Vliv komplexity prostředí a rizika predace na utváření společenstev v malých stojatých vodách

Diplomová práce

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Anotace:
The effects of habitat complexity and predation risk on community assembly in small standing waters are reviewed. Both lethal and nonlethal predator effects are discussed. Emphasis is put on the role of omnivorous predators which do not fit into the standard food chain model. The review is complemented by a manuscript intended for publication in Freshwater Biology. It reports the results of a mesocosm experiment focusing on the effects of both lethal and nonlethal predation risk, induced by the invasive crayfish species *Orconectes limosus*, and habitat complexity on the abundance and biomass of macroinvertebrates in a naturally assembled community.

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Cíl práce:

Provést mesokosmový experiment zaměřený na vyhodnocení souběžného vlivu komplexity prostředí a rizika predace na utváření společenstev v malých stojatých vodách. Experiment bude probíhat na experimentální ploše vybudované na výukové zahradě PF JU a bude zahrnovat studium přímých i nepřímých vlivů predátora. Práce bude vycházet z hypotéz a předběžných výsledků získaných v rámci bakalářské diplomové práce.

Obsah

Úvodní komentář

Společenstva malých stojatých vod

Pojmem malé stojaté vody rozumíme rybníky, jezírka a jiné mělké přírodní i uměle vytvořené nádrže. Od velkých stojatých vod se odlišují kromě velikosti také trvalostí a složením společenstva. Wellborn, Skelly & Werner (1996) popsali gradient velikosti vodních nádrží, na němž je možné pozorovat změny ve složení společenstva. Nejmenší nádrže, například louže a tůně, jsou zpravidla dočasné a obývají je pouze rychle se vyvíjející organizmy, například tiplice (Diptera: Tipulidae), komáři (Culicidae), pakomáři (Chironomidae) a pakomárcovití (Ceratopogonidae), ale i organizmy přizpůsobené k přežití období bez vodního sloupce. To mohou být kromě některých pakomárů a dalších skupin vodního hmyzu i někteří plži a mlži, často vybavení operkulem nebo schopníschopností hloubit si úkryty v substrátu. Tímto způsobem se umí před vyschnutím chránit také někteří raci a larvy vážek (Williams, 2006). Tito živočichové mohou být kořistí predátorů, kteří nejsou ve svém životě omezeni pouze na jedno lokální stanoviště (například brouci a ploštice, kteří mohou přelétat mezi vodními prostředími). Větší nádrže jsou stálejší, ale bývá pro ně charakteristické periodické vysychání. V takovém prostředí mohou žít i větší organizmy s delším vývojem; nalezli bychom zde např. zdrojové populace vodních brouků, ploštic a vážek nebo například chrostíky (Williams, 2006). Vrchol potravního řetězce v těchto vodních nádržích tvoří bezobratlí predátoři. Společenstvům velkých trvalých nádrží (jezer) představující opačný konec spektra než malé dočasné nádrže pak dominují rybí predátoři. Velcí bezobratlí v nich přežívají pouze tam, kde jsou před jejich dosahem chráněni (například zarostlý litorál – Diehl, 1992; Kolář, 2013).

Faktory ovlivňující utváření společenstev malých stojatých vod

Na utváření společenstev malých stojatých vod se podílejí biotické i abiotické faktory (Brönmark & Hansson, 2005). Malé stojaté vody mají svá specifika, například kolísající teplotu, velké množství světla v poměru k objemu, kolísavou koncentraci minerálních látek apod. Tyto faktory určují překryv s fundamentální nikou různých organizmů, které by dané stanoviště mohly osídlit. Jejich absence pak může být důsledkem nepříznivých biotických faktorů, například konkurence (Begon, Townsend & Harper, 2005) nebo predace (Eitam & Blaustein, 2004).

To by naznačovalo, že stanoviště, které je brzy po svém vzniku osídlené predátorem, bude mít jiné složení společenstva než totožné stanoviště, které je predátory osídleno později. Tuto úvahu popsal Chase (2003) jako teorii mnoha rovnovážných bodů. V teorii mnoha rovnovážných bodů je konečná podoba společenstva závislá nejen na abiotických faktorech a zdrojích kolonizátorů, ale také na pořadí, v jakém různé druhy kolonizují nové stanoviště. Murrell, Ives & Juliano (2014) např. ukázali, že stáří stanoviště a sezóna (určujícím faktorem ovlivňujícím průběh kolonizace je vlastně fenologie kolonizátorů) také mohou mít vliv na výslednou podobu společenstva. Naproti tomu teorie jednoho rovnovážného bodu předpokládá, že výsledná podoba společenstva je odvoditelná ze znalosti abiotických faktorů a zdrojů kolonizátorů v okolí nově vzniklého habitatu. Chase (2003) poskytuje empirické příklady podporující obě hypotézy.

Variabilní charakteristika prostředí (například brzký nárůst makrofyt nebo přítomnost členitého dna) je jedním z faktorů, které mohoumůže mít v souladu s teorií mnoha rovnovážných bodů zásadní dopad na budoucí vývoj místního společenstva. Dalším takovým faktorem je riziko predace, které může vzniknout již ve zrovna utvořené malé stojaté vodě (například přeletem dravých brouků či ploštic nebo migrací raků). Těmto faktorům se proto věnuji podrobněji níže.

Vliv struktury prostředí

Někteří autoři rozlišují strukturu prostředí, anglicky „habitat structure", a složitost prostředí, anglicky „habitat complexity" (Kovalenko, Thomaz & Warfe, 2011). V rámci své diplomové práce používám pro zjednodušení termín struktura prostředí k popisu obecného konceptu, který v sobě zahrnuje oba pojmy, tj. pokrývá jak prostorově členité prostředí, tak diverzitu tvarů a rozmístění prostorových prvků.

Struktura prostředí je klíčový faktor utvářející společenstva malých stojatých vod (Kovalenko et al., 2011). Ve sladkých vodách ji mohou tvořit vodní rostliny, substrát dna nebo organické zbytky a kořeny rostlin (Thomaz et al., 2008; Kovalenko et al., 2011; Walker, Wijnhoven & van der Velde, 2013). Ve stojatých vodách se v experimentech jako model struktury prostředí užívají kromě skutečných rostlin také jejich plastové modely (Burdett & Watts, 2009). Může jít o model obecný (Burdett & Watts, 2009; Soukup, 2013) nebo více či méně napodobující konkrétní rostlinu (Warfe & Barmuta, 2004; Verdonschot & Peeters, 2012).

Pokud je použita živá vegetace, není možné oddělit vlastní vliv struktury prostředí od dalších vlivů, zejm. poskytování potravních zdrojů pro herbivory nebo detritivory, a výsledky experimentů se tak se mohou lišit (Burdett & Watts, 2009). Podstatné je proto způsob zvýšení struktury prostředí dobře popsat a zohlednit při interpretaci výsledků.

Struktura prostředí má vliv na početnost a diverzitu vodních organizmů. Všeobecně vzato ve více strukturovaných prostředích očekáváme větší abundance a druhovou diverzitu bezobratlých. Například Walker *et al*. (2013) pozorovali v rámci jedné nádrže největší diverzitu i abundanci u dna a v prostorech zarostlých rákosem, o něco menší v dalších makrofyty zarostlých úsecích a vůbec nejmenší v otevřené vodě. Někdy tyto obecné předpovědi nemusí platit, například Burdett & Watts (2009) zjistili ve svém mesokosmovém experimentu, že počty pakomárů se v malých nádržích s přítomností přírodní vegetace zvyšují, ale celková diverzita bezobratlých klesá.

Ve stojatých vodách strukturu prostředí většinou představují porosty makrofytních rostlin (např. Walker *et al*., 2013), zatímco v tekoucích ji často zajišťuje strukturní diverzita dna (např. Brown, 2003). Různá složka struktury prostředí může vyvolávat podobné změny. Například Taniguchi & Tokeshi (2004) zjistili v dlouhodobém terénním experimentu pozitivní korelaci mezi strukturou prostředí danou charakterem dna potoka a abundancí různých zástupců jepic (Ephemeroptera). Diverzita dna potoka tak měla podobný efekt, jaký bychom očekávali díky přítomnosti makrofyt ve stojaté vodě.

Zmíněná pozorování mohou být důsledkem řady různých procesů. Zvýšená struktura prostředí například znamená více povrchů (větší fraktální dimenzi), na kterých mohou prospívat nárůsty perifytonu. Tímto způsobem navýšená primární produkce je pak potravou pro primární konzumenty a směrem vzhůru v potravní pyramidě (bottom up) zvyšuje abundanci jednotlivých organizmů (Taniguchi & Tokeshi, 2004).

Další vlastností strukturně složitých prostředí je značné množství mikrohabitatů různé velikosti. V těch mohou nalézt vhodný úkryt a potravu různé organizmy (Gotceitas & Colgan, 1989). Tímto způsobem struktura prostředí přispívá ke zvýšení druhové diverzity. Na druhou stranu jednolité porosty rostlin jednoho druhu komplexitu prostředí také zvyšují, ale poskytují uniformní podmínky, které mohou být vhodné jen pro několik málo určitých druhů. Podobně může komplexní prostřední sloužit predátorům jako místo pro útok ze zálohy (Flynn & Ritz, 1999), nebo jim umožnit obsáhnout rozsáhlejší část prostředí (Klecka & Boukal, 2014). Celkem lze tedy říci, že naše schopnosti interpretovat strukturu prostředí z hlediska

utváření společenstev jsou omezené (Kovalenko *et al.*, 2011). Navíc může docházet k neočekávaným důsledkům díky kombinaci vlivu struktury prostředí a rizika predace, kterému se detailně věnuji níže.

Vliv rizika predace

Důsledky přítomnosti predátora můžeme rozlišit podle mechanizmu, jakým ovlivňuje vznik společenstva, na vlivy letální (důsledky aktivní predace) a neletální (vyvolané pouhou přítomností predátora). V literatuře se někdy používají další obdobné termíny, jako např. konzumptivní a nekonzumptivní vlivy (McCauley, Rowe & Fortin, 2011). Letální vlivy můžeme dále rozdělit na přímé (predace na kořisti) a nepřímé (důsledky úbytku kořisti).

Přímým letálním vlivem je úbytek kořisti lovené predátorem, eventuálně snížení fitness kořisti, která byla napadena, ale unikla (Begon *et al.*, 2005). Přímý letální vliv predátora na kořist můžeme kvantifikovat například stanovením funkční odpovědi predátora (Holling, 1959) na kořist v laboratorním prostředí.

