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Vliv rizika predace a komplexity prostředí na trofické  
interakce ve vodním prostředí

Magisterská práce

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Anotace:

The thesis results of two laboratory experiments focusing on the impacts of predation risk, prey density and habitat complexity on predator-prey interaction strengths and predator metabolic rates, complemented by a brief review of the subject. The experimental system used in the first experiment consisted of cladoceran prey, larvae of three dragonfly species (*Sympetrum sanguineum*, *Libellula quadrimaculata*, *Ischnura cf. elegans*) as intermediate predators, and larvae of a large dragonfly species (*Aeshna* sp.) as a top predator. The second experiment investigated how predation risk influences metabolic rates of the intermediate predators.

Prohlašuji, že svoji diplomovou práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

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Vojtěch Kolář

## Poděkování

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Cíl práce:

1. Provést literární rešerši vlivu rizika predace způsobené vrcholovými predátory na trofické interakce v potravních sítích a shrnout základní teoretické koncepty týkající se daného jevu.
2. Provést laboratorní experimenty kvantifikující vliv vrcholového predátora na potravní chování 2-3 druhů představujících typické zástupce vodního hmyzu malých tůní bez ryb.
3. Získaná data vyhodnotit pomocí pokročilých statistických metod v programu R nebo Statistica.

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## Úvodní komentář

Nejdůležitější faktory ovlivňující utváření společenstev jsou po stálosti prostředí přítomnost predátorů a habitatová komplexita prostředí (Wellborn a kol. 1996). Poslední dva vlivy jsou proto jedněmi z nejčastěji studovaných proměnných v různých kombinacích a v různých prostředích (Kats a Dill 1998, Schmitz a kol. 2000, Kovalenko a kol. 2012, Klecka a Boukal 2014).

### Vliv přítomnosti predátora

Přítomnost predátorů hraje důležitou roli v utváření společenstev v terestrických ekosystémech (Schmitz a kol. 2000, Hawlena a Schmitz 2010) i ve vodním prostředí (Wellborn a kol. 1996, Schmitz a kol. 2000). Predátor může působit přímo na svou kořist nebo nepřímo skrze dopady úbytku této kořisti predátora na ostatní druhy včetně nižších trofických úrovní. Oba vlivy lze dále rozdělit na letální, t.j. aktivní predaci, a neletální, t.j. reakce kořisti na potkávání se s predátorem nebo signály o jeho přítomnosti (Wellborn a kol. 1996, O'Connor a kol. 2013).

Kořist v přítomnosti predátora či vystavená jeho kairomonům (tzv. *cues*) často uplatňuje nějakou strategii sloužící k obraně nebo úniku před predátorem (Kats a Dill 1998), např. morfologické adaptace jako ostny a obrnění (Hebert a Grewe 1985, Brönmark a Miner 1992, Beckerman a kol. 2007, Brönmark a Hansson 2012). Dále kořist využívá různé fyziologické adaptace (produkce jedu) či změny v životním cyklu jako pozdější líhnutí, produkci dormantních stádií nebo rychlejší dospívání (Lass a Spaak 2003). Predátor může vyvolávat i změny chování kořisti (Johnson 1991; Åbjörnsson a kol. 1997; Eklöv a van Kooten 2001; Bergström a Englund 2004; Oda a Hanazato 2008; Delclos a Rudolf 2011) či změnu denní aktivity (Tjossem 1990, Åbjörnsson a kol. 1997). Přítomnost predátora může vyvolávat i změnu chování jiných predátorů (Kopack a kol. 2015). Nakonec může přítomnost predátora a jeho kairomonů ovlivňovat také reprodukční chování potenciální kořisti, jak bylo ukázáno v případě ovipozice samic komárů do tůní s různou mírou rizika predace jejich larev (Blaustein 1998, Albeny-Simões a kol. 2014). Přítomnost predátora či příchod nového invazivního predátora může také ovlivňovat rozsah a intenzitu intraguild predace ve společenstvu (Grabowski a kol. 2008, Hall 2011, Anderson a Semlitsch 2015). Ve všech případech přitom záleží mimo jiné na strategii lovu a preferovaných mikrohabitátech predátora a kořisti: např. druhy obývající jiné mikrohabitáty se mohou

ovlivňovat jen minimálně (Ramos a Van Buskirk 2012, Boukal 2014, Anderson a Semlitsch 2015).

Vlivem přítomnosti predátora na kořist se věnovala řada autorů. Perločky v přítomnosti predátora více agreguje (Bergström a Englund 2004) nebo mění vertikální či horizontální migraci (Tjossem 1990, Ferrari a kol. 2010, Lass a Spaak 2003). Posun perlooček z vegetace do volné vody (horizontální migrace) může dále ovlivnit další bezobratlé jelikož ryby jsou pak nucené přejít na jinou kořist (Eklöv a van Kooten 2001). Některé druhy vážek mají na lokalitách s rybama sníženou aktivitu nebo se častěji schovávají do úkrytů (Johnson 1991, Wellborn a kol. 1996). Predátor může také vyvíjet větší tlak na určitou velikost kořisti a ta se zde pak může vyskytovat jen ve velké velikosti, jelikož všechny menší stádia byla od predátora (nejčastěji ryb) vyžrána (Johnson 1991). Nakonec kořist může v přítomnosti predátora zpomalovat růst to je dokázáno u vážek (Slos a Stock 2008, Culler a kol. 2014) nebo u menších ryb (Woodley a kol. 2003).

Zkoumání vlivu signálů z prostředí na chování kořisti se používá ke zjištění toho, podle čeho se kořist orientuje (Åbjörnsson a kol. 1997) nebo zda dokáže předem vycítit přítomnost predátora (Blaustein 1998). Kairomony se nejčastěji studují u rybích predátorů a sleduje se vliv na jejich kořist, např. jiné druhy ryb, rybí potěr (Mirza a Chivers 2003, Kopack a kol. 2015), korýše (Toscano a Griffen 2014) a hmyz (Tjossem 1990, Åbjörnsson a kol. 1997, Oda a Hanazato 2008, Slos a Stoks 2008). Další studie sledovaly vliv hmyzího predátora (Kats a Dill 1998, Albeny-Simões a kol. 2014) či vliv látek uvolňovaných z rozdrčených jedinců stejného druhu (Beckerman a kol. 2007, Sehr a Gall 2015).

