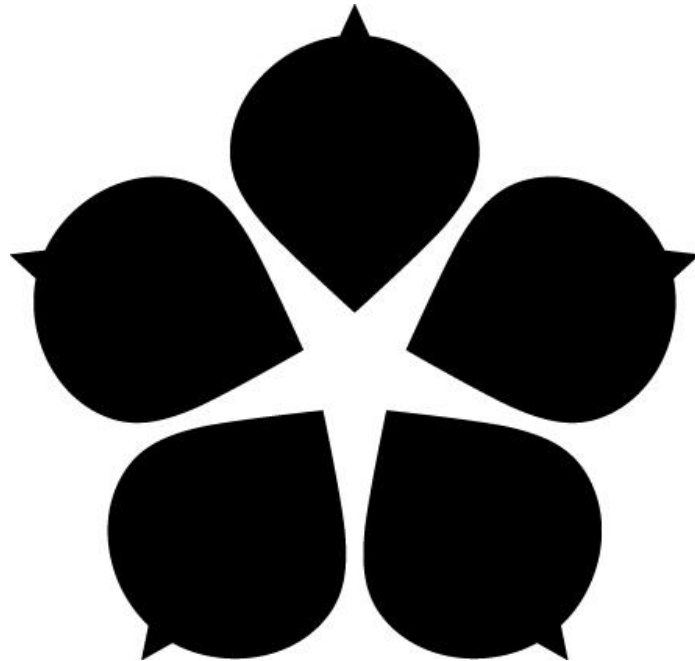


University of South Bohemia in České Budějovice
Faculty of Science



**Predation risk and habitat complexity modify
intermediate predator feeding rates and energetic
efficiencies in a tri-trophic system**

RNDr. Thesis

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Annotation

Many species show behavioural and physiological responses to predation risk, with important implications for their individual growth and development, but these responses may be context dependent. The goal of this study was to measure feeding and metabolic rates of intermediate invertebrate predators under different levels of predation risk and abiotic conditions to understand how they might affect their energetic efficiency, i.e. the ability to utilize available resources for individual growth. We ran laboratory experiments with larvae of three odonate species representing intermediate predators exposed to three levels of predation risk (none, visual cues, and visual and chemical cues) and two levels of habitat complexity (present vs. absent artificial submerged vegetation). We show that the response of intermediate predators to predation risk and its consequences for energetic efficiency are species specific and likely determined by their foraging and defense traits and their habitat domains.

Declaration [in Czech]

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

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České Budějovice, 16.1. 2020

Mgr. et Ing. Vojtěch Kolář

Contribution of the candidate

This thesis is based on the following publication:

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Vojtěch Kolář is the first and corresponding author of the paper based on his Master thesis. He with both co-authors conceived the experimental design and conducted the experiments. Together with Arnaud Sentis analysed the data with additional input from David Boukal. Finally, Vojtěch Kolář wrote the first draft of manuscript.

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Predation risk and habitat complexity modify intermediate predator feeding rates and energetic efficiencies in a tri-trophic system

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Abstract

1. To understand the effects of environmental changes on ecosystems, it is important to determine the factors and mechanisms influencing the strength of species interactions in food webs. However, joint effects of predation risk and additional environmental factors on species interaction strengths in multitrophic systems remain largely unexplored, leaving a substantial gap in our understanding of the links between local environmental characteristics and food web properties.
2. To fill this gap, we investigated the effects of habitat complexity and predation risk by top predatory dragonfly larvae (*Aeshna cyanea*) on feeding rates and energetic efficiency (i.e. the ratio of acquired and expended energy) of the larvae of three intermediate predatory odonate species (*Libellula quadrimaculata*, *Sympetrum sanguineum*, and *Ischnura elegans*) preying on cladocerans.
3. We hypothesised that predation risk would decrease the feeding rate, especially in the structurally simple habitat, and increase the metabolic rate of all intermediate predators. We also expected higher feeding rates of intermediate predators using aquatic vegetation as a perching site (i.e. *Sympetrum* and *Ischnura*) in the structurally complex habitat. Finally, we expected to observe habitat- and predation risk-dependent energetic efficiencies of the intermediate predators driven by changes in feeding and metabolic rates.
4. The effect of predation risk on feeding rates was species specific and differed between the structurally simple and complex habitat. Habitat complexity increased feeding rates but only in the absence of predation risk. Moreover, predation risk signalled by chemical cues significantly increased *Sympetrum vulgatum* feeding rates but did not influence the feeding rates of the two other intermediate predators.
5. Metabolic rates varied among the three intermediate predators but were not affected by predation risk. Estimated energetic efficiency decreased with intermediate predator body mass and depended, to a lesser extent, on the interactive effect of habitat complexity and predation risk.
6. Our results imply that the effects of habitat complexity and predation risk on trophic interactions are likely to be determined by traits related to foraging and defence of the intermediate predators and their habitat domains, and that energetic

efficiency is mainly determined by predator mass. Given that habitat complexity and predation risk can vary substantially across habitats, we conclude that it is important to consider habitat complexity and predation risk to better understand and predict the effects of environmentally driven variations on trophic interaction strength and metabolic rates that underlie the energetic efficiency of individual consumers. This has important implications for population and community dynamics as well as ecosystem functioning.

KEYWORDS

chemical cues, metabolic rate, Odonata, predator-prey interactions, trait-mediated effects

1 | INTRODUCTION

Species interaction strengths (i.e. the effect of one species on the abundance of a second species) are 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 strengths are given by the predator feeding rates relative to prey density (Berlow et al., 2004; Rall et al., 2012; Sentis, Hemptinne, & Brodeur, 2014; Wootton & Emmerson, 2005). At longer timescales, interaction strengths also depend on the predator's metabolic rate, which determines the energy demand of the predator and influences its energetic efficiency, defined as the ratio of feeding and metabolic rates (Binzer, Guill, Brose, & Rall, 2012; Gilbert et al., 2014; Rall, Vucic-Pestic, Ehnes, Emmerson, & Brose, 2010; Sentis, Morisson, & Boukal, 2015). Energetic efficiency underlies the dynamics and stability of food webs as it influences the energy flow between trophic levels and thus affects predator-prey oscillations and species persistence. Recent theoretical and empirical studies indicate that energetic efficiency declines with temperature. Metabolic demands may exceed ingestion rates and thereby lead to starvation and, ultimately, extinction of consumer populations at high temperatures irrespective of the amount of their resources (Binzer et al., 2011; Fussmann, Schwarzmüller, Brose, Jousset, & Rall, 2014; Petchey, McPhearson, Casey, & Morin, 1999; Sentis, Binzer, & Boukal, 2017; Vucic-Pestic, Rall, Kalinkat, & Brose, 2010). Investigating the effects of environmental drivers such as temperature on feeding and metabolic rates is thus important for predicting the dynamics and stability of ecological communities.