Nepřímé letální vlivy zahrnují důsledky úbytku kořisti usmrcené predátorem. Příkladem může být trofická kaskáda, tedy jev, kdy vrcholoví predátořiorganizmy vyšší trofické úrovně prostřednictvím predace nepřímo ovlivňují organizmy na nižších trofických úrovních, přičemž se u jednotlivých úrovní střídá pozitivní a negativní vliv (Begon *et al.,* 2005; O'Connor *et al.,* 2012). Trofickou kaskádu ve sladkých vodách nejčastěji způsobuje rybí predátor (Nyström, Svensson & Lardner, 2001; Vonesh *et al.*, 2009), ale mohou ji vyvolávat i bezobratlí predátoři (Stav, Blaustein & Margalit, 2000). V přírodních podmínkách navíc často působí několik predátorů zároveň. To může vést k posílení kaskádového efektu (Nyström *et al.*, 2001) nebo k jeho oslabení, například vlivem predace ve vlastní potravní gildě (tzv. intraguild predace). Dalším nepřímým letálním vlivem je změna konkurenční rovnováhy, kdy jinak konkurenčně schopnější kořist může být vytlačena v důsledku ztrát predací (Nyström *et al.*, 2001). Mezi nepřímé neletální vlivy také patří regenerace živin, kdy predátor konzumací biomasy a následným uvolňováním živin exkrecí urychluje koloběh živin (Stav *et al.*, 2000).

Neletální vlivy působené predátorem zahrnují například změnu chování kořisti (Resetarits, 2001), změnu jejího životního cyklu (Riessen, 1999; Riessen & Young, 2005) nebo výběr stanoviště daný vyhýbáním se predátorovi (Silberbush & Blaustein, 2011). Někteří kolonizátoři mají možnost vyhodnotit vhodnost stanoviště a podle toho se rozhodnout do něj vstoupit nebo jej opustit. Při rozhodování mohou využívat vizuální,

mechanické nebo chemické stopy. Klíčovou roli při tom hrají kairomony, což jsou charakteristické chemické stopy predátorů, které nechtěně vylučují do prostředí (Brönmark & Hansson, 2012).

Další kolonizátoři kladou vajíčka do vodního prostředí a umí posoudit vhodnost konkrétního habitatu pro jejich potomstvo. Obvykle se přitom vyhýbají prostředí s predátory. Blaustein (1999) stanovil následující podmínky pro silný evoluční tlak na vyhýbání se kořisti predátorům při rozmnožování: (1) nedospělá stádia jsou vysoce zranitelná detekovaným predátorem, (2) všechna vejce jsou kladena jako jedna snůška do jednoho prostředí, (3) kořist má během svého života jen málo reprodukčních cyklů (nejlépe jeden). Vyhýbání se prostředí s predátory při rozmnožování popsala celá řada studií (např. Leon, 1998; Stav *et al.*, 1999; Eitam *et al.*, 2002; Eitam & Blaustein, 2004). Příkladem mohou být komáři rodu *Culiseta* a *Culex* v mesokosmovém experimentu Eitama & Blausteina (2004), kteří ke kladení preferovali stanoviště bez predátora. Častým predátorem použitým ve studiích bývá ryba, které se při kladení vyhýbají např. komáři, pakomáři nebo také žáby (Vonesh *et al.*, 2009). Dále byly v roli predátorů studovány larvy vážek (Stav et al., 1999, 2000) nebo dravé ploštice (Blaustein *et al.*, 2004; Silberbush & Blaustein, 2011), které od rozmnožování nejčastěji odrazují různé komáry. Nakonec je nutno podotknout, že kolonizátoři vyhledávají k ovipozici prostředí s dostatkem živin (Fader & Juliano, 2014) a v malých stojatých vodách je přítomnost predátora signálem úživného a relativně stabilního prostředí. V některých případech je tedy evolučně výhodnější naklást vejce do prostředí, kde budou potomci vystaveni predaci, ale budou mít pravděpodobně dostatek času a potravy k vývoji (Sih, 1992; Albeny-Simões *et al.*, 2014)

V přirozeném prostředí predátoři vyvolávají letální i neletální vlivy současně. Pro pochopení jejich významu je ale nutné je od sebe experimentálně oddělit, například z těchto dvou důvodů: (1) Zatímco rozsah letálních vlivů predátora je většinou hustotně závislý (např. počet ulovených jedinců kořisti je určen typem funkční odpovědi predátora a množstvím kořisti, počet živin uvolněných predátorem v důsledku jeho predace je úměrný počtu ulovené kořisti, apod.), neletální vlivy predátora mohou mít rozsáhlý efekt nezávislý na hustotě (přítomnost několika jedinců predátora nemusí být kolonizátory rozeznána jako odlišná od přítomnosti stovek jedinců predátora – Eitam & Blaustein, 2004). (2) Neletální vlivy predátora mohou být vyvolány i uměle, například vpravením kairomonů – „cues" do prostředí nebo umístěním atrap predátora v prostředí (Bell *et al.*, 2011).

Interakce struktury prostředí a predace

Struktura prostředí má stejně jako riziko predace rozmanitý vliv na utváření společenstva. Vliv obou těchto faktorů se může v některých případech navzájem rušit nebo naopak posilovat. Struktura prostředí může poskytovat úkryt kořisti (Burks, Jeppesen & Lodge, 2001) nebo ovlivňovat úspěšnost lovu (Warfe & Barmuta, 2004). Na druhou stranu v strukturně složitých habitatech může kořist ztratit možnost detekovat riziko predace nebo se stát zranitelnější (Warfe & Barmuta, 2006; Klecka & Boukal, 2014). Jakým způsobem se bude vyvíjet společenstvo ve strukturovaném prostředí vystavené jak letálnímu, tak neletálnímu riziku predace současně není zatím možné odhadnout ani kvalitativně. Chybí totiž ucelená teorie, která by v sobě všechny tyto hlavní vlivy zahrnovala.

Všežravec jako vrcholový predátor

Všežravci přijímají jako potravu konzumenty i producenty (Brönmark & Hansson, 2005). Tím se vymykají klasickému chápání potravních vztahů s diskrétními trofickými úrovněmi standardního potravního řetězce (Nyström *et al.*, 2001).

Raci jsou příkladem bezobratlých všežravců, kteří mají zásadní vliv na utváření společenstva. Jsou schopni se v krajině šířit podél toků a tůní, živí se živou i mrtvou, rostlinou i živočišnou potravou, bezobratlými i obratlovci (Dorn & Wojdak, 2004). U nás i jinde ve světě jsou mnozí raci invazními druhy, které byly za různými účely vysazeny mimo svůj přirozený areál rozšíření nebo nevědomky zavlečeny (Geiger *et al.*, 2005). Jelikož mají raci klíčové postavení v trofické síti malých stojatých vod, může mít jejich rozšíření dalekosáhlé důsledky pro druhovou bohatost bezobratlých a pokryvnost makrovegetací (Usio *et al.*, 2009), úživnost a turbiditu (Dorn & Wojdak, 2004), apod. Navzdory obavám z narušení ekosystémů ztrátou původních druhů se ale ukazuje, že některé ekosystémové služby zastávají invazní raci stejně jako původní druhy (Ercoli *et al.*, 2014; Lagrue *et al.*, 2014). Pochopení jejich vlivu je komplikováno mimo jiné právě jejich omnivorií.

Příklad složitého působení raka na společenstvo představuje pozorování Dorna a Wojdaka (2004), kteří v tůních s rakem *Orconectes virilis* (Hagen, 1870) v terénním pokusu pozorovali posun ve druhovém složení perifytonu, úbytek plžů a pulců oproti tůním bez raka. Z počátku se zdálo, že rak vytváří trofickou kaskádu a zvyšuje biomasu perifytonu, ale později (následující sezónu) se rozdíly mezi kontrolními nádržemi a nádržemi s rakem setřely. Rak zde jednak přímo (selektivně) konzumoval perifyton, ale také lovil jeho spásače. Zároveň přispíval k regeneraci živin a tím dočasně podpořil růst perifytonu. Raci v souladu

s teorií optimálního příjmu potravy (optimal foraging theory, Stephens & Krebs, 1986) vyhledávají energeticky nejvýhodnější potravu. Jako všežravci mají široké potravní spektrum, v rámci kteréhož se mohou přizpůsobovat. To naznačuje, že (1) v rámci různých stanovišť mohou zaujmout různé trofické postavení a (2) mohou své trofické postavení měnit v čase podle dostupnosti různých složek potravy. Síla trofických interakcí vůči různým skupinám se také může měnit v rámci ontogeneze raka (Geiger *et al.*, 2005; Anastácio *et al.*, 2011). Navzdory tomu Johnston, Robson a Fairweather (2011) ukázali na čtyřech druzích invazních raků, že si mohou zachovávat charakteristickou trofickou pozici napříč sezónami, stanovišti a gradientem dostupnosti potravy. Podobně stálé potravní spektrum si během vývoje podle Chucholla (2012) zachovává i *Orconectes immunis* (Hagen, 1870).

Výsledek dlouhodobého působení těchto protichůdných vlivů v podobných situacích zatím není možné předpovědět, ale tento pokus přinejmenším naznačuje, že dopad raka nebo jiného všežravce jako vrcholového predátora se může měnit v čase. Raka rodu *Orconectes* jsem použil i ve svém experimentu.

Shrnutí

Struktura prostředí má významný vliv na utváření společenstev stojatých vod. Ovlivňuje zejména dostupnost mikrohabitatů (Gotceitas & Colgan, 1989), množství perifytonu a detritu (Taniguchi & Tokeshi, 2004) a sílu trofických interakcí (Flynn & Ritz, 1999; Klecka & Boukal, 2014). V kombinaci s rizikem predace, které je klíčové pro utváření potravních vztahů (Sih *et al.*, 1985), tak tvoří dvě zásadní složky určující vývoj společenstev v malých stojatých vodách. Raci jsou hojně studovaní zejména pro jejich invazní potenciál (Dorn & Wojdak, 2004; Usio *et al.*, 2009). Jako všežravci mají na utváření společenstev kombinovaný vliv, který zatím není uspokojivě popsán (Dorn & Wojdak, 2004; Lagrue *et al.*, 2014).