Chemické složení kairomonů řady predátorů není známo, ale zřejmě se mění i mezi blízkými příbuznými druhy (Brown a kol. 2003, Lass a Spaak 2003, Brönmark a Hansson 2012). U ryb se jedná nejčastěji o hypoxantin-3(*N*)-oxid, u čolků o tetrodotoxin, u předožábřích plžů o navenon A, B a C a haminol A a B. U dalších predátorů je známa pouze atomová hmotnost řetězce (od několika Daltonů až po několik stovek kDa) a některé přítomné chemické skupiny (Brönmark a Hansson 2012). Kairomony bez ohledu na přesné složení časem degradují a ztrácí na účinnosti (Oda a Hanazato 2008). Životností kairomonů se zabývali Van Buskirk a kol. (2014), kteří v experimentu s pulci vystavenými kairomonům vážek zjistili, že nezáleží, zda používají čistou laboratorní vodu nebo vodu z rybníku. Pulci na přidání vody od vážek reagovali vždy sníženou aktivitou. Kairomony přestaly účinkovat nebo si na jejich přítomnost pulci zvykli a zvýšili svou aktivitu na normální úroveň přibližně po 35 hodinách. Rozkladu kairomonů kromě mikroorganismů zřejmě napomáhá také UV světlo (Chivers a kol. 2013) a fyzikálně-chemické vlastnosti vody (Lass a Spaak 2003,

Ferrari a kol. 2010). Vzhledem k náročnosti studia kairomonů v terénních experimentech není ani dostatek informací o tom, na jaké vzdálenosti působí. Wisenden (2008) zjistil, že dva druhy kaprovitých ryb reagovaly na kairomony z kůže konspecifických jedinců na vzdálenost přibližně 2 m, u vodního hmyzu tento jev nebyl vůbec studován.

### **Vliv komplexity prostředí**

Komplexita prostředí tj. prostorové členění prostředí či rozmístění prostorových prvků v prostoru rozděluje je důležitým faktorem ovlivňujícím vztah predátora a kořisti v terestrických (Hurlbert 2004, Langellotto a Denno 2004) i vodních ekosystémech (Wellborn a kol. 1996, Kovalenko a kol. 2012, Tokeshi a Arakaki 2012). Komplexita prostředí může být buď horizontální (typ a struktura dna, přítomnost prohlubní apod.) nebo vertikální (Kovalenko a kol. 2012, Tokeshi a Arakaki 2012). Vertikální členění prostředí může být dosaženo různými způsoby, např. přítomností kořenů stromů (Kovalenko a kol. 2012), emerzní a submerzní vegetace (Lombardo 1997, Eklöv a van Kooten 2001, Warfe a Barmuta 2004, Hartel a kol. 2007, Canion a Heck 2009, Delclos a Rudolf 2011, Scheinin a kol. 2012, Fischer a kol. 2013, Klecka a Boukal 2014, Anderson a Semlitsch 2015), korálového útesu či porosty škeblí (Grabowski a kol. 2008, Tokeshi a Arakaki 2012). Důležitá je především míra komplexity (jeho fraktálnost) prostředí (Scheinin a kol. 2012).

Komplexitu prostředí lze při zkoumání jejího vlivu dobře manipulovat v laboratorních i terénních experimentech (Canion a Heck 2009). Vlivem ponořené vegetace jako nejtypičtějšího prvku vytvářejícího strukturované prostředí ve sladkých stojatých vodách na chování predátorů i kořisti se proto zabývala celá řada autorů (ryby: Eklöv a van Kooten 2001, Hartel a kol. 2007, Scheinin a kol. 2012; obojživelníci: Anderson a Semlitsch 2015; vodní bezobratlí: Delclos a Rudolf 2011, Fischer a kol. 2013, Klecka a Boukal 2014). Někdy vegetace nemusí mít na úspěšnost lovu predátora a přežívání kořisti žádný vliv (Lombardo 1997, Delclos a Rudolf 2011, Fischer et al. 2013, Klecka a Boukal 2014, Anderson a Semlitsch 2015). Většina studií ale potvrdila, že přítomnost vegetace obvykle poskytuje kořisti úkryt nebo zhoršuje schopnost predátora kořist najít a snižuje tak predanční tlak (Klecka a Boukal 2014). Naopak vyčkávajícím predátorům (např. štika *Esox lucius*) může poskytovat úkryt, ze kterého poté chytají kořist, a predátorům využívajícím vegetaci k přístupu do vodního sloupce může poskytovat místo podporující jejich lov. Klecka a Boukal (2014) posledně zmíněný mechanismus pojmenovali jako *anti-refuge* efekt. Zjistili,



že vegetace zvyšovala mortalitu perlooček rodu *Simocephalus* predovaných larvami vážek rodu *Coenagrion*, *Platycnemis* a *Sympetrum*.

Komplexita prostředí nejen mění chování jedinců, ale ovlivňuje také abundance druhů. Ve složitějším prostředí je více druhů díky efektu rozrůznění nik a větší povrchové ploše podobně jako ve vztahu *species-area* popisujícím závislost mezi velikostí zkoumané plochy a počtem přítomných druhů (Hurlbert 2004, Kovalenko a kol. 2012, Tokeshi a Arakaki 2012). To ovšem není zohledněno ve většině studií zkoumajících vliv komplexity na úspěšnost predátora. Canion a Heck (2009) toto zohlednili ve svém laboratorním pokusu, kdy s větší hustotou vegetace zvyšovali i množství predátorů a kořisti stejně jako v přirozených podmínkách. Vyšlo jim, že nejvíce kořisti bylo sežráno v treatmentu bez vegetace, naopak tři rozdílné hustoty vegetace se mezi sebou v množství zkonzumované kořisti nelišily. Ukázali tak, že hustší vegetace nechrání kořist před predátorem, jelikož obou druhů je tam vždy přibližně stejně a tedy je stejná i míra predace a také že predáční schopnost neklesá s hustší vegetací.

### **Vliv přítomnosti predátora na metabolismus**

Vliv přítomnosti predátora na rychlost metabolismu kořisti byl zatím zkoumán jen okrajově. Většina studií zjistila, že predátor snižuje rychlost metabolismu jeho kořisti např. u pavouků rodu *Hasarius* (Okuyama 2015), sarančí rodu *Melanoplus* (Hawlena a Schmitz 2010), larev vážek druhu *Enallagma vesperum* Culler a kol. 2014, ). Na druhou stranu množství přijaté energie se u perlooček rodu *Daphnia* v přítomnosti kairomonů od ryb snižuje (Beckerman a kol. 2007) nebo králíci druhu *Sylvilagus aquaticus* při vystrašení predátorem snižují množství přijatého kyslíku a také rychlost srdečního tepu (Smith a kol. 1981). Metabolická odezva na přítomnost predátora může být ovlivněna i teplotou. Culler a kol. (2014) zjistili, že šidélka druhu *E. vesperum* mají při nižších teplotách stejně rychlý metabolismus, ať tam rybí predátor je či není. Rychlost metabolismu se však výrazně lišila ve vyšších teplotách, kde šidélka s predátorem měli metabolismus rychlejší