However, little is known about how biotic (e.g. predation risk) and abiotic factors (e.g. habitat complexity) jointly influence short- and long-term trophic interaction strengths. Species are embedded within communities and the strengths of predator-prey interactions depend on direct interactions between the predator and its prey but also on indirect interactions mediated through one or more species (Schmitz, Krivan, & Ovadia, 2004; Sentis, Gémard, Jaugeon, & Boukal, 2017; Werner & Peacor, 2003). While food-web studies traditionally focus on trophic interactions in single predator-prey

systems, multiple studies have highlighted the importance of indirect density- and trait-mediated effects for the strengths of species interactions in ecological communities (Davenport & Chalcraft, 2013; McCoy, Stier, & Osenberg, 2012; Okuyama & Bolker, 2007; Ousterhout, Graham, Hasik, Serrano, & Siepielski, 2018; Werner & Peacor, 2003). For instance, prey are typically less active and feed less in the presence of a predator or its cues (Hawlena & Schmitz, 2010b; Stoks & McPeck, 2003; Trussell, Ewanchuk, & Bertness, 2003), which may indirectly alter species interaction strengths between the prey and their resources.

Predation risk can also influence the metabolic rates of the prey, which together with the effects on feeding rates may have consequences for short- and long-term interaction strengths. Respiration rate (a proxy of metabolic rate) of prey can increase (Beckerman, Wieski, & Baird, 2007; Hawlena & Schmitz, 2010a; Okuyama, 2015; Slos & Stoks, 2008; Steiner & Van Buskirk, 2009) or decrease under predation risk (Antoń, Kierat, & Czarnoleski, 2018; Barry & Sval, 2013; Handelsman et al., 2013; Smith, Sims, & Vich, 1981). Lowered metabolic rates correspond to reduced prey activity and slower growth that trades off against lower predation mortality (Handelsman et al., 2013). Higher respiration rates reflect faster development as a life-history response to predation risk (Beckerman et al., 2007) or a short-term behavioural response as the individuals attempt to escape from the predator. This behaviour, known as the fight-or-flight strategy, is energetically costly (Okuyama, 2015; Slos & Stoks, 2008). Moreover, the initial fight-or-flight response may be replaced by long-term reduction of growth and lowered metabolic rates (Steiner & Van Buskirk, 2009). This suggests that metabolic responses to predation depend on predator and prey identities and on the balance between prey activity and metabolic demand. Nevertheless, only few studies simultaneously investigated the effects of predation risk on the prey feeding and metabolic rates (Beckerman et al., 2007) or directly quantified the effect of predation risk on some measure of growth or energetic efficiency (McPeck, Grace, & Richardson, 2001; Stoks, De Block, Van de Meutter, & Johansson, 2005; Trussell, Ewanchuk, & Matassa, 2006), which hinders our understanding on how predation risk affects long-term trophic interaction strengths.

Additional abiotic factors can also profoundly alter species interaction strengths. Among them, habitat complexity (i.e. diversity, size, density and arrangement of structural elements, for details see Tokeshi & Arakaki, 2012) can affect species interactions in both terrestrial (Hurlbert, 2004; Langelotto & Denno, 2004) and aquatic ecosystems (Wellborn, Skelly, & Werner, 1996). The effect of habitat complexity in freshwater food webs, e.g. due to the presence of submerged vegetation, is context dependent. It can range from decreased predator feeding rates when prey can use vegetation as a refuge (e.g. Fischer, Zanotti, Castro, Quiroga, & Vargas, 2013; Klecka & Boukal, 2014; Warfe & Barmuta, 2004), to no effect (e.g. Delclos & Rudolf, 2011; Fischer et al., 2013; Klecka & Boukal, 2014), to increased hunting efficiency of sit-and-wait/ambush predators (Convey, 1988; Eklöv & van Kooten, 2001; Savino & Stein, 1989), and phytophilous predators. For example, some odonate larvae use submerged vegetation as perching sites to access the water column (Klecka & Boukal, 2014). Overall, these previous studies suggest that the effect of habitat complexity on feeding rate depends on the changes in habitat use of prey and predators, which, in turn, influence predator-prey encounters and predator foraging success.

In summary, current experimental evidence suggests that predation risk and habitat complexity can influence the prey feeding and metabolic rates. To understand the consequences for short- and long-term interaction strengths in multitrophic ecological communities, we experimentally investigated the joint influence of predation risk, and habitat complexity on the feeding rate of three intermediate predators using predatory odonate larvae and cladoceran prey as a model system. We also measured the resting metabolic rates of these three intermediate predators in the presence and absence of top predator chemical cues. We then combined data on feeding and metabolic rates to calculate the energetic efficiency (= consumer relative growth rate; Rall et al., 2010) of the intermediate predators. Our choice of the trophic position of the study organisms was motivated by the apparent prevalence of intermediate predators in food webs (Williams & Martinez, 2000). Using published data on 24 aquatic food webs, we found that intermediate predators account for 60% (range 31–88%) of all species in each food web, which contrasts with only 16% (range 0–43%) of top predators (Table S1).

Our study focuses on small fishless pools in which aeshnid dragonfly larvae are the top predators that often co-occur with other smaller dragonfly and damselfly species. Previous studies on dragonflies and *Daphnia* used as prey showed that *Daphnia* respond primarily to predators that co-occur with them in nature (Stoks, Govaert, Pauwels, Jansen, & De Meester, 2016; Stoks & McPeck, 2003). Moreover, other invertebrates can modify their behaviour, growth and development in response to chemical cues released by aeshnid larvae (e.g. Johansson, 1993; Schaffner & Anholt, 1998; Stoks & McPeck, 2003; Šupina, Bojková, & Boukal, 2016). We thus expected that the intermediate predators used in our study can sense and respond to stimuli provided by aeshnid predators. We hypothesised that: (1) predation risk caused by late-instar larvae of the dragonfly *Aeshna cyanea* will decrease the feeding rates of all three intermediate predators; (2) the feeding rate of phytophilous predators

(*Ischnura elegans* and *Sympetrum sanguineum*) will increase whereas the feeding rate of the benthic predator (*Libellula quadrimaculata*) will remain constant in the more complex habitat; (3) the effect of predation risk on feeding rates will be weaker in the more complex habitat; (4) predation risk will increase or decrease metabolic rates of the intermediate predators; and (5) these four mechanisms will jointly affect the energetic efficiency of the intermediate predators. By testing these five hypotheses simultaneously, our study highlights the importance of accounting for the interacting effects of biotic and abiotic factors to better understand and predict interactions across multiple trophic levels.