V literatuře zabývající se utvářením společenstev zatím chybí ucelená práce, která by současně vyhodnotila letální i neletální vlivy predace všežravým predátorem v kombinaci se strukturou prostředí na utváření společenstev, zatím chybí. Vliv struktury prostředí popsala řada laboratorních (Warfe & Barmuta, 2004; Burdett & Watts, 2009; Klecka & Boukal, 2014) i terénních (Taniguchi & Tokeshi, 2004; Thomaz *et al.*, 2008; Walker, Wijnhoven & van der Velde, 2013) experimentů a pozorování. Studium vlivu rizika predace se soustředilo hlavně na ovipozici (Stav *et al.*, 2000; Eitam et al., 2002; Blaustein *et al.*, 2004), a to zejména v kontrolovaných podmínkách. Zkoumán byl také celkový vliv predátora na biomasu kolonizátorů (Usio *et al.*, 2009) nebo jen jeho neletální vlivy, např. na rychlost růstu pulců

(Werner & Anholt, 1996) nebo abundanci kolonizátorů (Soukup, 2013). Některé práce se snažily rozlišit neletální a letální vlivy působící zároveň (Vonesh *et al*., 2009), další zkoumaly vliv struktury prostředí na efektivitu predace (Warfe & Barmuta, 2004; Canion & Heck, 2009) nebo trofické vztahy více predátorů (Flynn & Moon, 2011), nebo společný vliv struktury prostředí a neletální přítomnosti predátora na utváření společenstva (Soukup, 2013).

Na základě těchto prací mohu vyslovit hypotézu, že letální přítomnost raka bude snižovat abundanci a biomasu zejména bentických skupin (např. Nyström *et al.*, 2001; Jackson *et al*., 2014). Předpokládám také, že neletální vlivy na celkovou abundanci a biomasu vzniklého společenstva budou slabé nebo zanedbatelné, jelikož kolonizátoři nedokáží přítomnost tohoto invazního druhu zaznamenat. Dále usuzuji, že ve struktuně bohatším prostředí bude větší biomasa a abundance kolonizátorů, zejména primárních konzumentů.

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Habitat complexity and predation risk effects on community assembly in small standing waters

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Summary

1. Predation risk and habitat complexity are two key factors that shape community assembly in small standing water bodies, but their joint impact has never been examined.

2. We ran a full factorial experiment in which we have jointly manipulated habitat complexity and predation risk to test their effects on community assembly in small (~50 L volume) mesocosms. To distinguish between consumptive and non-consumptive predation risk effects, we used three levels of predation risk that consisted of no predators, one caged and one free-ranging Eastern crayfish (*Orconectes limosus*). To investigate the role of habitat complexity, we added artificial plant models in half of the replicates of each predation risk treatment. After 29 days, we evaluated the abundance, biomass and size spectra of the colonizing macroinvertebrates in the treatments.

3. The resulting communities were taxonomically depauperate. The five main groups colonizing our experimental containers included Chironomidae, Culicidae, Ephemeroptera (*Cloeon dipterum*), Coleoptera (mainly *Hydroglyphus geminus*) and Gastropoda (*Gyraulus albus*).

4. While the experimental treatments had almost no effect on the abundance of any of the five main taxonomic groups, we found multiple effects of both habitat complexity and predation risk on total biomass and size spectra. This suggests that abundance estimates commonly used in community studies and biodiversity inventories are likely to miss important information on the processes structuring natural communities.

5. Both habitat complexity and predation risk altered biomass and abundance of individual groups differentially. Habitat complexity increased biomass of Ephemeroptera. Gastropoda and Chironomidae had lower total biomass in containers with predation risk. Indirect and direct effects of the crayfish predator altered the community structure similarly.

6. Our results suggest that the join effect of habitat complexity and predation risk can often be deduced from separate studies of habitat complexity effects and predation risk effects. Our results also have implications for conservation biology and management of invasive species: they indicate that the main impact of omnivorous invasive crayfish, such as the one used in our study, could be deduced from widely known effects of top predators on prey communities.

Introduction

Community assembly in small water bodies is a rapid process, easily influenced by a number of factors. Most important drivers of community assembly include local species pool (Leibold *et al.*, 2004), dispersal rates (De Bie *et al.*, 2012), colonization history (Drake, 1991; Chase, 2003), local population density (Leibold *et al.*, 2004), environmental productivity (Chase, 2010), and abiotic conditions such as temperature. Besides these, habitat complexity and predation risk have been widely recognized to influence community assembly dramatically. Both of them can also be manipulated for various purposes such as disease prevention (Lou & Zhao, 2011) or invasive species control (Alexander *et al.*, 2014).

In small standing waters, the presence of a top predator in the early stage of community assembly may cause a number of direct (consumptive) and indirect (non-consumptive) effects (McCauley, Rowe & Fortin, 2011). The direct effects most commonly include reduction of prey abundance (Leon, 1998; Stav, Blaustein & Margalit, 2000), emergence of a trophic cascade (Stav *et al.*, 2000; Nyström *et al.*, 2001; O'Connor *et al.*, 2012), apparent competition and changes in competition mediated by selective predation (Holt, Grover & Tilman, 1994; Stav *et al.*, 2000; Nyström *et al.*, 2001). The indirect effects often involve habitat selection and oviposition preferences of the potential prey (Stav, Blaustein & Margalith, 1999; Eitam, Blaustein & Mangel, 2002; Eitam & Blaustein, 2004; Blaustein *et al.*, 2004; Silberbush & Blaustein, 2011) or changes in both prey and predator behaviour (Werner & Anholt, 1996; Burks, Jeppesen & Lodge, 2001; Resetarits, 2001).

Despite the fact that direct and indirect effects always occur simultaneously in nature, it is crucial to distinguish them. Some indirect effects can be density independent. For example, ovipositing insects may only determine if a predator is present but may not be able quantify the associated predation risk (Eitam & Blaustein, 2004). On the other hand, direct effects are mostly density dependent; for example, the amount of prey eaten increases with on number of feeding predators. Moreover, indirect effects such as predator avoidance can be evoked artificially, e.g., by adding predator cues into the environment (Bell *et al.*, 2011).

While both direct and indirect predator effects contribute to top-down community control (Hairston, Smith & Slobodkin, 1960), habitat complexity changes often influence the community on a bottom-up basis (Diehl, 1992). In particular, increasing habitat complexity in small water bodies can enlarge surface area and create more niches, thus increasing

abundance and species richness (Diehl, 1992; Kovalenko, Thomaz & Warfe, 2011). Because predator avoidance while ovipositing may be outweighed by resource availability (Albeny-Simões *et al.*, 2014), complex habitats might be preferred regardless of predation risk.

Habitat complexity can therefore play an important role of mediating the strength of top-down control. It commonly provides refuges for prey and lessens predation efficiency of some predators (Canion & Heck, 2009; Flynn & Moon, 2011), while providing cover and improving prey detection or increasing the habitat domain for others (Klecka & Boukal, 2014). Indirect effects of predation can also be magnified by increased habitat complexity: the joint effect of trophic cascade and increased surface area can facilitate numbers of primary consumers (Diehl, 1992). On its own, increased habitat complexity usually leads to an overall increase in the abundance of species (Burdett & Watts, 2009) and biodiversity (Walker, Wijnhoven & van der Velde, 2013). Both predator presence (Resetarits 1996) and more complex habitats (Boven et al. 2008) can be suggestive of longer-lasting water bodies and influence habitat selection of species with longer developmental times.

Assessing the intensity of the effects related to predation risk or habitat complexity is often based on comparing abundances across taxonomical or trophic groups. Moreover, many important traits change with individual body size (Werner & Gilliam, 1984). Since macroinvertebrates span one to several orders of magnitude of length during their ontogeny, their diet and trophic interactions can change rapidly (Rudolf & Rasmussen, 2013). Body size alone can therefore be used as a good proxy of functional differences (Rudolf *et al.*, 2014). Hence a combination of abundance-based and size-structured approaches to comparison of different communities seems beneficial (Gilljam *et al.*, 2011) and a comparison of abundances and size structure can provide further information important for better understanding of ecological data (Woodward *et al.*, 2005). In particular, the state of the community is often described by a size spectrum, i.e. the allometry describing the relationship between individual body size and abundance (Sheldon, Prakash & Sutcliffe, 1972).

Both field studies (e. g. Thomaz *et al.*, 2008; Walker *et al.*, 2013) and laboratory experiments (e. g. Burdett & Watts, 2009; Klecka & Boukal, 2014; Grutters *et al.*, 2015) examined the influence of habitat complexity on community assembly. Some of them focused on the modifying effect of habitat complexity on trophic interactions (Flynn & Ritz, 1999; Warfe &

Barmuta, 2004; Canion & Heck, 2009; Flynn & Moon, 2011). The effect of omnivorous predators has been the subject of recent studies (Dorn & Wojdak, 2004). To our knowledge, however, no study has examined both direct and indirect effects of an omnivorous predator on community assembly along a habitat complexity gradient.

To fill this gap, the main purpose of this study was to assess abundances, biomass and size spectra of colonizing organisms in a mesocosm experiment in order to: (1) identify direct and indirect effects of predation by an omnivorous invasive crayfish *Orconectes limosus* on prey assemblages, (2) examine the effect of increased habitat complexity on community assembly, and (3) investigate how habitat complexity modifies direct and indirect predator effects on community assembly. We chose this predator because invasive species have the potential to influence their environment dramatically (Sakai *et al.*, 2001) and crayfish are frequent invaders in small standing waters in continental Europe and elsewhere (Geiger *et al.*, 2005). They are omnivorous and, to varying degrees, simultaneously interact with multiple trophic levels (McCann & Hastings, 1997; Nyström *et al.*, 2001). Whether trophic positions of crayfish change regularly and how much this affects their ecosystem function and, consequently, their impact as top predators on community assembly, remains uncertain (Anastácio *et al.*, 2011; Johnston, Robson & Fairweather, 2011).

Methods

Experimental protocol

We conducted the outdoor mesocosm experiment at the main campus of the University of South Bohemia in České Budějovice (Czech Republic, GPS: N 48°58'31", E 14°26'48"). It was conducted as a 3×2×3 full factorial design with three levels of experiment duration (10, 20 or 29 days) in 94 half-buried, cylindrical plastic containers (approximate volume 45 L, water depth ~25 cm). Only the last sampling (N=34) was analysed in this study. We used two levels of habitat complexity with artificial plants present (N=17) or absent (N=17) and three levels of predation risk with a free-ranging (N=12), caged (N=12) and absent predator (N=10). Eastern crayfish (*Orconectes limosus*) obtained from experimental populations maintained at the Faculty of Fisheries and Protection of waters of the University of South Bohemia was used as a model predator (carapax length: 17–24 mm, wet weight: 0.94–3.58 g). Empty cages were put in the pools with free-ranging or no crayfish. Thus we obtained 6 treatments with 5–6 replicates each. The containers were arranged in 4 double rows. All treatments (including the experimental duration) were distributed evenly across the double rows but randomly within each row.