Snížený metabolismus kořisti může být zapříčiněn několika vlivy a mít různou dobu trvání. Dlouhodobě snížený metabolismus kořisti za přítomnosti predátora může odpovídat snížení aktivity, nižšímu příjmu potravy a celkovému zpomalení rychlosti vývoje (Culler a kol. 2014). Na druhou stranu může kořist dlouhodobě snížit svojí aktivitu, ale zrychlit metabolismus, aby rychleji dokončila vývoj a tím snížila šance na svou predaci i za cenu menší velikosti těla v dospělosti (Abrams a Rowe 1996). Akutním zrychlením metabolismu

při vycítění kairomonů predátora se naopak kořist připravuje na útěk (tzv. *fight-or-flight* odpověď: Slos a Stoks 2008, Okuyama 2015). Toto zvýšení metabolické aktivity může při častém opakování ve spojení se sníženou aktivitou jedince a sníženému příjmu energie přinejmenším hypoteticky vést až ke zvýšenému riziku vyhladovění.

Vliv přítomnosti predátora na metabolismus kořisti může být navíc pozměněn přítomností vegetace. Woodley a Peterson (2003) zjistili, že množství spotřebovaného kyslíku bylo vyšší u ryb, které byly bez vegetace a zároveň viděly na predátora než v kontrolní nádrži bez kairomonů. Naopak ryby, které byly schované ve vegetaci a přítomnost predátora jim signalizovaly pouze kairomony, spotřebovaly stejně kyslíku jako ryby v kontrolní nádrži bez kairomonů.

## Shrnutí

Mezi nejdůležitější faktory ovlivňující utváření společenstev a strukturu potravních sítí jsou přítomnost predátora a habitatová komplexita. Celá řada experimentálních studií se zabývala přímými vlivy predátora anebo jeho kairomonů na kořist, zatímco jeho nepřímým vlivům na nižší trofické úrovně zatím nebyla věnována dostatečná pozornost. Například vliv přítomnosti predátora na rychlost metabolismu nebyl zatím u vodního hmyzu moc studován, jelikož většinou se spíše studuje aktivita či životní cyklus kořisti jako celek. Z dosavadních studií nejen na vodním hmyzu přitom vyplývá, že metabolickou odezvu na přítomnost predátora u konkrétních druhů je velmi těžké předpovědět. Vzhledem k možnému současnému ovlivnění aktivity a rychlosti metabolismu kořisti se tak naskýtá otázka, jak predátor ovlivňuje její energetickou účinnost, definovanou jako poměr přijaté a vydané energie (Sentis et al. 2015). Vliv predace na mortalitu, aktivitu a rychlost metabolismu kořisti přitom může být významně ovlivněn komplexitou prostředí, která je v malých stojatých vodách typicky určena množstvím a druhovým složením submerzní vegetace.

Cílem mé studie proto bylo objasnit vliv současného působení rizika predace a komplexity prostředí na příjem potravy a metabolismus vybraných druhů vodního hmyzu. V pokusu jsem riziko predace simuloval pomocí chemických, resp. chemických a vizuálních stimulů přítomnosti larev vážek rodu *Aeshna* a habitatovou komplexitu měnil pomocí plastových maket vegetace. Sledoval jsem příjem potravy (2 hustoty kořisti 50 a 150; směs zooplanktonu rodů *Daphnia* a *Simocephalus*) u fokálních predátorů zahrnujících larvy vážek třech různých druhů: vážky čtyřskvrnné *Libellula quadrimaculata* lovicí výhradně na dně a dvou fytofilních druhů vážek: vážky rudé *Sympetrum sanguineum* a šidélka většího *Ischnura*

cf. *elegans*. V druhém experimentu jsem pak zjišťoval efekt přítomnosti kairomonů na rychlost metabolismu fokálních predátorů.

Předpokládal jsem v souladu s publikovanými výsledky, že v jednoduchém prostředí bez maket vegetace budou larvy fytofilních druhů méně úspěšné v lovu kořisti (zvláště při nižší hustotě kořisti) a že komplexita prostředí nebude mít vliv na bentického predátora. Dále jsem předpokládal, že riziko predace sníží příjem potravy fokálních predátorů. V prostředí s vegetací a rizikem predace jsem předpokládal vyšší či stejné množství zkonsumované kořisti jako v prostředí bez predátora s tím, že vegetace poskytne fokálním predátorům úkryt a jejich rychlost predace se tak nezmění. Ve druhém experimentu jsem předpokládal, že rychlost metabolismu bude u všech tří druhů vyšší v přítomnosti kairomonů (akutní *fight-or-flight* odpověď). Předpokládal jsem, že zvířata vystavená vlivu predátora v komplexním prostředí budou mít vyšší energetickou účinnost než zvířata vystavená vlivu predátora v prostředí bez umělé vegetace. Nejvyšší energetickou účinnost pak budou mít zvířata nevystavená predáčnickému tlaku.

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# Habitat complexity and predation risk modify predator-prey interaction

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## Abstract

Feeding and metabolic rates are of paramount importance for the stability of populations and communities, energy flows in ecosystems and functional consequences of biodiversity loss. However, little information exists about how multiple abiotic and biotic factors jointly influence the strengths of predator-prey interactions and metabolic rates of prey. To fill this gap, we performed a laboratory experiment to investigate the effects of habitat complexity, prey density and predation risk by large dragonfly larvae (*Aeshna* sp.) on short-term interaction strengths (i.e., feeding rate) of the larvae of three odonate species (*Sympetrum sanguineum*, *Libellula quadrimaculata*, *Ischnura* cf. *elegans*). We found that, for all predator species, interaction strength decreased with prey density. Predation risk significantly decreased interaction strength in *S. sanguineum* whereas it had no effect on the feeding rates of the other two predators. Finally, vegetation significantly increased interaction strengths but only in predation risk free treatment. We also investigated the impact of predation risk (i.e., chemical cues from *Aeshna* larvae) on the metabolic rates of the larvae of the three focal odonate species. Metabolic rate was affected by species identity, but not by predation risk. In conclusion, we show that the effects of vegetation and predation risk on intermediate predators are context-dependent and interactive. Our study thus suggests that it is important to take into account multiple factors to better understand and predict environmentally driven variations in trophic interaction strength and metabolic rates that underlie the energetic efficiency of individual consumers.