2 | MATERIAL AND METHODS

2.1 | Experiments

Experiments were conducted at the Institute of Entomology in České Budějovice (Czech Republic) in July and the first half of August 2014. We used late-instar larvae of three odonate species as intermediate predators, which dominated the populations in the field at the time of the experiment: *Libellula quadrimaculata* (F-1 instar, 0.22 ± 0.02 g wet weight, 17.5 ± 0.6 mm total body length, 5.0 ± 0.1 mm head width, all data as mean \pm SD), *Sympetrum sanguineum* (F-0 instar, 0.13 ± 0.02 g wet weight, 14.5 ± 1.2 mm total body length, 4.9 ± 0.2 mm head width), and *Ischnura elegans* (F-0 instar, 0.027 ± 0.003 g wet weight, 13.1 ± 1.1 mm body length without caudal lamellae, 3.2 ± 0.1 mm head width). As top predator we used larvae of the dragonfly *Aeshna cyanea* (F-0 instar, 0.86 ± 0.20 g wet weight, 40.1 ± 2.3 mm body length, 8.0 ± 0.2 mm head width). All these species (hereafter referred to only by the genus name) are common in small standing waters in central Europe.

The dragonfly larvae used in this study are all sit-and-wait predators occupying different habitat domains: while *Libellula* is a benthic predator, *Sympetrum* often access prey in the water column by perching on the vegetation and using it as a hunting site (Cham, 2012; Klecka & Boukal, 2014). *Ischnura* can use aquatic vegetation as a hunting site (Convey, 1988) and is also known as an early coloniser of newly formed habitats without vegetation (Corbet, 2004), indicating that it can also hunt efficiently in structurally simple habitats. Moreover, the latter two intermediate predators (*Ischnura* and *Sympetrum*) use visual stimuli, while the first one (*Libellula*) mostly uses mechanical stimuli for prey detection (Corbet, 2004; Reborja, Piersanti, & Gaino, 2004). Finally, the top predator *Aeshna* is a phytophilous sit-and-wait, ambush predator that uses mainly visual stimuli to detect prey and inhabits mostly vegetated pools. We used a mixture of *Daphnia magna* and *Simocephalus* sp. (Cladocera: Daphniidae) as prey with standardised body size obtained by passing them through mesh sizes of 710 and 850 μ m and keeping the intermediate size fraction. The prey are active filter feeders occupying the water column and the benthic zone. Top and intermediate predators were collected in small ponds and pools in south Bohemia, where they often coexist. Prey populations, established from individuals collected in fishponds near České Budějovice, were maintained on

Chlorella vulgaris (Chlorellales: Chlorellales) in the laboratory and in outdoor tanks. Prior to experiments, all predators were acclimated to laboratory conditions in a walk-in temperature controlled room ($20 \pm 1^\circ\text{C}$, 17:7 hr light:dark cycle) for at least 2 days and fed daily *ad libitum* with a mixture of *D. magna* and *Simocephalus* sp. (for intermediate predators) or commercially available chironomid larvae (for top predators). Intermediate 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.). Experiments were performed in a temperature-controlled incubator (Lovibond BSK ET 650, water temperature $19.4 \pm 0.3^\circ\text{C}$, daylight conditions). Intermediate predators were exposed to predation risk cues (see below for details) for 6 hr in the first experiment and for 2.5 hr in the second experiment.

Each individual *Aeshna* larva was kept in a large plastic cup filled with 0.3 L of aged tap water. Water containing the chemical cues was prepared 1 hr before the experiment as follows: half of the water (i.e. 0.15 L) from 12–20 individual *Aeshna* rearing cups was collected together in one container, then thoroughly mixed, filtered through a 0.5-mm sieve to remove large particles, and diluted with aged tap water to obtain a concentration of *Aeshna* cues at c. 20% of the rearing cup concentration following standard methods outlined in Brönmark and Hansson (2012). The missing volume of each rearing cup was then refilled with aged tap water. Each top predator was rested for at least 48 hr before it was used again.

2.1.1 | Experiment 1: Effects of predation risk and habitat complexity on intermediate predator feeding rates

We first examined if the intermediate predators responded (i.e. changed their feeding rate) differently to visual and/or chemical cues in different environmental contexts. To this end we used a full factorial design (Figure S1) by crossing two levels of habitat complexity (i.e. presence and absence artificial aquatic plants) with three levels of predation risk (no top predator, top predator chemical cues, and caged top predator with added chemical cues; respectively referred hereafter as *risk-free*, *chemical cues*, and *visual and chemical cues* treatments). Experimental arenas consisted of plastic jars (length 12.5 cm, width 8 cm, height 19 cm) filled with 1 L of aged tap water (or 1 L of the water with chemical cues, see above), lined with 1–2 cm of fine crystalline sand (diameter < 1 mm). Two artificial plants with 4 leaves (stripes, c. 7 cm long and 2 cm wide, made of green plastic fabric, mesh size c. 3 mm) were added in each arena in the *complex habitat* treatment.

Intermediate predators were starved in the rearing cup at the experimental temperature 12 hr before the experiment to standardise their hunger levels. They were not acclimated to predation risk cues before the experiment. At the onset of the experiment, 150 prey were individually introduced in the experimental arenas. Two hours later, one intermediate predator (immediately followed by a caged top predator in the visual risk treatments) was randomly introduced

in each arena. Each caged top predator was placed in a transparent, impermeable plastic box ($8.5 \times 9.5 \times 8$ cm) filled with 0.45 L water and placed on top of the experimental arena, so that the bottom of the box reached 5 cm below the water surface. The same empty box was used in treatments without visual cues. Intermediate predators were allowed to feed on prey for 6 hr. Afterwards, all predators were removed and surviving prey counted. Natural prey mortality was assessed in control treatments without predators. We performed 4–9 replicates for each treatment except controls, for which we did 3–5 replicates (Table S2).

2.1.2 | Experiment 2: Effect of predation risk on the metabolic rate of intermediate predators

To measure the effect of predation risk on respiration rate as a proxy of metabolic rate, we used the Unisense® MicroOptode oxygen probe with the software SensorTrace Basic v3.2.3 (Unisense®) following Sentis et al. (2015). Respiration was measured in sealed glass chambers (mean \pm SD volume: 57.5 ± 1.6 ml) filled with distilled water conditioned by adding purified salt (sera®, 0.14 g L^{-1}) to achieve the conductivity of c. $200 \mu\text{S cm}^{-1}$ representative of field conditions (Sentis et al., 2015). Intermediate predators were starved and acclimated to temperature experimental conditions 48 hr prior to the experiment. We used a subset of randomly selected intermediate predators that were first used in Experiment 1. Individuals were used 3 or more days after Experiment 1 and were randomly assigned to one of the predation risk treatment. It was not possible to test the effects of other factors from Experiment 1 due to limitations set by the size and shape of the glass chambers. For the *predation risk* treatment with chemical cues, we produced top predator chemical cues using the same protocol and concentration as in Experiment 1.