The containers were filled with tap water a day before the experiment and the bottom of each container covered with fine gravel. The experiment began on the morning of 5 August 2014 by adding the artificial vegetation, predators (where appropriate), and 0.5-L aliquots of a well mixed inoculum of both phyto- and zooplankton into each pool. The inoculum was obtained with a ring plankton net (20 cm in diameter, mesh size 40 μm) from a nearby pond (Dubenský rybník, N 48°58'48", E 14°22'36").

Water temperature was monitored 5 cm above the bottom in 15 randomly chosen containers using HOBO® Pendant® Temperature dataloggers (Onset Computer Corporation, Massachusetts, USA) during the experiment. It ranged between 11.6 and 28.2 °C (mean ± SE: 18.0 ± 2.9 °C). The weather changed dramatically after the first 8 days (21.9 ± 2.5 °C), when it got cold for the rest of the experiment (16.8 ± 1.7 °C). Extreme temperatures were rare (Appendix S3). Daily minima and maxima differed by 5.6 ± 2.3 °C.

We did not observe temperature stress signals in the predators (based on fish stress signals, Nakata *et al.*, 2002) during their daily visual control, and hence assume that the effects on the community assembly in our experimental system could be extrapolated to natural

conditions. Crayfish were not fed to prevent the input of external nutrients into the environment. Free-ranging crayfish had ingested food visible when we inspected their abdomen. We therefore presume that they fed readily on colonizers, although we have no information on the composition of their diet. Caged crayfish fed very rarely, presumably on organisms that swam or crawled into their cages.

We sampled the replicates destructively after 29 days (on 4 September 2014) by filtering the entire content of each container through a 200 µm net. Predator cages and artificial vegetation were washed in the container prior to sampling and taken out. Gravel was washed thoroughly in the container using the initial volume of water and then added tap water. No more macroinvertebrates were observed in the gravel after this procedure. The samples were stored in 80% ethanol and all aquatic macroinvertebrates (N=7918) were identified to one of the 5 taxonomic groups that essentially corresponded to different trophic levels and habitat domains: Coleoptera (predatory, benthic), Diptera: Culicidae (primarily detritivorous, occupying water column), Diptera: Chironomidae (primarily detritivorous, benthic), Ephemeroptera (primarily herbivorous, benthic/phytophilous), and Gastropoda (herbivorous, benthic). We further identified part of larval Coleoptera to genus (Galewski, 1998) all other Coleoptera, Ephemeroptera and Gastropoda to species (Ložek, 1956; Rozkošný, 1980).

In community assembly studies, all Chironomidae are often considered as a uniform group of generalist detritivores (Burdett & Watts, 2009; Walker et al., 2013; Lagrue et al., 2014). We therefore did not identify Chironomidae larvae further and treated them all as one group for the purpose of this study.

Measurements of body size

Body length (without antennae and abdominal appendages) of each individual was measured either by hand (Coleoptera, Ephemeroptera, N=674) or using a semi-automated procedure (Diptera, N=5749) by analysing pictures taken with computer assisted stereomicroscope Olympus SZX10 (magnification 3.15×) in Quick Photo Camera 2.3 (PROMICRA, s.r.o., Czech Republic) software. The resulting binary image was then processed in the NIS-Elements software (Laboratory Imaging, Ltd., Czech Republic) to identify individuals in the photographs and FIJI plugin "analyse skeleton" (Polder et al., 2010) was used to measure their length. Shell diameter was measured as a proxy for size in Gastropoda.

To correct for possible errors of the semi-automated procedure, we re-measured by hand a subset of individuals spanning the minimum-maximum length interval (Chironomidae: N=103, range 0.6–17.3 mm; Culicidae: N=107, range 1.2–8.6 mm). We then approximated the error of the automated measurement, expressed as a function of measured length, with polynomial regression and compared polynomials of increasing degree and identified the best model based on the smallest AIC value. We then corrected the automated measurements by the estimated length-dependent measuring error (Appendix S2).

We used published length-mass equations to estimate dry mass for each individual. When multiple equations were available, we chose one according to criteria established by Benke et al. 2010. We were not able to find published equations for some specific taxa. In that case, we chose equations for the morphologically and taxonomically closest taxa (Table 1 and Table S1). Coleoptera larvae (*Hydroglyphus geminus*, N=1495) changed shape due to their preservation in ethanol. Therefore, instead of measuring them, we classified them into three size categories corresponding with larval instars, dried 10 individuals of each instar at 60 °C for 8 hours, weighed them to the nearest 0.001 mg, and assigned the average weight to all individuals of the same instar.

Statistical analyses

All analyses were carried out in R version 3.0.2 (R Core Team, 2013). Overall biomass and abundance were calculated separately for each of the five groups in every container. Data contained few zero values but were overdispersed, so we chose generalized linear model with negative binomial distribution to interpret the abundance changes in different treatments (predation risk × habitat complexity) (Ver Hoef & Boveng, 2007). Interactions and factors were eliminated in a stepwise algorithm to reach a final model with the lowest AIC. To meet the requirements of the model (integer values), we used rounded weight in µg in the biomass analysis.

To analyse size spectra we used standardized major axis regression because both the dependent and independent variables were measured with error (Warton *et al.*, 2002). In the analysis we focused on the size ranges in which abundance declined with body mass. To identify this range, a histogram of weights was first plotted for each combination of

taxonomic group and treatment to create size classes. We then split the data in two parts and used only the size range above the body size at which the maximum frequency was reached. We then took the mean size of each size class used in the histogram and its frequency and log-transformed their values prior to analysis. We used the package smatr-3 to fit the allometries linking body size and abundance and to compare the slopes of the resulting size spectra between individual treatments (Warton *et al.*, 2012). At this point, no method is able to compare size spectra that are a function of two independent factors, and we thus considered each of the six treatments as independent.

Results

The diversity in our samples was low (Table 1). Only one beetle, the small predatory diving beetle *Hydroglyphus geminus*, reproduced in the experimental containers. Adults of this species were also abundant, although we also found a few individuals of other Dytiscidae and Hydrophiloidea. Ephemeroptera were represented by only one species, *Cloeon dipterum*, a grazer that mainly feeds on periphyton (Zahrádková *et al.*, 2009). Only one species of snail appeared in the samples, the herbivorous *Gyraulus albus*, which was most likely introduced with the zooplankton inoculum. Larvae of Culicidae most probably belonged to a single genus, *Culex*. Its larvae are highly mobile suspension feeders who most often filter the water column for microalgae and fine detritus (Merritt, Dadd & Walker, 1992). Chironomidae were represented by at least 6 different species in the samples, some of which were predatory (P. Soukup, unpublished data), but we treat them for simplicity as one group in this paper.

Effect of habitat complexity and predation risk on abundances

We observed no significant effects of either predation risk or habitat complexity on abundances of all groups except adult Coleoptera (Fig. 1 and Table 2). Adult Coleoptera were significantly less abundant in the presence of a caged predator (negative binomial GLM: coefficient = -0.77 ± 0.39, P = 0.046). They were moreover less common in treatments with free predator and more common in the complex habitat treatments, both effects were of similar, opposing magnitude but neither was significant (negative binomial GLM, free predator: coefficient = -0.49 ± 0.38, P = 0.19, habitat complexity: coefficient = 0.47 ± 0.31, P = 0.13).

Effect of habitat complexity and predation risk on total biomasses

Habitat complexity and predation risk significantly affected total biomass of all groups except Coleoptera adults and larvae (Fig. 2 and Table 3). Coleoptera larvae could not be fitted with appropriate model, but visual inspection of data suggests a slight negative effect of the interaction of habitat complexity and predation risk posed by a free-ranging predator. Biomass of Ephemeroptera was higher in treatments with habitat complexity (negative binomial GLM: coefficient = 1.88 ± 0.24, P < 0.001) and did not change significantly across the gradient of predation risk. On the other hand, biomass of Culicidae and Gastropoda decreased in complex habitats irrespective of the predation risk, but the effect was not

significant (negative binomial GLM, Culicidae: coefficient = -0.86 ± 0.53, P = 0.11, Gastropoda: coefficient = 0.54 ± 0.35, P = 0.13).

Predation risk lead to significant changes in biomass only in Gastropoda and Chironomidae. Biomass of Gastropoda was significantly lowered by predation risk and the impact of both caged and free-ranging predator was comparable (negative binomial GLM, caged predator: coefficient = -1.20 ± 0.44, P = 0.006, free-ranging predator: coefficient = -1.23 ± 0.44, P = 0.005). The results for Chironomidae were most complex and indicative of a non-additive effect of habitat complexity and predation risk. In the absence of predation risk, their biomass was lower in the complex habitat although the effect was not significant (negative binomial GLM, habitat complexity: coefficient = -1.15 ± 0.87, P = 0.19). The impact of predation risk was opposite in the treatments with and without added complexity: biomass decreased under predation risk and the impact of both caged and free-ranging predator was comparable in the absence of artificial plants (negative binomial GLM, caged predator: coefficient = -2.25 ± 0.83, P = 0.007, free-ranging predator: coefficient = -2.06 ± 0.83, P = 0.013), while the presence of both caged and free-ranging predator increased the biomass when the artificial plants were present (negative binomial GLM: interaction between habitat complexity and caged predator: coefficient = 2.56 ± 1.18, P = 0.029, interaction between habitat complexity and free predator: coefficient = 2.10 ± 1.18, P = 0.074). Again, the impact of both caged and free-ranging predator was comparable although the latter effect was marginally insignificant.

Size structure analysis

Habitat complexity and predation risk significantly affected the slopes of size spectra of Culicidae, Chironomidae and Gastropoda (Fig. 3, Table 4). The analyses of Coleoptera and Ephemeroptera did not produce sensible data due to low number of individuals (P. Soukup, unpublished data). All size spectra slopes were significantly negative and their mean estimate ranged between -0.60, -1.59 for Chironomidae, -4.95, -1.67 for Culicidae and -0.56, -2.63 for Gastropoda. That is, the size spectra of Gastropoda were shallower than those of the Chironomidae but steeper than those of the Culicidae. Moreover, the confidence intervals for the estimates of the mean slopes were considerably larger for Culicidae than for the other two groups, indicating lower reliability of the results.