## Introduction

Species interaction strength (i.e., the effect of one species on the abundance of a second species) is of paramount importance for the stability of populations and communities, energy flows in ecosystems and functional consequences of biodiversity loss (Berlow et al. 2009). At short timescales, trophic interaction strength is closely related to the predator feeding rate (Berlow et al. 2004, Wootton and Emmerson 2005, Rall et al. 2012). At longer timescales, interaction strength also depends on the predator metabolic rate, which determines the energy demands of the predator and is therefore crucial for its energetic efficiency, i.e., balance between feeding and metabolic rates (Rall et al. 2010, Binzer et al. 2012, Gilbert et al. 2014, Sentis et al. 2015).

Recent empirical and theoretical studies reported that energetic efficiency is crucial for the dynamics and stability of food-webs as it influence energy flow between trophic levels and thus predator-prey oscillation as well as predator survival. For instance, recent studies indicated that, when temperature increases, metabolic demands may exceed ingestion rates and thereby lead to consumer starvation and, ultimately, extinction (Petchey et al. 1999, Rall et al. 2010, Binzer et al. 2011, Fussmann et al. 2014). Altogether, these results demonstrate that investigating the relative scaling of biological rates is important for predicting the dynamics and stability of ecosystems. However, very little is known about how biotic and abiotic factors jointly influence the strength of predator-prey interaction and predator metabolic rates, which jointly determine the predator energetic efficiency.

Habitat structure and complexity influence the strength of predator-prey interactions in terrestrial (Hurlbert 2004, Langelotto and Denno 2004) and aquatic ecosystems (Wellborn et al. 1996). However, studies quantifying the influence of vegetation on predator-prey interactions in fishes (Eklöv and van Kooten 2001, Hartel et al. 2007, Scheinin et al. 2012), amphibians (Anderson and Semlitsch 2015), and invertebrates (Delclos and Rudolf 2011, Fischer et al. 2013, Klecka and Boukal 2014) reported contradicting results: vegetation can decrease predator feeding rate by providing a refuge to the prey (Warfe and Barmuta 2006, Stoner 2009, Fischer et al. 2013, Klecka and Boukal 2014, Anderson and Semlitsch 2015), have no effects on the predator feeding rate (Lombardo 1997, Johnson 2006, Delclos and Rudolf 2011, Fischer et al. 2013, Klecka and Boukal 2014, Anderson and Semlitsch 2015), or increase the hunting efficiency of sit-and-wait, ambush predators (e.g., pike *Esox lucius*) and of phytophilous predators that spend most time on or within submerged vegetation (e.g., many odonate larvae, Klecka and Boukal 2014). Individuals may also

respond differently to chemical cues in the presence of vegetation. For instance, Woodley and Peterson (2003) found that fish prey have higher metabolic rate when exposed to chemical and visual cues, but this effect was not significant in presence of vegetation.

Species are embedded within communities and their fate depends on the consequences of changes in the nature and strength of intraspecific and interspecific interactions (Petchey et al. 1999, Gilbert et al. 2014). These interactions can be direct (such as feeding) or indirect (trait-mediated effects). A well-known indirect effect is the influence of predation risk on prey behavior and life history traits. Prey have evolved a vast array of strategies in responses to predation risk which can be both olfactory (e.g. chemical cues) and/or visual (Kats and Dill 1998, Ferrari et al. 2010). For instance, *Daphnia* exposed to chemical cues develop defensive spines and thicker exoskeleton to reduce predation by *Chaoborus*. They also mature at larger or smaller body size compared to non-exposed *Daphnia* (Hebert and Grewe 1985, Lass and Spaak 2003, Beckerman et al. 2007, Ferrari et al. 2010, Brönmark and Hansson 2012). Juvenile *Carassius carassius* exposed to pike cues develop a deeper body, which could be a defensive mechanism against gape-limited predator (Brönmark and Miner 1992, Ferrari et al. 2010). However, the influence of a top predator on the behavior and feeding rates of intermediate predators (i.e. prey of the top predator) remains largely unexplored and it is not clear if the direct effects of predation risk on herbivorous prey mentioned above also apply to intermediate predators.

Moreover, several studies investigated the effects of predation risk on prey respiration rate (a proxy of energy consumption) and found different and sometimes opposite results. Some studies showed that grasshoppers, spiders and tadpoles under predation risk have higher metabolism rates (Slos and Stoks 2008, Steiner and Van Buskirk 2009, Hawlena and Schmitz 2010, Okuyama 2015) whereas studies on daphnia and swamp rabbits found the opposite pattern (Smith et al. 1981, Beckerman et al. 2007). Culler et al. (2014) studied the metabolic rate on aquatic insect and found that the damselfly *Enallagma vesperum* metabolic rate is not significantly influenced by the presence of fish predator at low temperature. However, metabolic rates differed in higher temperature: exposed damselflies had a higher metabolism than non-exposed ones. As a consequence of lower metabolic rates, prey are less active and feed less, which tends to slow down their development but reduces the likelihood of being eaten by the predator. On the other hand, elevated metabolic rates could signal the fight-or-flight response of individuals anticipating a meeting with a predator (Slos and Stoks 2008, Okuyama 2015).

The results mentioned above indicate that animals respond differently to the presence of predators; a unified framework is missing. In addition, most of the studies mentioned above investigated the effects of predation risk on herbivorous prey. Little is known about how a top predator (sometimes acting as intraguild predator) affects the behavior, physiology and feeding rate of intermediate predators and how these effects modulate the strength of the interactions between the intermediate predators and their prey and the energetic efficiency of the intermediate predators.

To fill this gap, we experimentally tested how the presence of artificial vegetation modifying habitat complexity and of predation risk from a top predator (larvae of *Aeshna* sp.) jointly influence the numbers of cladoceran prey eaten by three intermediate predators (*Ischnura* cf. *elegans*, *Libellula quadrimaculata* and *Sympetrum sanguineum*). We also measured the resting metabolic rate of these three predators in the presence and absence of chemical stimuli indicating the presence of the top predator. We hypothesized that: (1) predation risk will decrease the feeding rates of intermediate predators, (2) in the simpler habitat without vegetation, phytophilous predators (*I.* cf. *elegans* and *S. sanguinerum*) will be less successful in hunting of prey, especially at lower prey density, (3) presence of vegetation will have a weaker or no effect on the benthic predator (*L. quadrimaculata*), (4) in treatments with vegetation, feeding rate will be similar with and without predation risk as the vegetation provide a refuge for the intermediate predator, and (5) predation risk (i.e. chemical cues) will increase metabolic rates of the intermediate predators due to risk-induced stress.