Oxygen concentration was measured just before the introduction of the individuals and the chambers were then immediately sealed with a hermetic glass plug. After 150 min, water was mixed for c. 15 s using a magnetic stirrer to homogenise the samples and oxygen concentration was measured again. The duration of the experiment was chosen to generate sufficient contrast between blank controls and chambers containing odonate larvae and to avoid any artefacts due to excessive oxygen depletion (mean relative oxygen depletion \pm SD: controls $1.25 \pm 2.83\%$, *Ischnura* $3.64 \pm 2.50\%$, *Libellula* $15.8 \pm 4.77\%$, *Sympetrum* $13.3 \pm 5.57\%$). Each intermediate predator was then weighed to the nearest 0.0001 g using a Kern® ABT microbalance to account for the effects of body weight on respiration rate (see statistical analyses below). We performed 14–39 replicates for each treatment (Table S3). For each predation risk treatment, possible background oxygen depletion was assessed in controls without larvae ($n = 69$, 3–10 controls per day, see Table S3 for details). Oxygen consumption in $\mu\text{mol min}^{-1}$ was converted to J hr^{-1} following Weiss (1970).

Replicates of each treatment in each experiment were run in a predetermined random order. Numbers of replicates differed between days because we occasionally lacked the cladoceran prey or some intermediate predators died or moulted.

2.2 | Statistical analyses

All statistical analyses were performed using R software, version 3.3.2 (R Core Team, 2016). Linear mixed effect models (LMMs) were used to analyse the effects of habitat complexity, intermediate predator identity and predation risk on the feeding rates (Experiment 1) and the effects of chemical cues and intermediate predator identity on the metabolic rates (Experiment 2) of the intermediate predators. Linear mixed effect models were implemented in the *lme4* package version 1.1-12 (Bates, Mächler, Bolker, & Walker, 2015) and Kenward–Roger F-tests were computed using the *anova* function from the *pbkrtest* package version 0.4-7 (Halekoh & Højsgaard, 2014).

2.2.1 | Experiment 1: Effects of predation risk and habitat complexity on intermediate predator feeding rates

We quantified feeding rate (i.e. short-term interaction strength) as the proportion of prey eaten. The proportions were first arcsine square-root transformed to linearise the binomial distribution of the proportion of prey eaten by the intermediate predators (Gotelli & Ellison, 2004). The LMM was then used to test the effects of intermediate predator identity, habitat complexity, predation risk, and their interactions on the arcsine square-root transformed proportion of prey eaten. Experimental date was included as a random effect to account for possible temporal variation in experimental conditions. Body mass of each intermediate predator was included

in the model as a covariate to account for potential mass-dependent effects. We found significant prey mortality in the controls (mean \pm SD = 4.32 ± 4.78 individuals) although it did not differ among habitat complexity and predation risk treatments (LMM, $p > 0.05$). We therefore corrected the data by subtracting the mean number of dead prey in the controls from the number of eaten prey. The most parsimonious model was determined by the sequential deletion of the least significant explanatory parameters (or interaction terms) from the saturated model with all fixed effects and possible interaction terms (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). The final model contained only explanatory variables that had a significant effect or were part of significant interaction terms ($p < 0.05$). Fixed effects were tested using F-tests for which the degrees of freedom for the fixed effects were adjusted for statistical dependence using the Kenward–Roger method. *Post-hoc* Tukey tests (*lsmeans* package version 2.25; Lenth, 2016) were used to determine significant differences among treatment means.

2.2.2 | Experiment 2: Effect of predation risk on the metabolic rate of intermediate predators

Effect of predation risk on metabolic rate was analysed with LMMs and Kenward–Roger F-test. Metabolic ecology theory proposes that metabolic rate scales with body size with an exponent of 0.75 (West, Brown, & Enquist, 1997). We thus used log-transformed mass-corrected rate of oxygen consumption (i.e. O_2 consumption $\cdot g^{-0.75}$) as the response variable. The analysis further included cues, species identity, and previous exposure to predation risk in