With a few exceptions, slopes of the size spectra within each group were not significantly different between treatments. Size spectrum of Chironomidae was significantly steeper in the

structurally simple habitat with a caged predator, mainly due to the absence of larger individuals, than in other treatments (Fig. 3A). We found a similar result in Culicidae, where only the size spectrum in structurally simple habitat with a free-ranging predator was significantly shallower than the size spectrum in complex habitat with a caged predator (Fig. 3B). We obtained a largely opposite results for Gastropoda, in which the size spectrum in structurally simple habitat with a free-ranging predator was significantly steeper than the size spectra in both complex and simple habitats with a caged predator (Fig. 3B).

Discussion

Community assembly in aquatic habitats is driven by a number of interacting biotic and abiotic factors. Among others, both predation risk and habitat complexity can alter early stages of community assembly beyond what could be predicted from species composition in neighbouring habitats and abiotic factors (Chase, 2003, 2010; Hein & Gillooly, 2011). Previous studies dealt separately with habitat complexity (Taniguchi & Tokeshi, 2004; Burdett & Watts, 2009; Walker *et al.*, 2013) or predation risk (Stav *et al.*, 2000; Dorn & Wojdak, 2004; Usio *et al.*, 2009; Vonesh *et al.*, 2009) as a determining factor for community assembly in small water bodies. Research on community assembly combining these two factors is, however, scarce and mostly focuses on underlying mechanisms such as predation effectivity (Jordan & McCreary, 1996; Flynn & Ritz, 1999; Warfe & Barmuta, 2004) as opposed to overall patterns in community assembly. To fill this gap, our manipulative experiment focused on differences in the structure of colonizing macroinvertebrates in a small freshwater habitat.

As trophic interactions change with body size (Rudolf & Rasmussen, 2013; Rudolf *et al.*, 2014), our emphasis was on the changes in size structure of the community. We characterized the community structure by total abundance, total biomass and size spectra of the main taxonomic groups, thereby focusing mainly on overall traits of the resulting community rather than using the more widespread biodiversity approach. Most importantly, while the experimental treatments had almost no effect on the abundance of any of the five main taxonomic groups (with the exception of adult Coleoptera, but their numbers were generally low), we found multiple effects of both habitat complexity and predation risk on total biomass and size spectra. This suggests that abundance estimates commonly used in community studies and biodiversity inventories are likely to miss important information on the processes structuring natural communities.

We also found that the responses of the five main taxonomic groups to habitat complexity and predation risk were not uniform. However, we found no evidence of non-additive effects of both factors except in the responses of total biomass in Chironomidae, but the effect was clearly minor. We therefore discuss the effects of habitat complexity and predation risk for the five main taxa separately.

Habitat complexity affected the abundances and biomass of each group differentially, suggesting that the response of taxa to habitat complexity during community assembly in our study was idiosyncratic. We did not detect any significant effect of habitat complexity on abundance of any taxonomic group except adult beetles, for which the effect was positive but not significant, possibly due to a low number of replicates. Presence of macrophytes is characteristic of longer lasting pools (Boven *et al.*, 2008) with more predators (Resetarits, 1996; Wellborn, Skelly & Werner, 1996). In our experiment, most adult beetles were the small predatory diving beetles *Hydroglyphus geminus*, which occupy a wide range of habitats ranging from temporary puddles to *Sphagnum* peat bogs (Boukal *et al.*, 2007). Our results suggest that they might be more attracted to complex habitats.

The effect of habitat complexity on total biomass and size spectra differed between groups. In the presence of habitat complexity, the biomass of Ephemeroptera (*Cloeon dipterum*) increased, while the biomass of Gastropoda (*Gyraulus albus*), Chironomidae and Culicidae decreased, although the latter effect was always insignificant. The increase in Ephemeroptera biomass was most likely due to increase in food supply and hence faster growth of the indiduals, because the models of macrophytes increased surface area on which peripython, consumed by herbivore scrapers such as *Cloeon*, may grow (Walker *et al.*, 2013). This bottom-up effect seems characteristic of plants, either artificial or real: Taniguchi & Tokeshi (2004) used bottom substrate heterogeneity as a measure of habitat complexity and observed larger numbers of smaller individuals of Ephemeroptera in more complex habitats, while maintaining nearly constant biomass along the complexity gradient.

While the apparent decrease in biomass of Gastropoda in complex habitats is difficult to explain, given that they feed on decaying biomass as well as periphyton and hence often prefer complex habitats with macrophytes (e. g. Walker *et al.*, 2013), the lower biomass of Culicidae and Chironomidae in containers with artificial plants could be due to slightly lower abundance (compare Figs 1 and 2), different species composition, later oviposition, slower growth, or predation risk by predatory Coleoptera colonizing slightly more the complex habitat in the experiment. It is possible that diving beetles in our experiment preyed occasionally on *Culex* as in (Klecka & Boukal, 2012) and the larvae grew more slowly as a behavioural response to predation risk (see also below). Size spectra analysis suggested that

at least some of the size spectra of Culicidae in the complex habitat were steeper than in the simple habitat, providing further support for our hypothesis. The results for Chironomidae were more complex and affected also by predation risk, and we discuss them in detail below.

Our results suggest that the primary mechanism driving the differences in community assembly between simple and complex habitats was the increased availability in periphyton in the complex habitat. Intermediate predators attracted to the complex habitat might have also influenced abundance and biomass of organisms on lower trophic levels. In sum, we hypothesize that habitat complexity alone might not be overly important for species colonizing small and potentially temporary water bodies. It is likely that macrophytes serve other functions beyond increasing the complexity that make habitats with aquatic plants more suitable for various groups of macroinvertebrates (Grutters *et al.*, 2015).

We focused purely on the role of habitat complexity and therefore did not use live plants in the experiment, which could have affected our results (Grutters *et al.*, 2015). For example, Burdett & Watts (2009) observed no effect of plastic models of vegetation on the abundance of Culicidae and Chironomidae but found more Culicidae and Chironomidae in complex habitats with live plants, indicating that plant-animal interactions determine the suitability of vegetated water bodies for these groups. Walker *et al.* (2013) observed no preference of Chironomidae for any specific plant, suggesting that this interaction is general rather than species specific. Culicidae and Chironomidae probably benefit from fine detritus produced by plants decay, which provides their food source (Merritt *et al.*, 1992).

Effect of predation risk

Similar to habitat complexity, predation risk had very limited impact on abundance but a strong impact on total biomass and size spectra. Moreover, its effect on each group was different and the differences between caged and free-ranging predators were at most minor, suggesting that the responses of taxa to the top predator *Orconectes limosus* were dominated by non-lethal and indirect effects of predation risk and that the lethal effects were either negligible or compensated by bottom-up facilitation (Stav *et al.*, 2000).

We did not detect any positive effects of predation risk. There were significantly fewer adult beetles in habitats with predation risk posed by a caged predator. In the free predator treatment there were also fewer beetles, but the effect was not significant. The biomass of

Chironomidae and Gastropoda was significantly smaller in habitats exposed to predation risk by either caged or free predator, but their abundance did not differ from treatments with no predation risk. Moreover, the effect of predation risk appeared to buffer the effect of habitat complexity in Chironomidae biomass.

These results could arise from three different processes: (P1) Individuals colonized all treatments randomly and avoided predation (otherwise the results for free-ranging predators would have differed from the other two predator treatments) but grew at slower rates (Nyström *et al.*, 2001); or (P2) habitats with predators were colonized later when the costs of perceived predation risk became similar to the costs of competition, or (P3) colonization rate increased disproportionately in predation risk habitats due to positive cues (Albeny-Simões *et al.*, 2014), predation was selective towards larger individuals, and their growth was slower than in the absence of predators due to competition or predation risk.

In response to predator cues prey can become less active (Resetarits, 2001), attempt to hide (Gotceitas & Colgan, 1989; Oram & Spitze, 2013; Klecka & Boukal, 2014) or change energy allocation in favour of development as opposed to growth (Werner & Gilliam, 1984; Werner & Anholt, 1993). This alteration in behaviour may result in individuals growing slower and sometimes reaching smaller sizes altogether (as in process P1). Free predators release cues by actively feeding. These cues can be evaluated as undesirable due to increased risk of predation (Eitam *et al.*, 2002; Eitam & Blaustein, 2004; Blaustein *et al.*, 2004) but also desirable indicating a habitat suitable for conspecifics to colonize in numbers (Albeny-Simões *et al.*, 2014). Moreover, predators can promote nutrient regeneration and cause trophic cascade favourable for quickly developing grazers (Stav *et al.*, 2000). These effects could cause higher colonization rate compensated by size-selective predation and create a community with equal abundance, lower biomass and steeper size spectra slope (as in process P3). In what follows, we discuss the relevance of these processes for the main groups found in our study.

Avoidance of nonlethal fish predator by beetles was described by Resetarits (2001) who observed fewer egg cases in addition to fewer adults. Although we found fewer adults, we did not detect any effect on beetle larvae and therefore cannot make any inference on oviposition site selection. It is possible that adult Coleoptera moved between containers and stayed more often in habitats without predator cues released by both caged and free-ranging

predator. Total biomass of beetles did not change among treatments, possibly because larger species were less alarmed by predator cues and compensated the loss of biomass caused by fewer smaller beetles moving to habitats perceived as less dangerous.

Biomass of Chironomidae and Gastropoda decreased in the presence of predation risk caused by both caged and free-ranging predator in the simple habitat. Usio *et al.* (2009) reported no effect of crayfish predation on abundance of Chironomidae except for Tanytarsini. Creed & Reed (2004) found fewer Chironomidae, especially large ones, in river enclosures with crayfish. Since we detected no effect on abundance, the lower biomass must have been caused by a change in their average size. We found fewer large individuals in caged predator treatment in simple habitat in line with process P1 defined above, but not in other treatments.

Interestingly, the effect of predation risk on chironomid biomass was opposite in the complex habitat. Since most Chironomidae larvae are detritivores or algivores, the increase in periphyton availability in complex habitats might have provided more food resources and removed the negative effect of competition and override the effect of predation stress (process P3). Crayfish in our experiment were small (body length: 34–53 mm) but still large enough to prefer the largest Chironomidae larvae (body length > 3.15 mm, which represents the 75th percentile in our data) as their prey, since they commonly feed on larger prey (for example Trichoptera larvae, Usio *et al.*, 2009). However, size spectra analysis did not indicate the absence of large Chironomidae in free predator treatments, suggesting that they were preyed upon randomly. Free-ranging crayfish, unlike caged ones, could also disturb the sediment and help release nutrients from detritus (Creed & Reed, 2004). This could hide the impact of selective predation on the slope of the size spectrum. Finally, late colonization of free-ranging predator treatment (process P2) could also lead to lower biomass compared to other treatments. However, this would be noticeable in the size spectra analysis as a shift along a common slope, which we did not observe. It appears that the lower Chironomidae biomass in response to predation risk cause by a free-ranging predator was caused solely by stress-induced growth reduction.