## Materials and Methods

### Laboratory experiments

Experiments were conducted in a climatized incubator (Lovibond BSK ET 650, water temperature  $19.4 \pm 0.3^\circ\text{C}$ , 18L:6D photoperiod) at the Institute of Entomology in České Budějovice in summer 2014. The intermediate predators used in this study were dragonflies (Odonata) *Libellula quadrimaculata*, *Sympetrum sanguineum* (Libellulidae) and *Ischnura* cf. *elegans* (Coenagrionidae). The top-predator was *Aeshna* sp. (Aeshnidae). All these predators are common species of standing water in middle Europe. We used a mixture of *Daphnia magna* and *Simocephalus* sp. (Cladocera: Daphniidae) as prey. The prey and predators were collected in small ponds and pools in south Bohemia. The predators have largely the same sit-and-wait hunting strategy but occupy different habitat domains: *L. quadrimaculata* is a benthic predator whereas *S. sanguineum* and *I. cf. elegans* hunt in the water column and are phytophilous (Dolný et al. 2008, Klecka and Boukal 2014). The prey (*D. magna* and *Simocephalus* sp.) are active filter feeders occupying the water column. Before the beginning of the experiments, animals were acclimated in the laboratory for at least two days for acclimatization and fed daily *ad libitum* with mosquito larvae or a mixture of *Daphnia magna* and *Simocephalus* sp. The predators were kept individually in plastic cups filled with 0.1 L of aged tap water with a piece of willow moss (*Fontinalis antipyretica*) or peat moss (*Sphagnum* sp.).

#### *Experiment 1. Effects of predation risk, vegetation, and prey density on predator-prey interaction strength*

Intermediate predators were starved and acclimated at the experimental conditions 12 hours before the experiment. We used a full factorial design with two prey densities (50 and 150 ind.L<sup>-1</sup>), presence/absence of artificial vegetation, and three levels of predation risk: no top predator (risk-free treatment), caged top predator with added chemical cues (visual+chemical cues treatment), and top predators cues (chemical cues treatment). We used two “plants” with 4 leaves (length ca. 7 cm, width ca. 2 cm) made of green plastic fabric (mesh size ca. 3 mm).

Top predator was enclosed in a cage (transparent plastic box, length 6 cm, width 4 cm, height 7 cm, with 0.4 L volume placed on top of the experimental vessel, so that the bottom of the predator box reached 5 cm below water surface). For the chemical cues we used 2-day old water from 12 larvae of *Aeshna* sp., which were individually reared in plastic

cups filled with 300 ml of aged tap water. To collect the chemical cues, we took half of the water in each cup and then refilled the missing volume in the cup with aged tap water. We used 0.2 L filtered (0.5 mm sieve) water with cues for each experimental arena with predation risk represented by chemical stimuli, i.e. we diluted the concentration of the *Aeshna* cues to ca 20% of the value in the rearing cups.

Experimental arenas consisted of plastic jars (length 12.5 cm, width 8 cm, height 19 cm) filled with 1 L of aged tap water and lined with 1-2 cm of fine crystalline sand (diameter < 1 mm) (see Sentis et al. 2015). *Daphnia magna* and *Simocephalus* sp. were standardized by body size (0.5–1.5 mm). Prey were counted individually and introduced in the experimental arenas. Two hours later, the intermediate predators and top predator (or its chemical cues) were introduced in the arena and allowed to feed on prey under continuous light conditions. After 6 hours, all predators were removed and surviving prey counted. Natural mortality of prey was assessed in control treatments without predators. We performed 6–9 replicates for each treatment except controls, in which we did only 4–5 replicates (Table S1).

#### *Experiment 2. Effect of predation risk on the metabolism rate of intermediate predators*

To measure respiration rate (a proxy of metabolic rate) we used Unisense© MicroOptode oxygen probe with the software SensorTrace Basic v3.2.3 (Unisense©) following mostly the protocol in Sentis et al. (2015). Respiration was measured in sealed glass chambers (volume 57 ml) filled with distilled water conditioned by adding purified salt (sera©, 0.17 g.L<sup>-1</sup>) to achieve the conductivity of 200 µS.cm<sup>-1</sup> representative of field conditions (Sentis et al. 2015). Intermediate predators were starved and acclimated to experimental conditions 48 hours prior to the experiment. For the predation risk, we used top predator chemical cues following the same procedure and using the same concentration as in Experiment 1. The concentration of oxygen was measured just before the introduction of intermediate predator larvae. The glass chambers were then immediately closed with a hermetic glass plug. After 150 minutes, we measured oxygen concentration again (Sentis et al. 2015). The experiment was performed at 20.6±0.8°C. Before each oxygen measurement, water was mixed using a magnetic stirrer. Each larva was then weighed to the nearest 0.0001 g using a Kern© ABT microbalance. We performed 14–39 replicates in each treatment (Table S2). Possible background oxygen depletion was determined in 69 controls without larvae in each treatment. Oxygen consumption in µmol.min<sup>-1</sup> was converted to J.hour<sup>-1</sup> following Sentis et al. (2015).

## **Statistical analysis**

### *Experiment 1. Effects of predation risk, vegetation, and prey density on predator-prey interaction strengths*

Binomial GLM with logit link function was used to test the effects of vegetation, predation risk, prey density, and their interactions on the proportion of prey eaten by the intermediate predators. We detected model overdispersion and thereby corrected the standard errors using a quasi-GLM model (Zuur et al. 2009). Analysis of deviance was subsequently performed using an F-test. Post-hoc tukey tests (lmeans package; Lenth 2015) were used to determine significant differences among treatment means. Mortality in the controls without intermediated predators was significant but did not differ between vegetation, prey density and predation risk treatments. We therefore corrected data by subtracting the mean number of dead prey individuals found in the controls.

### *Experiment 2. Effect of predation risk on metabolism of intermediate predators*

Effect of predation risk on metabolic rates was analyzed with linear regression model. The analysis included cues and species identity as predictors. Metabolic ecology proposes that, for most animals, metabolic rate scales with body size with an exponent of 0.75 (West et al. 1997), which we used as a correction factor in our analysis. We also tested an exponent of 0.6 that we found to be more appropriate for dragonflies (V. Kolář et al., unpublished data). Mean oxygen depletion from control treatments (with and without chemical cues) was deducted from measured values in each treatment. All statistical analyses were performed using R software, version 3.1.2 (R Development Core Team, 2014).