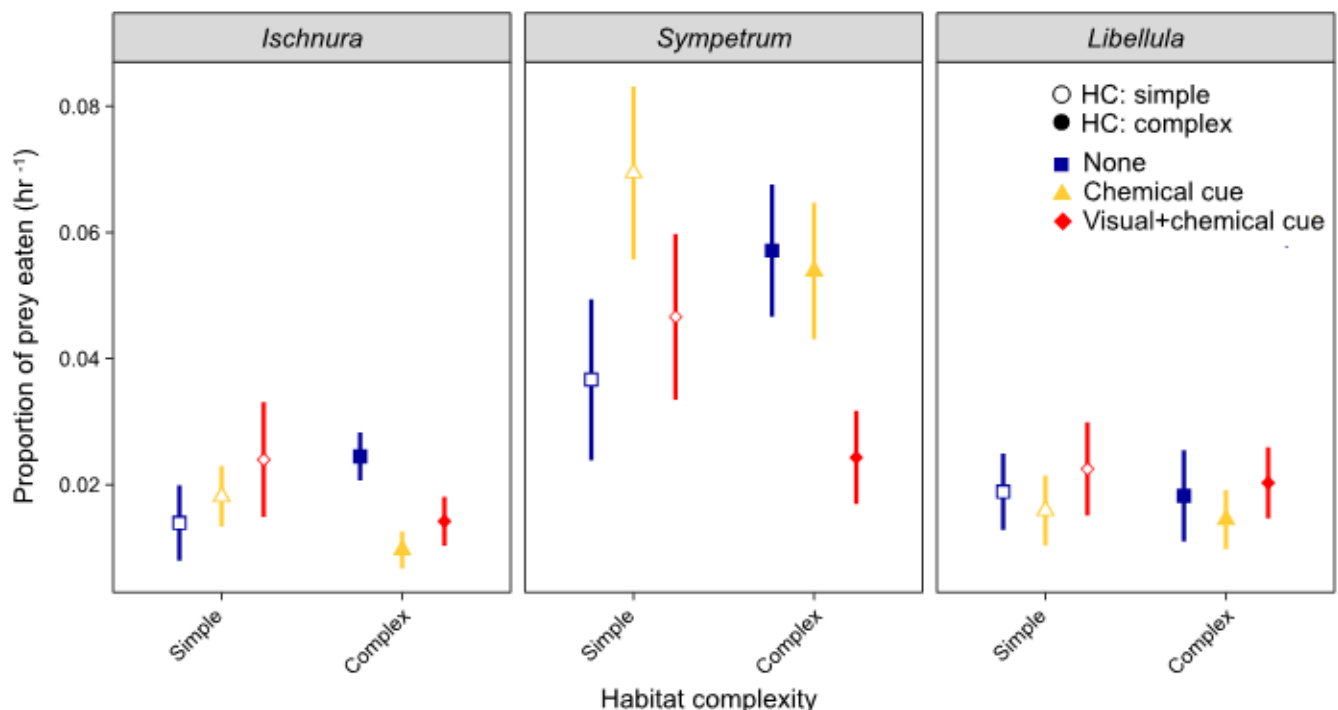


FIGURE 1 Proportion of prey eaten per hour (mean \pm SE) for the three intermediate predators in simple (empty symbols) or complex (filled symbols) habitats and at three predation risk treatments: no risk (squares), predator risk signalled by chemical cues (triangles), and predator risk signalled by visual and chemical cues (diamonds)

Experiment 1 as predictors and day of experiment as a random effect to account for potential temporal variations in experimental conditions. Previous exposure of intermediate predators to predation risk, including its possible interactions with predator cues and species identity, did not have a significant effect on their metabolic rate (LMM, $F_{1, 91.9} = 0.04$, $p = 0.85$) and was thus excluded from final analyses.

Oxygen depletion in controls was low relative to the metabolic activity of the intermediate predators, but varied significantly among dates and chemical cue treatments (with chemical cues: range -0.025 to 0.117 $J\ hr^{-1}$, mean \pm $SD = 0.023 \pm 0.027$ $J\ hr^{-1}$, without chemical cues: range -0.029 to 0.028 $J\ hr^{-1}$, mean \pm $SD = -0.001 \pm 0.014$ $J\ hr^{-1}$). We therefore deducted daily mean depletion measured in controls from all values in each respective treatment (with and without chemical cues) on that day.

2.3 | Energetic efficiency of the intermediate predators

We used our empirical data on metabolic and feeding rates to estimate the predator energetic efficiency $EE = (\omega F) / RMR$, which corresponds to the *per capita* energy intake rate relative to metabolic rate (Gilbert et al., 2014; Rall et al., 2010; Sentis, Binzer, & Boukal, 2017). Here, F is the *per capita* intake rate ($J\ hr^{-1}$), equal to the *per capita* biomass feeding rate ($mg\ hr^{-1}$, calculated as number of eaten prey multiplied by average prey body weight (mean \pm SE: $18.7 \times 10^{-5} \pm 2.01 \times 10^{-5}$ g wet mass) multiplied by a weight-energy conversion factor [1 mg wet mass = 7 J (Peters, 1983)], ω is the assimilation efficiency [0.85 for carnivores (Peters, 1983)], and RMR is the resting metabolic rate ($J\ hr^{-1}$) measured in Experiment 2.

Mean feeding and metabolic rates were used to calculate the energetic efficiency of each intermediate predator in each experimental treatment. We assumed that (a) habitat complexity had no effect on the metabolic rates and (b) metabolic rates under the combined visual and chemical predation risk cues are closely approximated by metabolic rate measurements from the chemical cue treatment, because it was not logistically possible to test for either mechanism in Experiment 2. To illustrate the amount of uncertainty in the energetic efficiency estimates, we used the standard errors associated with the estimates of each parameter to calculate the 95% confidence intervals (CIs) of energetic efficiency for each treatment using the law of propagation of uncertainty (package *propagate* version 1.0-4; Spiess, 2014). We were not able to analyse the data on energetic efficiency by an LMM and therefore compared energetic efficiencies based on their 95% CIs. If the CIs do not overlap, the differences between the means are not significantly different at $p = 0.05$ level. Finally, we analysed the effect of intermediate predator body mass on energetic efficiency with linear regression using a dataset including the mean energetic efficiency estimate from each treatment as the response variable and the mean wet weight of each intermediate predator as the explanatory variable.

3 | RESULTS

3.1 | Experiment 1: Effects of predation risk and habitat complexity on intermediate predator feeding rates

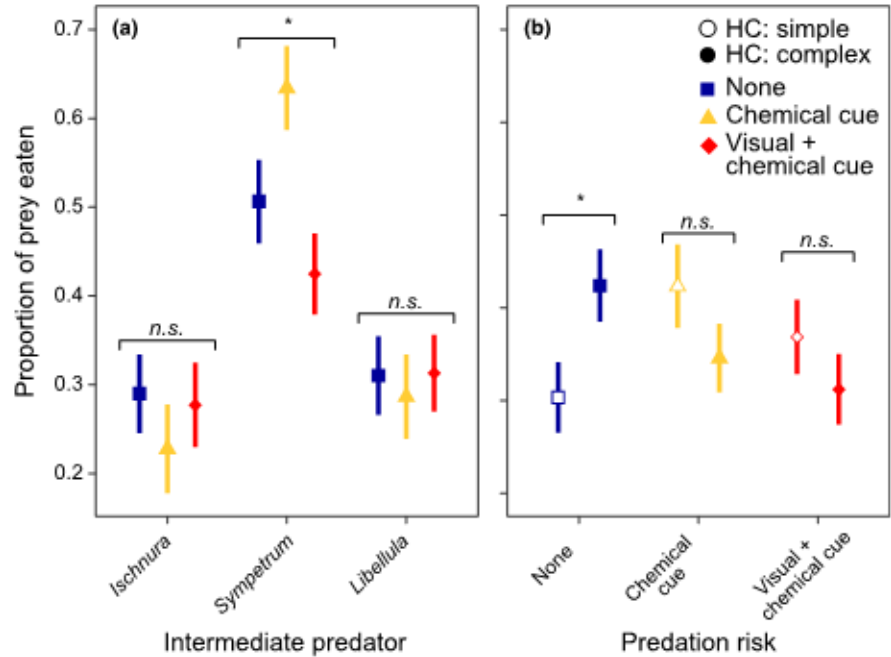
The proportion of prey eaten differed markedly between species and treatments (Figure 1, Table 1). Data were best described by a model in which proportion of prey eaten was explained by one main effect (identity of the intermediate predator) and two-way interactions between habitat complexity and predation risk, and between intermediate predator identity and predation risk (Table 1). In other words, the effects of intermediate predator identity, predation risk, and habitat complexity on feeding rate were not independent.