Crayfish prey on snails and can control their population (Nyström et al., 2001; Dorn & Wojdak, 2004). In our experiment, however, Gastropoda abundance remained comparable in all treatments. Since we probably introduced the Gastropoda with plankton inoculum, they must have been distributed evenly among all containers. Turner & Montgomery (2003)

demonstrated that *Physa acuta* activity and growth can decrease dramatically in the presence of fish predator cues, and this behaviour was most pronounced in 1 m radius, which is larger than the size of our containers. Given that the biomass in both caged and free-ranging predator treatments decreased similarly, we conclude that the decrease in Gastropoda biomass was most likely caused by predator avoidance behaviour that led to slower growth, corresponding with our process P1, and the direct effect of predation was negligible.

Impact of environmental conditions and colonization rates

Community assembly in our study might have been driven mainly by stochastic processes (Chase, 2010). Although we found almost 8000 individual macroinvertebrates in total, abundances of most groups were much lower than in another similar experiment we ran in 2012 (P. Soukup et al., unpublished data). Temperature dropped rapidly after 8 days of the experiment (Appendix 3) and it rained every other day (P. Soukup, personal observation). These weather conditions could have decreased colonization rates. It is possible that some of the insignificant effects, e.g. those of habitat complexity, would become apparent if the experiment ran longer or in a warmer period.

Due to unfavourable weather conditions, crayfish activity might also have decreased. Low temperatures can lower their activity, but are certainly less harmful than high temperatures (Paglianti & Gherardi, 2004).

Moreover, a few randomly occurring large individuals could have altered the slopes of size spectra in Culicidae and Gastropoda. As a consequence, some of the significant differences that we found between the size spectra in individual treatments could have been driven by such random influences. It is therefore conceivable that neither habitat complexity nor predation risk affected the slopes of the size spectra in these two groups in our experiment, and that any differences should be ascribed to random effects.

Our study highlights the need for future studies to link the stochastic community assembly theory (Chase, 2010) to research on size-structured communities and to identify conditions under which differences in size spectra provide reliable signals of the effects of some underlying drivers on community assembly and dynamics.

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Tables

Table 1: Abundances, size ranges and length-mass relationships for the taxa observed in the experiment.

Order	Family	Species	N	Body length		Length-mass equation
				range (mm)	mean (mm)	
Coleoptera adults	Dytiscidae	*Agabus bipustulatus*	1	10.5	10.47	2
		Rhanthus suturalis	3	10.5–11.9	11.41	2
		Hydroglyphus geminus	92	2.1–2.9	2.45	2
		Graptodytes pictus	1	2.7	2.69	2
		Hydroporus augustatus	1	3.0	3.03	2
	Haliplidae	*Haliplus* sp.	3	2.9–3.3	3.01	3
	Helophoridae	*Helophorus* sp.	21	2.4–3.6	2.86	1
	Hydrophilidae	*Enochrus melanocephalus*	1	4.3	4.28	4
		Hydrobius fuscipes	5	3.3–8.0	6.63	4
		Helochares obscurus	2	3.2–5.6	4.39	4
		Anacaena lutescens	7	2.1–3.4	2.77	4
	Hydraenidae	*Limnebius truncatelus*	2	2.4–2.8	2.61	1
Coleoptera larvae	Dytiscidae	*Hydroglyphus geminus*	1495	0.0028–0.077*	0.04*	–
Diptera	Chironomidae	all	2240	0.6–15.8	2.65	6
	Culicidae	all	3509	1.2–8.1	2.92	8
Ephemeroptera	Baetidae	*Cloeon dipterum*	222	0.5–3.2	1.41	9
Pulmonata	Planorbidae	*Gyraulus albus*	313	0.7–6.2	1.61	13

Details for taxa observed in the experiment. When "all" is given for species the group was not determined to species level. N = number of individuals. *weight data, based on mean weights for each instar. Equation numbers as in Table S1.

Table 2: Abundance analysis details.

Taxon	Final model	(Intercept)	Habitat complexity (H)	Caged predator (P1)	Free predator (P2)	Habitat complexity × Caged predator (H*P1)	Habitat complexity × Free predator (H*P2)
Ephemeroptera	const.	(1.88 ± 0.24)***	–	–	–	–	–
Gastropoda	const.	(2.22 ± 0.13)***	–	–	–	–	–
Chironomidae	const.	(4.19 ± 0.19)***	–	–	–	–	–
Culicidae	const.	(4.63 ± 0.23)***	–	–	–	–	–
Coleoptera adults	H+P	(1.55 ± 0.32)***	0.47 ± 0.31	(-0.77 ± 0.39)*	-0.49 ± 0.38	–	–
Coleoptera larvae	const.	(3.78 ± 0.16)***	–	–	–	–	–

Final models, their coefficients for respective taxonomic groups. Models: const. = no effect of predator or habitat complexity; H = only habitat complexity effect; P = only predator effect; H+P = habitat complexity and predator effect; H*P = habitat complexity, predator and their interaction effect. Coefficients (log values; non transformed output of the model) are given as expected value ± 1 SE. Intercept = value of model coefficient for the treatment without predators or vegetation; all other values represent differences from the intercept. Significance levels: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; #, $P < 0.1$.

Table 3: Biomass analysis details.

Taxon	Final model	(Intercept)	Habitat complexity (H)	Caged predator (P1)	Free predator (P2)	Habitat complexity × Caged predator (H*P1)	Habitat complexity × Free predator (H*P2)
Ephemeroptera	H	(3.10 ± 0.49)***	(2.65 ± 0.69)***	–	–	–	–
Gastropoda	H+P	(7.97 ± 0.37)***	-0.54 ± 0.35	(-1.20 ± 0.44)**	(-1.23 ± 0.44)**	–	–
Chironomidae	H*P	(9.03 ± 0.61)***	-1.15 ± 0.87	(-2.25 ± 0.83)**	(-2.06 ± 0.83)*	(2.56 ± 1.18)*	(2.10 ± 1.18)[#]
Culicidae	H	(9.06 ± 0.38)***	-0.86 ± 0.53	–	–	–	–
Coleoptera adults	const.	(8.67 ± 0.30)***	–	–	–	–	–
Coleoptera larvae	–	–	–	–	–	–	–

Final models, their coefficients and predictions for respective taxonomic groups. All symbols and coefficients as in Table 2. For Coleoptera larvae, the dispersion parameter estimate was extremely high ($\theta = 1.4 \times 10^{11}$) and it was apparent that there is no effect of predator or habitat complexity.

Table 4: The effect of habitat complexity and predation risk on size spectra of the most abundant taxonomic groups in the experiment.

Taxonomic group	Habitat complexity	Predation risk	c_1 (CI 95%)	c_2 (CI 95%)
Chironomidae	no	none	0.62 (0.41, 0.83)	-0.62 (-0.80, -0.48)[a]
	no	caged	-1.40 (-2.36, -0.43)	-1.59 (-2.37, -1.07)[b]
	no	free	0.19 (-0.08, 0.46)	-0.60 (-0.78, -0.45)[a]
	yes	none	0.08 (-0.55, 0.71)	-0.94 (-1.70, -0.52)[ab]
	yes	caged	-0.05 (-0.64, 0.55)	-1.13 (-1.63, -0.78)[ab]
	yes	free	0.51 (0.20, 0.81)	-0.62 (-0.84, -0.45)[a]
Culicidae	no	none	-2.63 (-4.57, -0.67)	-3.98 (-6.36, -2.50)[ab]
	no	caged	-0.95 (-1.80, -0.10)	-2.47 (-3.60, -1.70)[ab]
	no	free	-0.17 (-0.95, 0.61)	-1.67 (-2.82, -0.99)[b]
	yes	none	-2.15 (-3.04, -1.27)	-2.84 (-3.73, -2.16)[ab]
	yes	caged	-3.91 (-6.36, -1.47)	-4.95 (-7.99, -3.07)[a]
	yes	free	-2.73 (-4.43, -1.03)	-3.74 (-5.93, -2.34)[ab]
Gastropoda	no	none	-1.55 (-2.38, -0.73)	-2.34 (-3.67, -1.49)[ab]
	no	caged	0.13 (-0.13, 0.40)	-0.56 (-0.82, -0.38)[a]
	no	free	-2.34 (-3.35, -1.32)	-2.63 (-3.82, -1.81)[b]
	yes	none	-0.12 (-0.44, 0.20)	-1.02 (-1.70, -0.61)[ab]
	yes	caged	-0.71 (-1.13, -0.30)	-1.11 (-1.54, -0.80)[a]
	yes	free	-0.54 (-1.13, -0.05)	-1.13 (-1.87, -0.7)[ab]

