and molluscs. The importance of phosphates was confirmed by the predictive model, which explained a high percentage of the variance. The tolerance of *A. heteroclita* to water pollution was much higher, and its oxygen requirements lower, than those recorded in Germany (Grosser et al. 2001). This discrepancy might be caused by differences in the sampled habitat types; lotic habitats sampled in Saxony (Germany) might not be polluted to a similarly high level as some of the lenitic habitats (fish ponds) included in our study. As a species that prefers lenitic habitats (Mann 1955, Košel 1989, Koperski 2006), *A. heteroclita* cannot be evaluated on the basis of lotic habitats only and the indication value and saprobe optimum of *A. heteroclita* should be re-evaluated to α-mesosaprobity, according to Košel (1989).

Habitat preferences of less common and rare leech species (cluster B)

Alboglossiphonia striata and *A. hyalina* have not been frequently reported from the Czech Republic (Schenková et al. 2009). In this study, only a few specimens were recorded, and therefore, it was impossible to better describe their ecology.

Piscicola geometra is a common species in Central Europe: the small number of specimens recorded in this study was due to the sampling method used. Mann (1955), who used a similar sampling method for stagnant as well as running water, also reported *P. geometra* as a comparatively rare leech. The small number of our records might also have led to its positioning in the cluster analysis near to the rare species *A. striata* and *A. hyalina*, however, with a different ecology. *Piscicola geometra* spends most of its life attached to its fish host, and is therefore more frequently found during fish catches. Water quality was not a limiting factor for the occurrence of this leech, but recorded numbers were probably underestimated in comparison to its actual numbers (Grosser et al. 2001). In spite of few records, our results were similar to those in Grosser's study (Grosser et al. 2001).

The remaining six species within cluster B were all very rare during sampling. A small number of records of *G. nebulosa* in lotic habitats and an even smaller number in lenitic habitats, with the exception of the dominance of 25 specimens at one site, enabled us to calculate predictive models and visualise box plots.

Due to its semi-aquatic lifestyle, *Haemopis sanguisuga* was usually found on the edge of water or on the shore during sampling: the most typical habitat was a pond with soft shores at higher altitudes. *Haemopis sanguisuga* was always found as a single specimen per site, except in one case, where 20 copulating individuals at one site were found. *Haemopis sanguisuga* was predominantly found in lenitic habitats, not only during this sampling but also in previous studies (Schenková et al. 2009, and unpublished data). Concerning its altitude preferences, we recorded this species preferentially at higher altitudes, similar to findings by Košel (1982), rather than in lowlands (Neubert & Nessemann 1999, Koperski 2006).

Piscicola haranti is a species only recently reported from the Czech Republic (Schenková et al. 2009). Three existing records are now supplemented by one new record from this study. Since these records belong to different river basins (Morava and Labe Rivers), the distribution of *P. haranti* is probably wider; imprecise determinations and confusion with *P. geometra* have previously lowered its numbers.

Hirudo medicinalis is a critically endangered leech species in the Czech Republic (Schenková & Košel 2005). The small populations are mostly weak and mutually isolated by various barriers. One site with a rather large population of *H. medicinalis* was included in our sampling site set, but from this single record, even general requirements could not be calculated. *Hirudo medicinalis* populations were depleted by collecting for medicinal purposes in the 19th century, and later by the deterioration in water quality as a consequence of industrial development at the beginning of the twentieth century. Changes in the agricultural landscape, such as the draining of pools, led to a decrease in the number of amphibians, which are hosts for juvenile leeches and *H. medicinalis* populations never recovered from these dramatic changes. Currently, even though water quality has improved and amphibians are more numerous, the availability of suitable habitats, irrespective of problems of leech dispersion, is still too low (Elliott & Kutschera 2011).

Dina lineata and *Erpobdella testacea* from the family Erpobdellidae are further species that are not commonly found in the Czech Republic. Similar to *Haemopis sanguisuga*, *Dina lineata* is the second species that is almost amphibian and it inhabits mostly shallow littoral areas or soft banks and is scarce in the Czech Republic. *Erpobdella testacea* should be expected in stagnant water (Neubert & Nessemann 1999), but our record from running water shows that its distribution should not be solely associated with this habitat type.

A few leech species whose occurrence could be expected within the study area remained completely undiscovered from various reasons. *Caspiobdella fadejewi* (Epshtein, 1961) from the family Piscicolidae and *Erpobdella nigricollis* (Brandes, 1900) from the family Erpobdellidae are relatively frequent, but both inhabit rather large rivers with slow current (Schenková et al. 2009) which were not included in the sampling. Absence of *Piscicola fasciata* Kollar, 1842 and *Piscicola respirans* Troschel, 1850 (family Piscicolidae) was probably caused by sampling method inappropriate to their collection (they live attached to the fish host) and their relative rarity. *Trocheta cylindrica* Örley, 1886 (family Erpobdellidae) was also not recorded, as it is an infrequent species with semiaquatic life. Nevertheless, gaining information about habitat preferences of these species is a challenging task for further research.

Conclusions

The results of this study show the fundamental importance of the type of habitat (lotic versus lenitic). Box plots visualising the preferred ranges of twelve physico-chemical variables of nine medium-frequent to frequent species, enabled a comparison between the species ecological requirements. Predictive models based on linear regression were produced for *Erpobdella octoculata*, *Erpobdella vilnensis*, *Glossiphonia complanata*, *Helobdella stagnalis*, *Alboglossiphonia heteroclita*, *Hemiclepsis marginata* and *Theromyzon tessulatum*. For each of the evaluated species, certain morphological and also chemical variables were relevant. For future surveys of the ecological demands of rare species, special sampling that focuses on their preferred habitats will be required.

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