Post-hoc analyses confirmed that the proportion of prey eaten significantly differed among intermediate predator species but the differences depended on the predation risk treatment. The phytophilous *Sympetrum* had significantly higher feeding rates compared to the other two species; feeding rate of *Libellula* and *Ischnura* did not differ significantly in any treatment (Table S4; Figure 2a). Contrary to our first hypothesis, predation risk almost never led to significantly lower proportion of prey eaten relative to the risk-free treatment in any of

TABLE 1 Values of the F statistics, degrees of freedom (df), and p values of the LMMs for the effects of habitat complexity, intermediate predator identity, predation risk, and their interactions on the arcsine square root proportion of prey eaten (a) and intermediate predator metabolic rate (b). Significant effects are in bold ($p < 0.05$)

Effect	Full model	Final model
(a)		
Habitat complexity (HC)	$F = 0.18$; $df = 1$; $p = 0.68$	$F = 0.02$; $df = 1$; $p = 0.88$
Intermediate predator (IP)	$F = 31.84$; $df = 2$; $p < 0.0001$	$F = 31.29$; $df = 2$; $p < 0.0001$
Predation risk (PR)	$F = 0.91$; $df = 2$; $p = 0.40$	$F = 0.76$; $df = 2$; $p = 0.47$
HC \times IP	$F = 0.46$; $df = 2$; $p = 0.64$	—
HC \times PR	$F = 4.96$; $df = 2$; $p = 0.009$	$F = 4.57$; $df = 2$; $p = 0.013$
IP \times PR	$F = 3.19$; $df = 4$; $p = 0.017$	$F = 2.92$; $df = 4$; $p = 0.025$
HP \times IP \times PR	$F = 3.19$; $df = 4$; $p = 0.34$	—
Weight of IP	$F = 1.36$; $df = 1$; $p = 0.25$	—
(b)		
IP	$F = 3.60$; $df = 2$; $p = 0.03$	$F = 3.50$; $df = 2$; $p = 0.03$
PR	$F = 0.24$; $df = 1$; $p = 0.63$	$F = 0.30$; $df = 1$; $p = 0.58$
IP \times PR	$F = 0.14$; $df = 2$; $p = 0.87$	—

FIGURE 2 Most parsimonious model of the effects of intermediate predator identity, predation risk, and habitat complexity on the proportion of prey eaten (arcsine square-root transformed data, mean \pm SE) for (a) the three intermediate predators at three predation risk treatments (no risk, predation risk signalled by chemical cues, and predation risk signalled by visual and chemical cues), and (b) in the presence or absence of artificial vegetation in the three predation risk treatments. Asterisk = significant differences ($p < 0.05$), n.s. = non-significant differences ($p > 0.05$). Symbols as in Figure 1



the three predators except for the phytophilous *Sympetrum* exposed to the combination of visual and chemical cues (Figure 2a). Contrary to our third hypothesis, we found that the effect of habitat complexity depended on the predation risk treatment: artificial vegetation did not have a significant effect in the two treatments with predation risk (although we detected a trend towards lower feeding rates in the more complex habitat), whereas it increased the proportion of prey eaten in the risk-free treatment (Table S4; Figure 2b). This increase of feeding rate in the complex habitat is in line with our second hypothesis. However, this effect was independent of intermediate predator identity whereas we expected an increase of feeding rate in the complex habitat only for the phytophilous predators *Sympetrum* and *Ischnura*.

3.2 | Experiment 2: Effect of predation risk on the metabolic rate of intermediate predators

We found that mass-corrected metabolic rates of the three intermediate predators differed among species as *Sympetrum* had significantly higher mass-corrected metabolic rates compared to *Libellula* and *Ischnura* (Figure 3). However, contrary to our fourth hypothesis, the rates were not influenced by predation risk or by the statistical interaction between intermediate predator species and predation risk (Table 1).

3.3 | Energetic efficiency of the intermediate predators

Estimated energetic efficiency differed among the three intermediate predators, being highest in *Ischnura* and lowest in *Libellula* (Figure 4). Energetic efficiency decreased steeply with body mass of the intermediate predators (linear regression: mean \pm SE parameter values: $y = 40.5 \pm 3.8 - (144.0 \pm 26.1)x$; adjusted $R^2 = 0.63$,

$F_{1,16} = 30.34, p < 10^{-4}$). The results were further indicative of, sometimes contrasting, trends in the joint dependence of energetic efficiency on predation risk and habitat complexity in *Ischnura* and

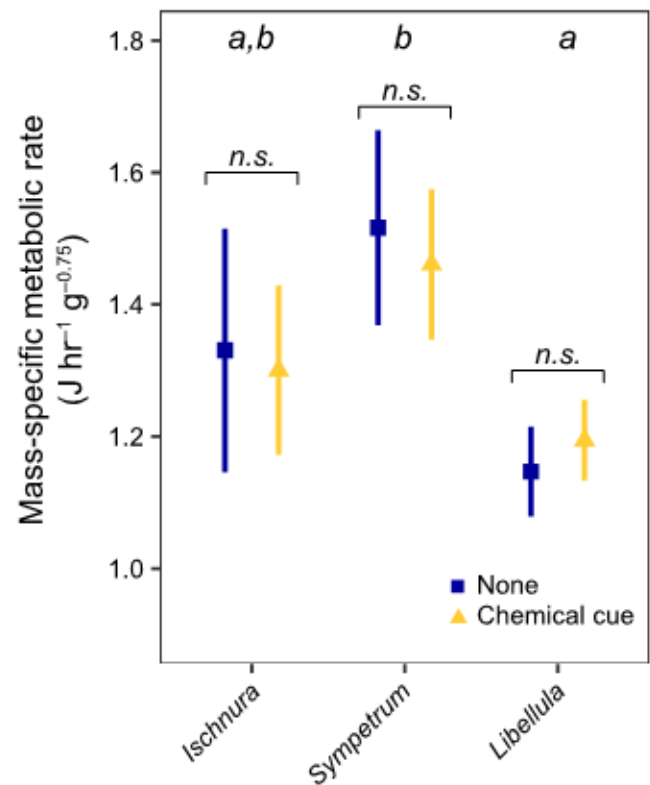


FIGURE 3 Effect of predation risk cues (squares = no risk, triangles = chemical cues) on mass-corrected metabolic rate (mean \pm SE) of each intermediate predator. Different letters denote significant differences among intermediate predators ($p < 0.05$); n.s. = non-significant differences ($p > 0.05$)