Results based on SMA regression with treatments as one factor. Coefficients: c_1 = elevation, c_2 = slope; 95% confidence intervals (minimum, maximum) are given in parentheses. Estimates of c_2 with different letters are significantly different from each other ($P < 0.05$). Results for Coleoptera and Ephemeroptera are omitted because SMA regression analysis did not produce sensible data due to low numbers of individuals (P. Soukup, unpublished data).
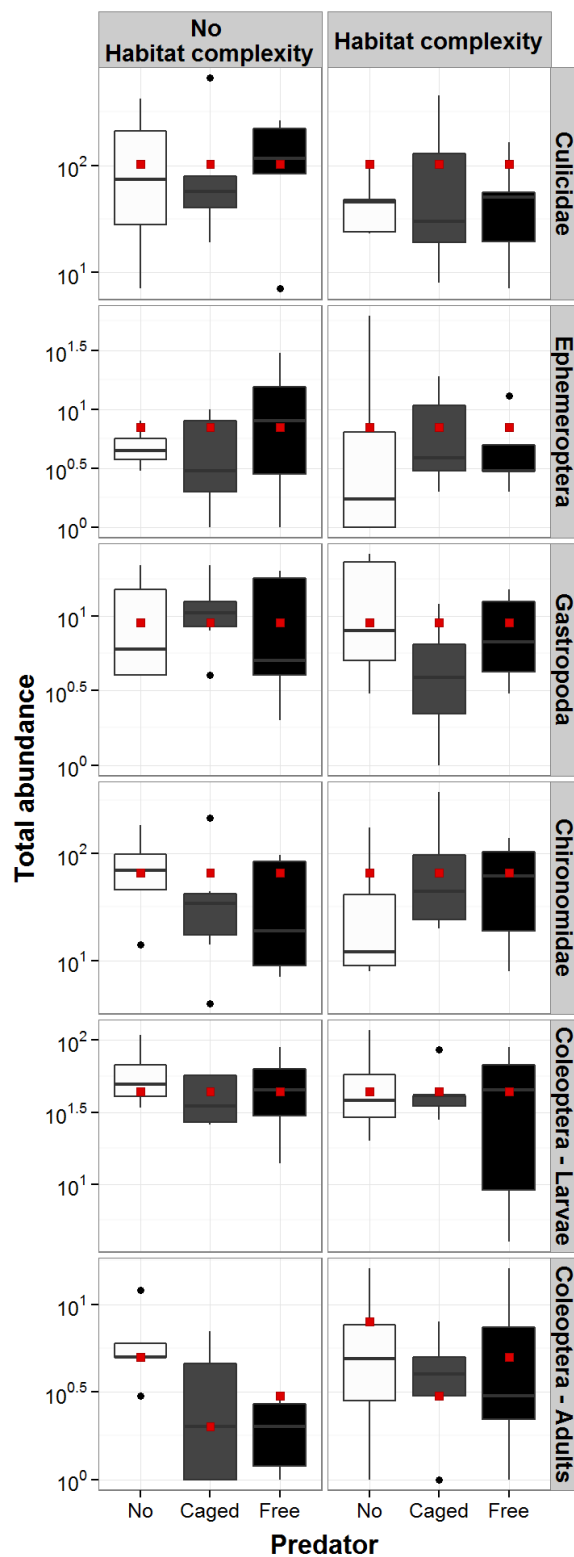
Figure 1: Changes in abundance of the main taxa in different treatments. Symbols: thick line = median, box = interquartile range, vertical lines = 1.5 times interquartile range, black circles = outliers, red squares = predicted values.
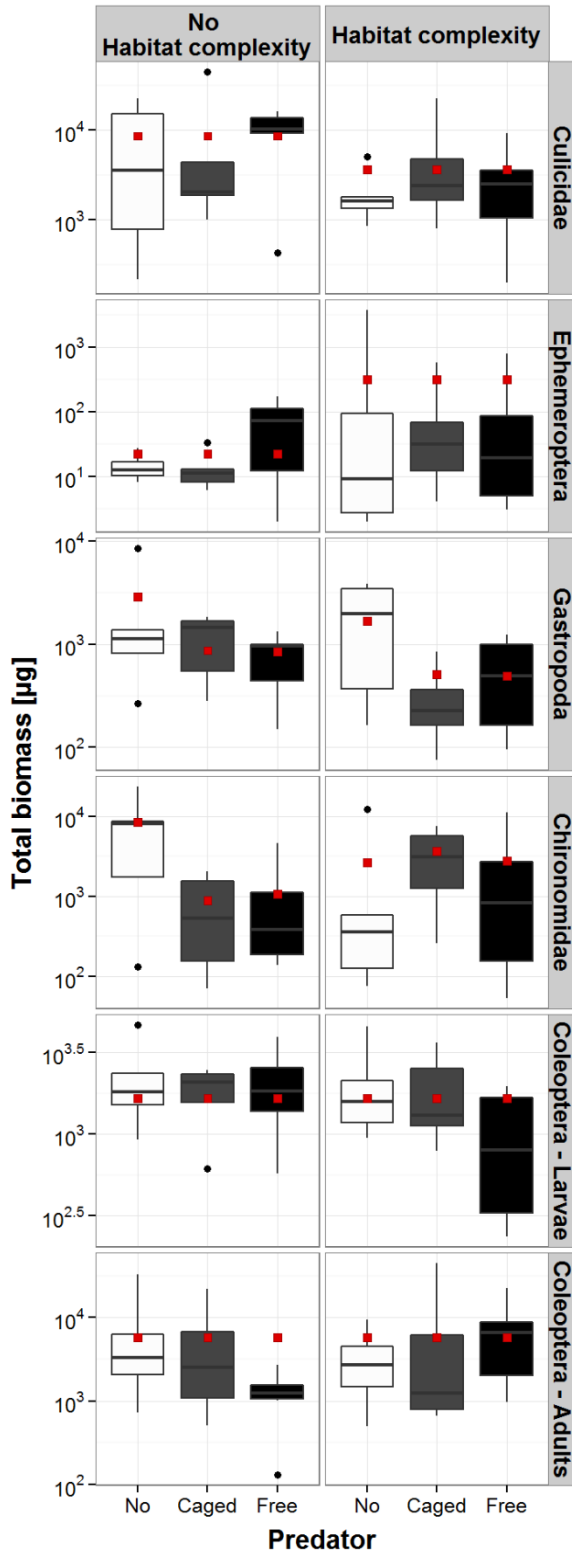
Figure 2: Changes in biomass of the main taxa in different treatments. Symbols as in Fig. 1. For Coleoptera larvae, where we had no model, mean is given instead of a predicted value.
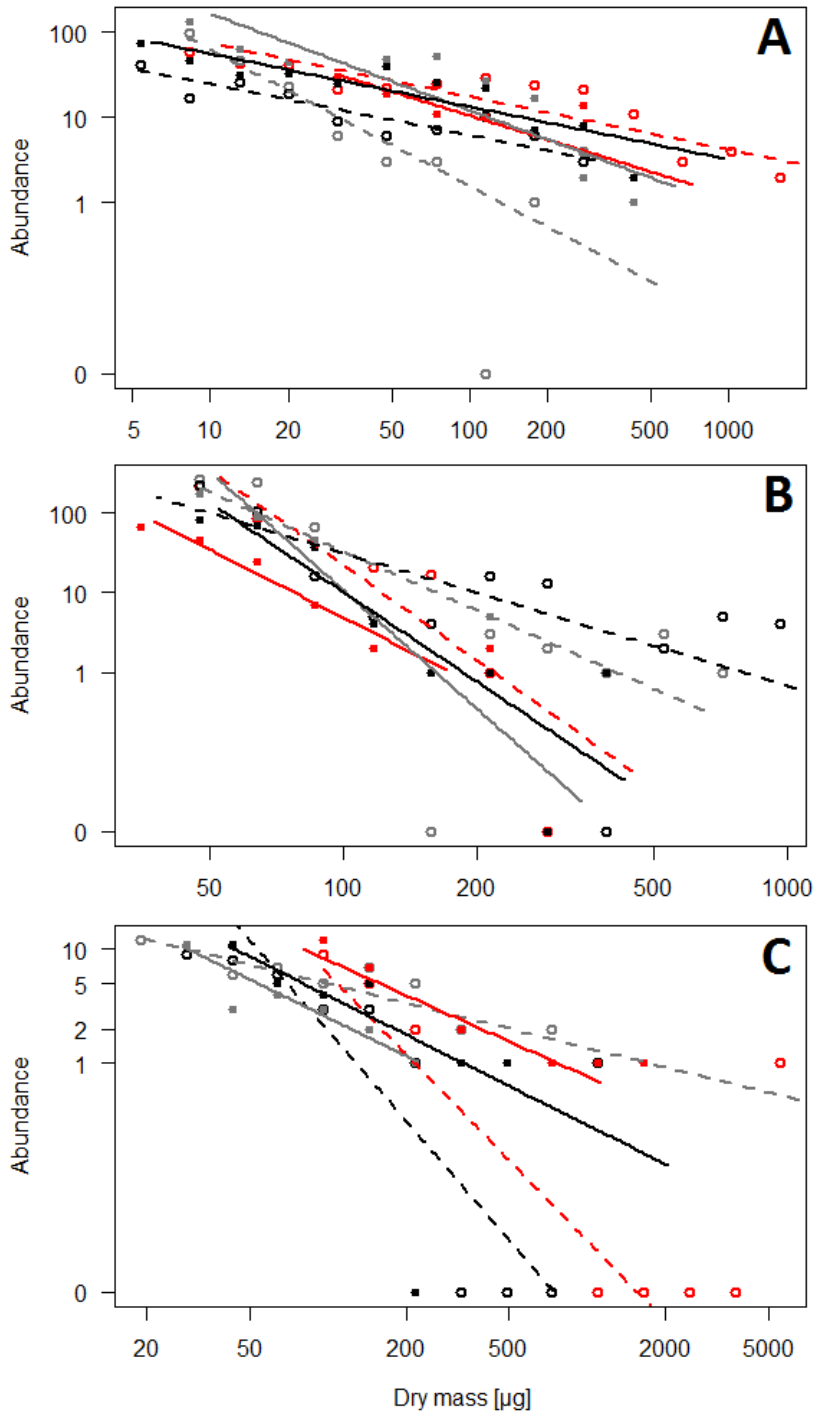
Figure 3: The effect of experimental treatments on the size spectra of (A) Chironomidae, (B) Culicidae and (C) Gastropoda. Circles represent data and lines the results of the SMA regression. Habitat complexity: open symbols and dashed lines = no artificial plants, full symbols and solid lines = added artificial plants. Predation risk: red = no predator, grey = caged predator, black = free-ranging predator. Zero abundance values were set arbitrarily at 0.01.

Appendices

Appendix S1: Published length-mass relationships relevant to our study.

Appendix S2: Automatic measurement error analysis.

Appendix S3: Temperature during the experiment.

Appendix S1: Published length-mass relationships relevant to our study.

Table S1: Overview of published length-mass relationship with details.