## Results

### *Experiment 1. Effects of predation risk, vegetation, and prey density on predator-prey interaction strength*

Trophic interaction strengths (i.e., the proportion of cladoceran prey eaten by intermediate predators) differed markedly between treatments (Fig. 1). Data were best described by a model in which interaction strength was significantly affected by prey density ( $F_{1,268}=7.3$ ,  $p=0.007$ ), intermediate predator identity ( $F_{2,264}=37.35$ ,  $p<0.0001$ ), interaction between vegetation and predation risk ( $F_{2,260}=3.35$ ,  $p=0.037$ ), and interaction between intermediate predator identity and predation risk ( $F_{4,268}=2.63$ ,  $p=0.035$ , Table 1). Interaction strength significantly decreased with prey density (Fig. 1). We found that the effects of predation risk on interaction strength were only significant for *S. sanguineum* and not for the other two intermediate predators. Nevertheless, for *S. sanguineum*, only the treatment with visual + chemical cues significantly decreased interaction strength compared to the control and chemical cue treatments. The analysis also indicated that, in the absence of predation risk, *S. sanguineum* has stronger interaction strengths (i.e., higher feeding rates) than the two other intermediate predators, while in the treatment with visual+chemical cues, interaction strengths were not significantly different between *S. sanguinerum* and *I. cf. elegans* (Fig. 1). Last but not least, vegetation significantly increased interaction strengths in the risk-free treatment, whereas it had no effect in the presence of predation risk represented by both chemical and visual+chemical cues (Fig. 1).

### *Experiment 2. Effects of predation risk on metabolic rate of intermediate predators*

Metabolic rates corrected for body mass of the three intermediate predators were not affected by intermediate predator identity ( $F_{2,117}=4.5$ ,  $p=0.013$ ) but not by predation risk ( $F_{1,116}=0.0002$ ,  $p=0.99$ , Table 2). *Sympetrum* larvae had significantly higher size-corrected metabolic rates compared to *L. quadrimaculata* and *Ischnura* (Fig. 2).

## Discussion

Species interaction strengths determine the stability of food webs (Berlow et al. 2004). A large body of literature has examined how environmental factors influence species interaction strengths and therefore affect entire communities. Most frequently, researchers have studied the effects of habitat complexity on trophic interaction strengths (Lombardo 1997; Eklöv & van Kooten 2001; Hartel et al. 2007; Anderson & Semlitsch 2015). Additional habitat structure that increases habitat complexity, usually tested in the form of added artificial vegetation as in our study, can provide refuges for prey or provide advantages for ambush predators (Klecka and Boukal 2014) and therefore respectively decreases or increases species interaction strengths.

Interaction strengths can also depend on predation risk from a top predator including fish (Tjossem 1990, Åbjörnsson et al. 1997, Mirza and Chivers 2003, Slos and Stoks 2008, Kopack et al. 2015), crustaceans in marine ecosystems (Grabowski et al. 2008, O'Connor et al. 2013), and insects in freshwater ecosystems (Steiner and Van Buskirk 2009, Albeny-Simões et al. 2014).

Few studies have focused on the joint effects of two or more factors on interaction strengths. Several studies addressed the question of how interaction strengths are affected by top predator's visual and/or chemical cues in habitats differing in complexity or productivity (i.e., differing in prey density). Others investigated the combined effects of predator species and habitat structure on prey behavior and trophic interaction strengths (Grabowski et al. 2008, O'Connor et al. 2013, Anderson and Semlitsch 2015). However, surprisingly little is known on the joint effects of the most common top-down and bottom-up regulatory mechanisms, i.e. the presence of different predation risk cues and varying prey density, on feeding rates of intermediate predators.

This leaves an important gap in our understanding of the links between environmental factors and community stability, because the joint effect of multiple factors may not simply be a sum of their individual effects. We therefore studied the combined effects of predation risk from a top invertebrate predator, habitat complexity and prey density on predator-prey interaction strengths in a cross-factorial laboratory experiment. We used three genera of medium-sized odonate larvae which represent common intermediate predators in fishless pools (Klecka and Boukal 2012).



Predation risk decreased interaction strength only in *S. sanguineum*, and we observed this effect only in the presence of both visual and chemical cues. This suggests that *S. sanguineum*, which is a more visual-oriented predator, reacts primarily to visual predation risk stimuli. We found no effect of predation risk on interaction strengths in both *I. cf. elegans* and *L. quadrimaculata*. The latter is a benthic predator, which lives on the bottom and sometimes digs into the substrate (Dolný et al. 2008, Klecka and Boukal 2012, 2014). This suggests that *L. quadrimaculata* can coexist with *Aeshna* sp. (if the latter cannot access the benthic habitat) due to non-overlapping habitat domains as shown in other studies on benthic predatory newts and phytophilous dragonfly larvae (Ramos and Van Buskirk 2012, Anderson and Semlitsch 2015).

Moreover, *L. quadrimaculata* larvae react only to mechanical and visual stimuli from prey within 1 cm (Rebora et al. 2004) and similar reaction distance could apply to predation risk. In our experiment, the distance between the intermediate and top predator was > 6 cm and mechanical stimuli, e.g., through water turbulences were excluded, because *Aeshna* larvae were isolated in a plastic box. This could explain why *L. quadrimaculata* and *I. cf. elegans* feeding rates did not change in the presence of *Aeshna* sp. Our results are in line with a study by Schaffner and Anholt (1998), who showed that *I. elegans* reacts only to free-ranging but not caged *Anax imperator* larvae. Finally, it is also possible that the concentration of cues used in our study was not high enough to influence *L. quadrimaculata* and *I. cf. elegans* feeding rates.

We found interactive effects of vegetation and predation risk on interaction strength, which suggests that these factors should be considered together to better understand trophic interactions in food webs. The presence of artificial vegetation significantly increased interaction strength for the three intermediate predator species, but only in the risk-free treatment. This result is in line with Klecka and Boukal (2014), who showed that phytophilous predators have a higher feeding rate in the presence than in the absence of vegetation. This suggests that vegetation provide an anti-refuge effect for the cladoceran prey when the intermediate predators are not under predation risk. Moreover, the interactive effects of vegetation and predation risk were not affected by prey density, which suggests that the effect of vegetation and predation risk on prey and/or predator behavior do not depend on prey density. The decrease of interaction strengths at higher prey densities is a general trend in predator-prey interactions (Rall et al. 2012, Sentis et al. 2012), usually caused by non-linear feeding rates of the predators.

Few studies examined the effect of predation risk on prey metabolic rates (Hawlena and Schmitz 2010, Culler et al. 2014, Okuyama 2015). Predation risk can increase the basal metabolic rate of the prey, which can be linked to a chronic response underlying faster development of the prey in the presence of predators or an acute response underlying the fight-or-flight behavioural reaction. On the other hand, predation risk can also decrease the metabolic rate of prey if its primary response is to slow down the developmental rate (Culler et al. 2014).