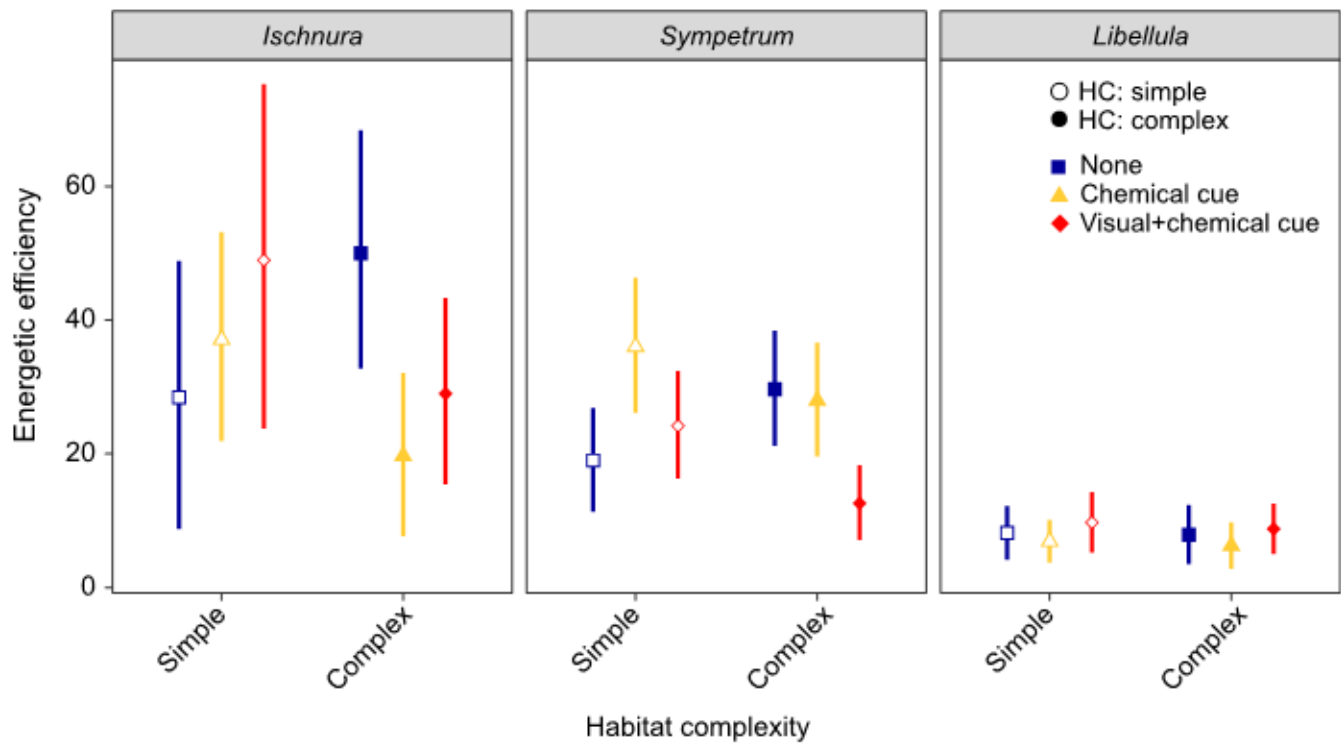


FIGURE 4 Effects of habitat complexity and predation risk on estimated energetic efficiencies (mean \pm 95% confidence interval) of the three intermediate predators. Symbols as in Figure 1

Sympetrum. Energetic efficiency of each intermediate predator was substantially affected by predation risk in only three cases, all in the complex habitat treatment: it was lower in *Sympetrum* exposed to either type of predator cues compared to the risk-free treatment and in *Ischnura* in the treatment with only chemical cues compared to the risk-free treatment, in which the 95% CI estimates did not overlap in the respective pairs of treatments (Figure 4). These results indicate that energetic efficiency may depend on the interactive effects of habitat complexity and predation risk which supports our fifth hypothesis.

4 | DISCUSSION

Revealing the effects of environmental factors on species interaction strengths is crucial to understanding the structure and dynamics of ecological communities (Duffy et al., 2007; Sents et al., 2014). However, only a handful of studies simultaneously investigated joint effects of multiple biotic and abiotic factors on the feeding rates and energetic efficiency of organisms (Miller, Matassa, & Trussell, 2014; Stoks, Swillen, & De Block, 2012; Stoks et al., 2005; Trussell et al., 2006), leaving a gap in our understanding on how environmental factors affects interaction strengths in the short and long terms, especially in intermediate predators, which account for the majority of species in food webs (see Table S1 for details). To fill this gap, we investigated the combined effects of predation risk and habitat complexity on intermediate predator feeding rate and energetic efficiency that both have importance

for community dynamics (e.g. Kovalenko, Thomaz, & Warfe, 2012; Wellborn et al., 1996).

4.1 | Impact of predation risk and habitat complexity on intermediate predator feeding rate

We found that the effects of predation risk on feeding rate depended on habitat complexity and differed among the intermediate predators. Contrary to our first hypothesis, the generalist *Ischnura* (found both on plants and on the bottom) and the benthic *Libellula* did not respond to predation risk. For *Sympetrum*, we only found an increase in feeding rate in response to chemical cues but no differences between the control without the predation risk and either predation risk treatment; these results are not consistent with observations that it responds primarily to visual predation risk stimuli (Corbet, 2004; Crespo, 2011). We propose that the lack of significant predation risk effect on the feeding rate of *Ischnura*, the other phytophilous predator, is due to its weak habitat overlap with the top predator in the field. *Ischnura* typically prefers early succession sites with few aquatic plants, while *Aeshna* larvae mostly occur in more densely vegetated habitats (Corbet, 2004). Our results would then mostly but not fully comply with the habitat domain concept (Schmitz, 2007), which posits that predators occupying different types of microhabitats or with different phenology interact less than predators sharing the same spatiotemporal domain. Non-overlapping habitat domains may also render the prey unreceptive to predation risk cues (Anderson & Semlitsch, 2016; Ramos & Van Buskirk, 2012; Schmitz, 2007).

Contrary to our second and third hypotheses, we found no systematic difference in the effect of habitat complexity on feeding rates between the benthic *Libellula* and the more phytophilous *Ischnura* and *Sympetrum*. These results could be due to the relatively small size of experimental arena (four times smaller volume than used in Klecka & Boukal, 2014), which could have made the zooplankton prey accessible also to the benthic predator and hence increase the overlap in the predator and prey habitat domains in the experiment. Alternatively, predator avoidance behaviour (e.g. predation-induced aggregation as in Bergström & Englund, 2004) could have altered spatial distribution of the prey in the experimental arenas; we were not able to track and quantify this behaviour. Nevertheless, we found that habitat complexity increased feeding rate in the treatments without predation risk (Figure 2b). This suggests that the intermediate predators used in this study are inherently more successful at hunting in more complex habitat, which has been reported in fish (Eklöv & van Kooten, 2001; Savino & Stein, 1989) and odonates (Convey, 1988; Klecka & Boukal, 2014), but this pattern is obscured by their behavioural responses to predation risk in our study.