No.	Order	Family	Species	Stage	N	Range (mm)	a	b	R^2	Source
1	Coleoptera	all	all	A	–	0.6–17.2*	0.0077	2.910	–	Benke et al. 2010
2		Dytiscidae	*Hydroporus* spp.	A	42	3.1–6.5	0.6180	2.502	0.71	Smock 1980
3		Haliplidae	*Peltodytes sexmaculatus*	A	17	4.4–6.0	0.0271	2.744	0.76	Smock 1980
4		Hydrophilidae	*Enochrus bicolor*	A	150	2.8–4.0	0.0150	3.012	0.89	Heydarnejad 2010
5	Diptera	Chironomidae	all	L	–	2.0–19	0.0011	2.730	0.96	Johnston & Cunjak 1999
6		Chironomidae	all	L	–	1.0–13.7*	0.0018	2.617	–	Benke et al. 2010
7		Chironomidae	NA	L	41	2.0–14.0	0.0047	1.820	0.50	Baumgärtner & Rothhaupt 2003
8		Culicidae	*Culex* sp.	L	36	1.4–7.1	0.0017	3.168	0.98	Quintana 1995
9	Ephemeroptera	Baetidae	all	L	–	1.8–8.5*	0.0053	2.875	–	Benke et al. 2010
10		Baetidae	*Cloeon dipterum*	L	1096	2.0–8.4	0.0010	3.680	0.95	Johnston & Cunjak 1999
11	Pulmonata	Pleuroceridae	*Elimia clavaeformis*	A	50	–	0.0123	3.984	0.96	Benke et al. 2010
12		Lymnaeidae	*Radix peregra/ovata*	A	8	2.0–12.2	0.0265	3.150	0.96	Baumgärtner & Rothhaupt 2003
13		Bithyniidae	*Bithynia tentaculata*	A	10	1.6–6.6	0.0106	3.660	0.95	Baumgärtner & Rothhaupt 2003

Equations given for "all" lower taxonomic groups are based on multiple equations, NA = species not determined. Coefficients a, b are given for allometric equation $DM = a \cdot L^b$, where DM = dry mass and L = body length. Stage levels: A = adult, L = larvae. N = sample size, R^2 = coefficient of determination. Missing values could not be acquired from source materials. * indicates that the value was not implicitly given but inferred from the published material.
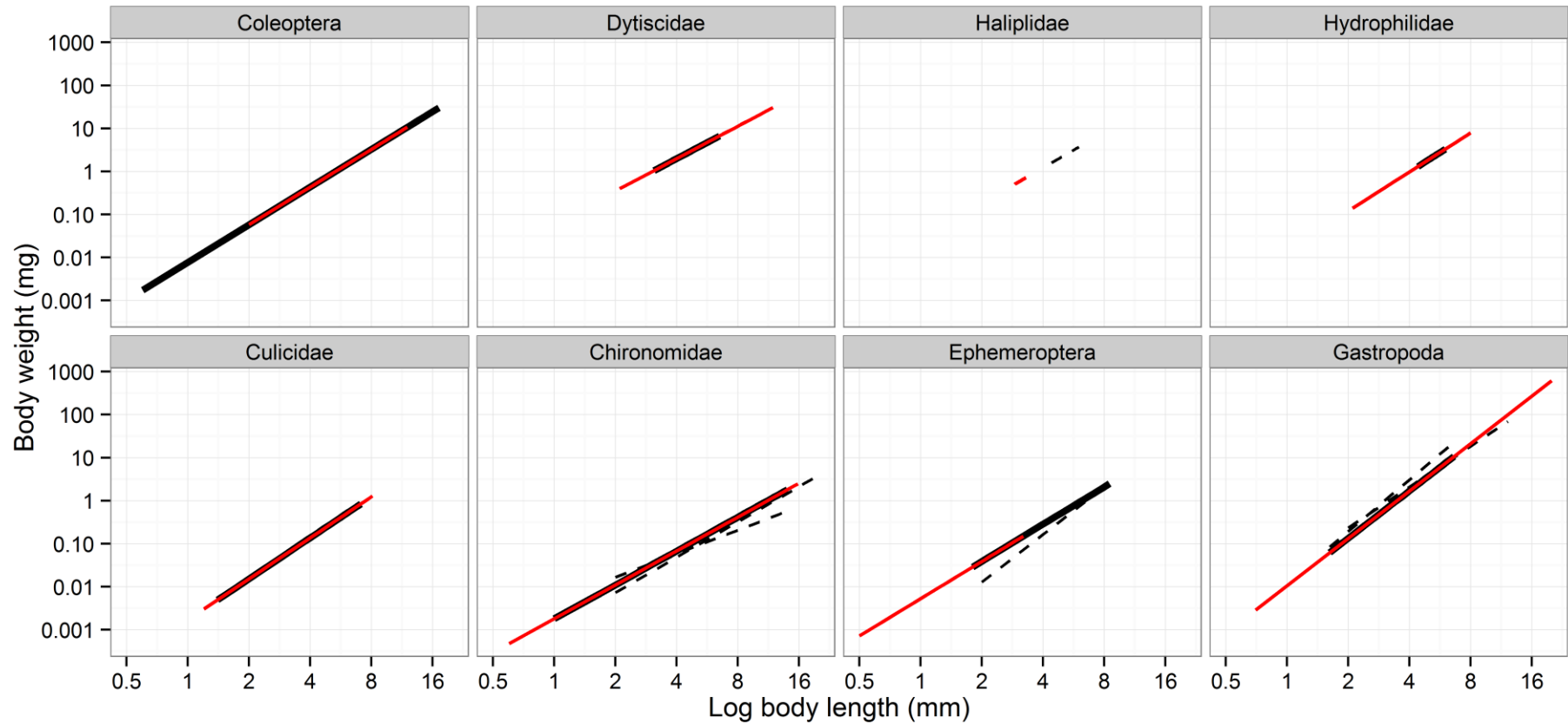
Figure S1: Comparison of length-mass allometries from Table S1 with our data. Each black line correspond to one equation. Dashed lines were not used in our study; thick black lines represent equations used in our study. Red lines depict the chosen allometry over the size range observed in our study. No thick black line in a panel = equation for a higher taxon was used instead.

The automatic measurement error ranged from -12.3% to +14.7% in Chironomidae and from -12.3% to +15.5% in Culicidae. The fitted polynomial provided a good fit for the data (Fig. S2) with no obvious biases (diagnostic plots not shown).
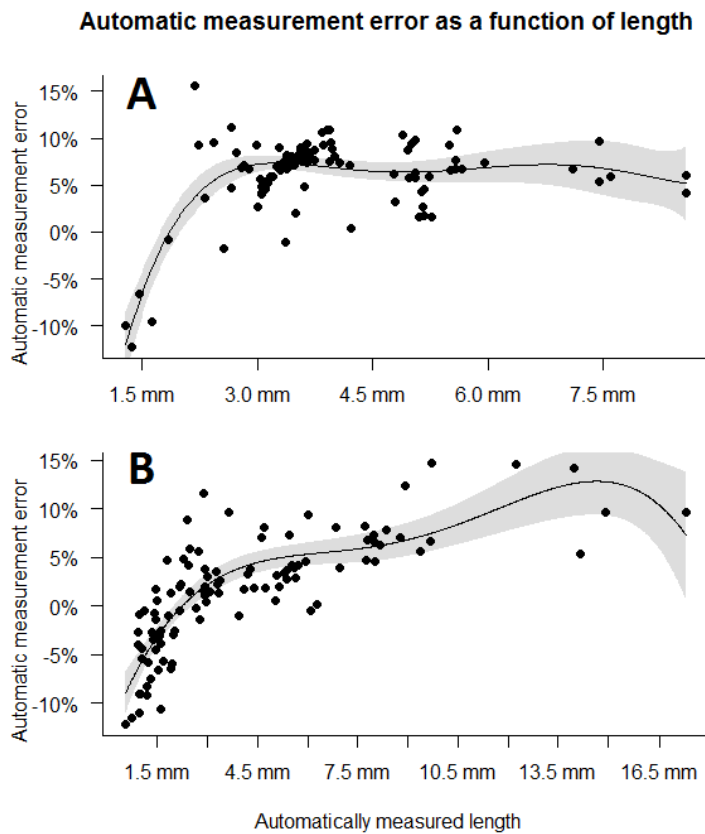

Figure S2: Automatic measurements error as a function of length (left) for Chironomidae (top) and Culicidae (bottom) and residuals as a function of fitted values (right). Black dots = measured individuals, black line = model prediction, grey interval = 95% confidence interval.
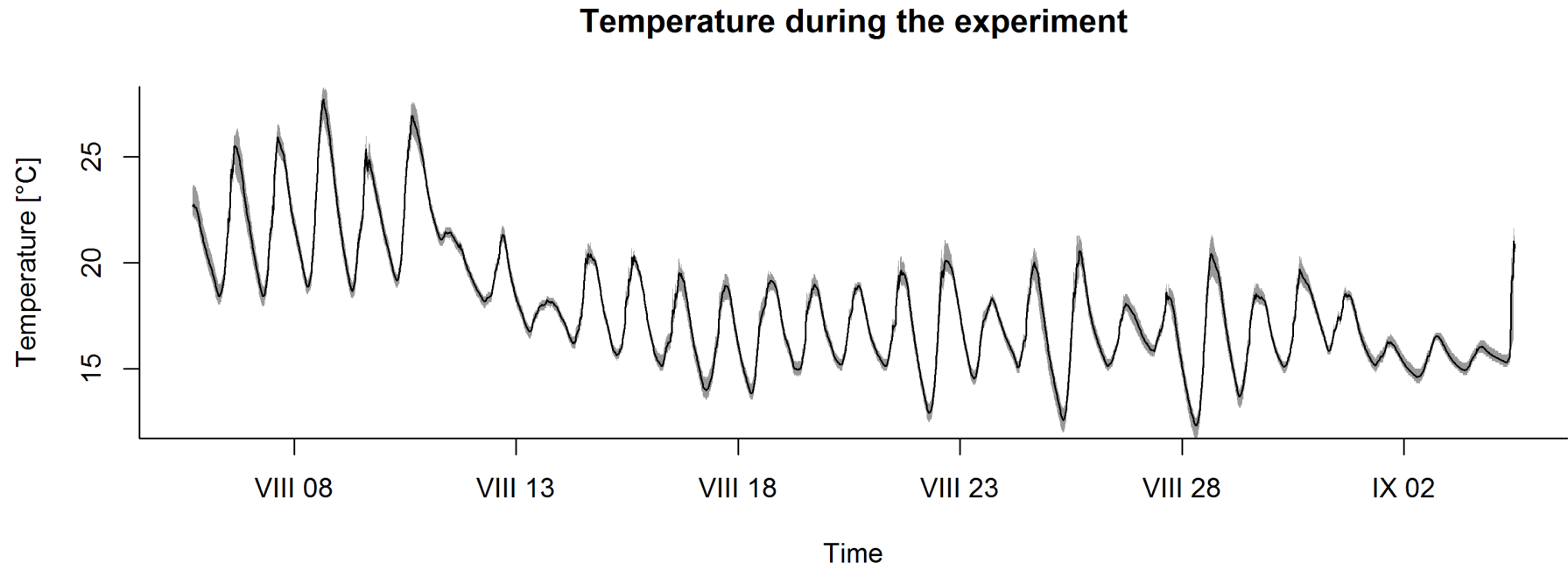
Temperature during the experiment

Figure S3: Temperature variation during the experiment. Data from all loggers are plotted (N=12). Symbols: black line = mean, grey area = range (minimum to maximum).