Previous studies of predation risk-dependent metabolic rates included only fish as predators (Slos and Stoks 2008, Culler et al. 2014). Here we found no effects of *Aeshna* sp. cues on metabolic rates of all three intermediate predators. This is surprising because other studies found elevated metabolic rates under predation risk (Steiner and Van Buskirk 2009, Hawlena and Schmitz 2010, Okuyama 2015). We conclude that the intermediate predators used in our study most likely do not respond to the chemical stimuli of *Aeshna* larvae unlike those of fish predators (Culler et al. 2014), because both metabolic rates and feeding rates of all intermediate predators were almost never different in the presence of chemical stimuli. It is also possible that the physiological response of intermediate predators to predation risk is very short (e.g., during a fleeing reaction) and afterwards they become accustomed to the cues (Lass and Spaak 2003, Wisenden 2008, Chivers et al. 2013, Van Buskirk et al. 2014). On the other hand, some experiments showed that prey can respond to very low cues concentrations (Mirza and Chivers 2003, Wisenden 2008, Chivers et al. 2013). Last but not least, the chemical composition and lifetime of most predator kairomones are unknown (Brönmark and Hansson 2012). If cues degrade rapidly, their effects on the prey will be low; however, chemical cues produced by *Aeshna* sp. larvae seem to have a more lasting effect, at least on *Cloeon dipterum* larvae (Šupina et al. 2015).

In conclusion, we showed that the effects of vegetation and predation risk on intermediate predators are context-dependent and interactive, because the net effect of vegetation on feeding rates changes with predation risk. Additional studies are needed to elucidate the role of these factors on the strengths of food web interactions and identification of key predator and prey functional traits such as habitat domain, ontogeny, foraging modes and previous exposure to predation risk cues (Boukal 2014, Anderson and Semlitsch 2015). Moreover, predation risk cues and more generally direct and indirect interactions in aquatic food webs may be affected by external input of toxins, hormone metabolites and heavy metals (Lass and Spaak 2003), which may further change predictions based on the assumption of more pristine natural conditions.

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VK, DSB and AS designed the study, VK ran the experiments, VK and AS analyzed the data with additional input from DSB, VK wrote the manuscript draft, and DSB and AS provided comments. We would like to thank Lucie Vebrová and Baptiste Jaugeon for help with the experiment. This work was supported by the Grant Agency of the Czech Republic (Project number: 14-29857S).

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# Figures

Fig. 1: Interaction strength (mean  $\pm$  1 SE) for the three intermediate predators at two prey densities (50, 150 ind.L<sup>-1</sup>) in three predation risk treatments: predator risk-free, predator risk signaled by chemical cues, and risk signaled by chemical and visual cues from top predator (larvae of *Aeshna* sp.). Effect of habitat complexity: artificial vegetation present (green) or absent (red).

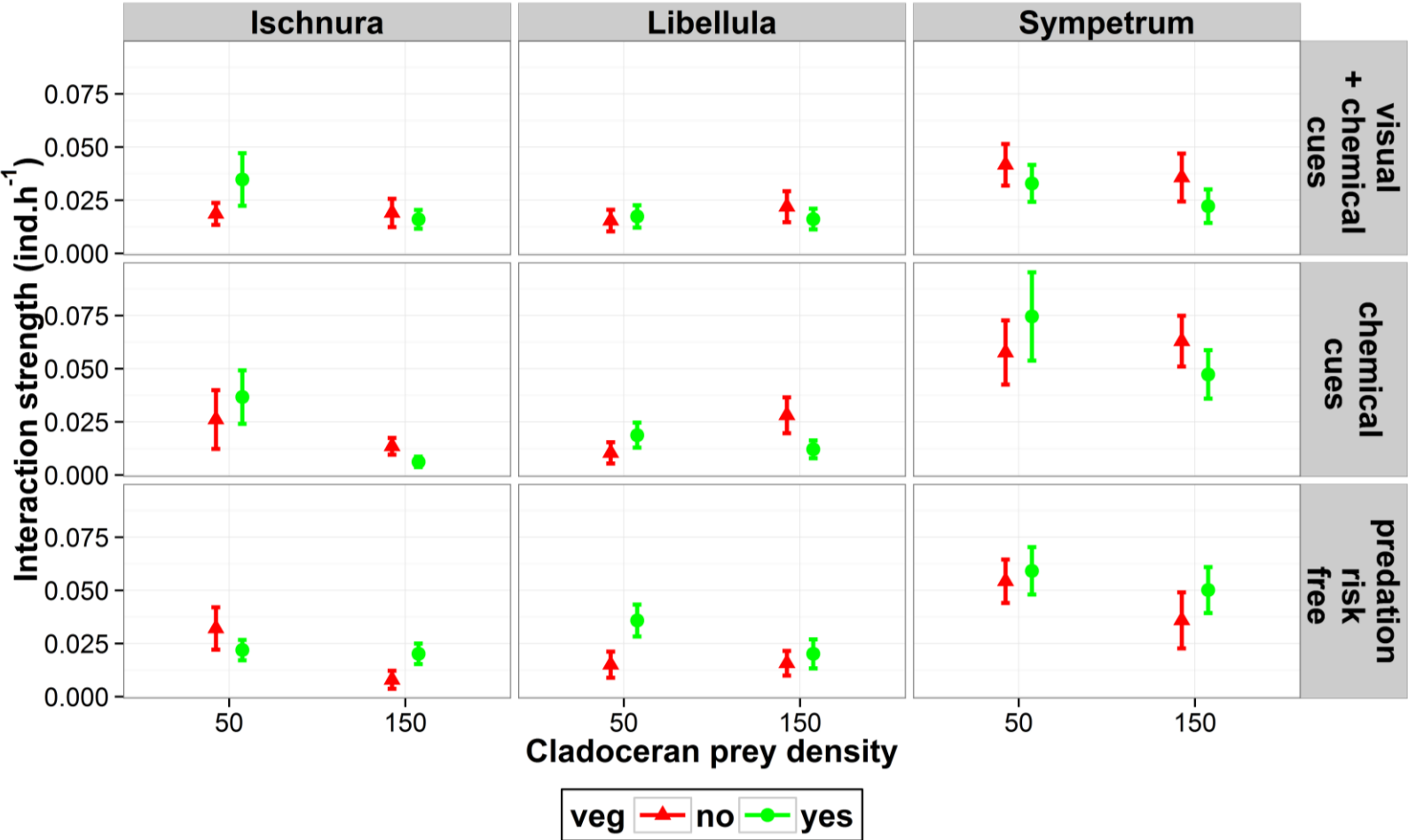
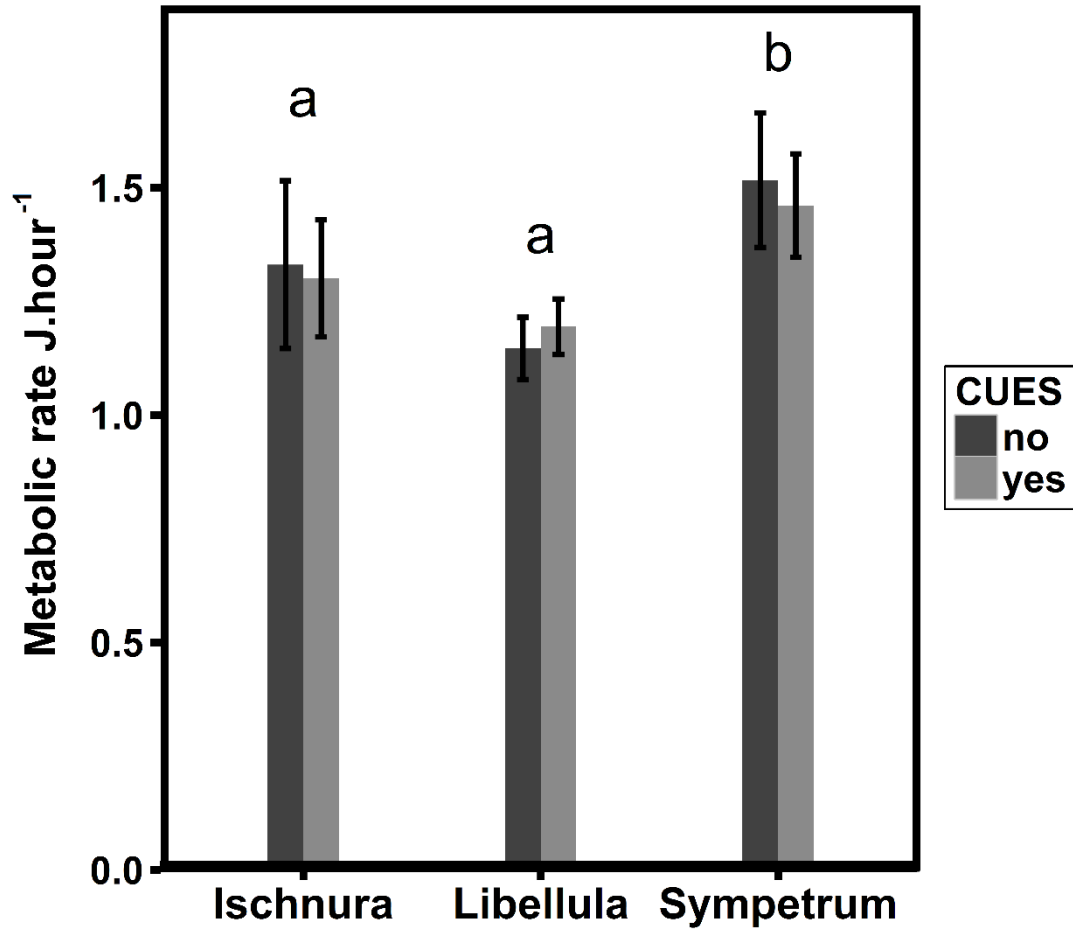