4.2 | Effects of predation risk on metabolic rates

Few studies directly measured the effect of predation risk on prey metabolic rates (reviewed in Hawlena & Schmitz, 2010a) and only three of them focused on intermediate predators (Culler, McPeck, & Ayres, 2014; Handelsman et al., 2013; Okuyama, 2015). Contrary to our fourth hypothesis, we found no effect of chemical cues on metabolic rates of all three intermediate predators. This response is consistent with previous studies showing that odonate predators respond primarily to visual predation risk stimuli (Corbet, 2004; Crespo, 2011). However, our result contrasts with previous studies that reported elevated metabolic rates under predation risk in tadpoles (Steiner & Van Buskirk, 2009), grasshoppers (Hawlena & Schmitz, 2010b), spiders (Okuyama, 2015), bivalves (Antoľ et al., 2018), and damselflies (Culler et al., 2014). Our experimental method for producing the chemical cues was comparable to those used in other studies investigating the effect of predation risk (Brönmark & Hansson, 2012). Our results thus suggest that the intermediate predators used in our study do not exhibit short-term responses to the chemical stimuli of *Aeshna* larvae fed with heterospecific prey, although we cannot exclude possible long-term effects on their growth and maturation as in Stoks et al. (2012) and Šupina et al. (2016). It is possible that, in our experiment, the intermediate predators were not exposed long enough for chemical cues to induce changes in their physiology and respiration rate. Nevertheless, previous studies found an effect of predation risk on metabolic rates even if the animals were exposed to predation risk for a short period of time and thus manifested an acute stress (e.g. Hawlena & Schmitz, 2010b; Okuyama, 2015).

4.3 | Effects of predation risk and habitat complexity on energetic efficiency

Changes in energetic efficiency affect the transfer of biomass across trophic levels in food webs and may lead to biodiversity changes,

e.g. in shifts in food web dynamics and/or extinction of some species (Berlow et al., 2009; Binzer, Guill, Rall, & Brose, 2016; Sentis, Binzer, & Boukal, 2017). In our experiments, energetic efficiency decreased with body mass of the intermediate predators because larger predators had disproportionately higher metabolic rates than feeding rates. This result is in line with previous studies showing that large predators are less efficient than smaller ones (Sentis, Binzer, & Boukal, 2017; Vucic-Pestic, Ehnes, Rall, & Brose, 2011; Vucic-Pestic et al., 2010).

Hawlena and Schmitz (2010a) proposed that prey stress associated with predation risk should lower energetic efficiency or growth efficiency (related to energetic efficiency, Text S1). Previous results on the effect of predation risk on growth efficiency are equivocal despite being limited to few taxa (Matassa & Trussell, 2015; Miller et al., 2014; Trussell, Ewanchuk, & Matassa, 2008; Trussell et al., 2006). For example, growth efficiency of various damselfly species remained unchanged (McPeck et al., 2001), declined (Stoks et al., 2005), or even increased (Stoks et al., 2012) when exposed to predation risk cues by aeshnid dragonfly larvae or predatory fish, probably due to the co-evolutionary history of the given predator-prey pair (Stoks & McPeck, 2003).

Energetic efficiency markedly decreased with predation risk only in *Ischnura* and *Sympetrum* in the complex habitat, but only in one of the predation risk treatments (with visual and chemical cues for *Sympetrum* and with chemical cues for *Ischnura*). We cannot directly compare these results to previous studies on predation risk and growth efficiency as none of them measured metabolic rates, while we did not directly measure growth efficiency. Nevertheless, significant changes in either measure of efficiency should become apparent in the other one (Text S1). Considering this link between these two efficiency metrics, our result is inconsistent with the study of Trussell et al. (2008) in which predation risk of green crab (*Carcinus maenas*) led to a stronger decline of growth efficiency of the snail *Nucella lapillus* in a habitat with low complexity than in habitats with high complexity. *Nucella* snails used complex habitats to make them less conspicuous to the green crabs and other potential top predators. Our results instead indicate a suppressed feeding activity of the intermediate predators when exposed to predation risk in a more complex and thus presumably less controllable habitat. The preferred hunting strategy of the top predator, i.e. the use of submerged vegetation as perching site for ambushing, could have led to an adaptive, habitat complexity-specific response in activity and decreased feeding rates of the intermediate predators. Moreover, our experiments ran on shorter timescales (2.5 and 6 hr) relative to the 18-day experiment by Trussell et al. (2008). Despite the lack of acute response, we cannot exclude that we would have detected a long-term response in energetic efficiency or growth rates if we ran the experiment longer as predator-induced metabolic response can depend on experimental duration. Previous studies showed that metabolic rate can change only a few minutes after exposure to predation risk as an acute stress response but can also change after several days or weeks when modifications of the activity, feeding and growth rates in response to predation

risk subsequently impact metabolic rate (Handelsman et al., 2013; Schaffner & Anholt, 1998).

Future studies comparing prey responses to predation risk over different timescales and quantifying multiple physiological and growth parameters are needed to reconcile these different observations. Our results imply that the effects of predation risk and habitat complexity on energetic efficiency are species specific and may be linked to habitat domain overlaps between the top and intermediate predators.

5 | CONCLUSIONS

We found that predation risk and habitat complexity significantly affected intermediate predator feeding rates. Metabolic rate was independent of predation risk and only linked to intermediate predator identity. By combining these results, we found that energetic efficiency of the three intermediate predators was more influenced by the identity of the intermediate predators than by habitat complexity and predation risk, suggesting that the latter two factors are less important than predator identity for long-term interaction strengths. Other studies are needed to confirm our findings, to test the role of other environmental variables and details of the experimental approaches, and to identify predator and prey traits such as habitat domains, foraging modes and anti-predator strategies (Anderson & Semlitsch, 2016; Boukal, 2014) that underlie the magnitude of trophic interaction strengths and energetic efficiency of intermediate predators. Moreover, future experiments should also investigate the effects of predation risk and habitat complexity on interactions between multiple prey and multiple predator species. For example, non-trophic interactions among predators such as interference or facilitation can modify the effects of environmental factors on trophic interactions (Sentis, Gémard, et al., 2017), and switching between different prey types could be altered by habitat complexity (e.g. Fischer et al., 2013).

Although we tested only three intermediate predator species, we found that larger predators are less efficient than smaller ones, which indicates that intermediate predator body mass is an important determinant of predator energetic efficiency. We thus conclude that intermediate predator feeding rates and energetic efficiencies depend primarily on intermediate predator identity and traits, but are little affected by habitat complexity or predation risk.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

V.K., D.S.B. and A.S. conceived the experimental design. V.K. conducted the experiments. V.K. and A.S. analysed the data with additional input from D.S.B. V.K., D.S.B. and A.S. wrote the manuscript.

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