Fig. 2: Effect of predation risk on mass-corrected metabolic rate (mean  $\pm$  1 SE) of each intermediate predator. Different letters denote significant differences among intermediate predators ( $p < 0.05$ ). Effect of chemical cues was not significant.



## Tables

Table 1: Analyses of deviance from the best candidate model for the effects of prey density, habitat complexity (i.e. vegetation presence), intermediate predator identity, predation risk, and interactions between top predator and habitat complexity and between top predator and intermediate predator on the proportion of prey eaten by the intermediate predators. Significant effects in bold.

<b>Effect</b>	<b>df</b>	<b>residual df</b>	<b>F</b>	<b>P</b>
Cladocera density	1	268	7.3	<b>0.007</b>
Habitat complexity	1	266	0.039	0.843
Intermediate predator species	2	264	37.35	<b>&lt;0.0001</b>
Predation risk	2	262	2.87	0.058
Predation risk × habitat complexity	2	260	3.35	<b>0.037</b>
Predation risk × intermediate predator species	4	256	2.63	<b>0.035</b>

Table 2: Results from the best linear model describing the effect of intermediate predator species and body weight on the metabolic rate. Significant effects in bold.

<b>Effect</b>	<b>df</b>	<b>residual df</b>	<b>F</b>	<b>P</b>
Intermediate predator	2	1.9±0.96	104.3	<b>&lt;0.0001</b>
Weight	1	0.43±0.43	46.4	<b>&lt;0.0001</b>

## Appendices

Table S1: Number of replicates  $N$  in Experiment 1 (Effects of predation risk, habitat complexity, and prey density on predator-prey interaction strength).

<b>Species</b>	<b>Prey density (ind.L<sup>-1</sup>)</b>	<b>Vegetation present</b>	<b>Predation risk cues</b>	<b><math>N</math></b>
<i>Ischnura</i>	150	Yes	Visual+chemical	7
		No	Visual+chemical	7
		Yes	Chemical	7
		No	Chemical	6
		Yes	None	8
		No	Predator risk free	6
	50	Yes	Visual+chemical	7
		No	Visual+chemical	7
		Yes	Chemical	8
		No	Chemical	6
		Yes	None	7
		No	None	8
<i>Libellula</i>	150	Yes	Visual+chemical	8
		No	Visual+chemical	8
		Yes	Chemical	8
		No	Chemical	7
		Yes	None	8
		No	None	8
	50	Yes	Visual+chemical	9
		No	Visual+chemical	8
		Yes	Chemical	8
		No	Chemical	8
		Yes	None	8
		No	None	8
<i>Sympetrum</i>	150	Yes	Visual+chemical	8
		No	Visual+chemical	8
		Yes	Chemical	7
		No	Chemical	6
		Yes	None	7
		No	None	7
	50	Yes	Visual+chemical	8
		No	Visual+chemical	8
		Yes	Chemical	8
		No	Chemical	7
		Yes	None	8
		No	None	7
Control	150	Yes	Visual+chemical	5
		No	Visual+chemical	5

	Yes	Chemical	5
	No	Chemical	4
	Yes	None	5
	No	None	5
50	Yes	Visual+chemical	5
	No	Visual+chemical	5
	Yes	Chemical	5
	No	Chemical	5
	Yes	None	5
	No	None	5

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Table S2: Number of replicates  $N$  in Experiment 2 (Effect of predation risk on the metabolism rates of intermediate predators).

<b>Species</b>	<b>Predation risk cues</b>	<b><math>N</math></b>
<i>Ischnura</i>	Yes	16
	No	16
<i>Libellula</i>	Yes	26
	No	29
<i>Sympetrum</i>	Yes	19
	No	14
Control	Yes	33
	No	34