

Department of Ecosystem Biology
Faculty of Science
University of South Bohemia



**Predation by aquatic insects: species traits and habitat
structure mediate predator-prey interactions**

Master Thesis

Bc. Jan Klečka

Supervisor: Ing. MgA. David Boukal, Ph.D.

**České Budějovice
2010**

Master Thesis

Klečka, J., 2010: Predation by aquatic insects: species traits and habitat structure mediate predator-prey interactions. MSc. Thesis, in English – 36 pp., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Annotation

In my thesis, I present results of experimental research on selective predation by aquatic insects, specifically on the role of predator and prey body size, microhabitat distribution, and selected behavioural traits on the strength of predator-prey interactions. The role of aquatic vegetation in mediating predator-prey interactions by providing refuges for prey or perching sites for predators was further addressed.

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

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své magisterské práce, a to v nezkrácené podobě elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách.

V Českých Budějovicích dne 29.4.2010

.....

Acknowledgement

I am obliged to my supervisor David Boukal for his kind help, fruitful discussions and comments on the manuscript. I thank Luboš Havlan, Markéta Nyklíčková and Irena Slámová for help in the field and in the lab. Last but not least, I would like to thank Irena Slámová and my family for patience and support during my work. This research was partly supported by Student Grant Agency of the Faculty of Science, University of South Bohemia (SGA2009/010).

Contents

1	Thesis summary	1
2	Manuscript I – Predation by aquatic insects: is body size the only trait that matters?	2
3	Manuscript II – Interactions of predator and prey traits determine prey mortality	13
4	Manuscript III – Vegetation alters predator-prey interactions of freshwater invertebrates by providing perching sites for predators	24
5	References	34

1. Thesis summary

- In my thesis, I address predator-prey interactions involving predatory aquatic insects and their, mainly invertebrate, prey. I have carried out a number of laboratory experiments to quantify prey selectivity of predatory aquatic insects and mortality of prey exposed to predators differing in body size and exhibiting contrasting foraging strategies. My approach is mainly comparative. Thus, I have focused on detecting patterns in selective predation of a wide spectrum of predators feeding on multiple prey species.
- Main questions I addressed were:
 - What predator and prey traits determine prey selectivity of predators and differential mortality of prey?
 - Does habitat structural complexity modify predator-prey interactions in freshwater food webs?
- I attempted to answer these questions in two experimental projects carried out since 2007. The results are presented in the form of three manuscripts (drafts of papers to be modified for publication) accompanied by a joint list of references.
 - Manuscripts I and II are based on a series of laboratory experiments with regionally dominant species of predatory aquatic insects of small fishless water bodies and their selected prey. The role of predator and prey traits as drivers of selective predation was of major interest.
 - Manuscript III presents results of a series of experiments which is a part of a larger project focused on the assembly of invertebrate communities of small water bodies. These laboratory experiments were focused on the question of importance of habitat structural complexity for predator-prey interactions.
- My results confirm that body size is a key factor affecting predation in aquatic insects, but other predator and prey traits also play a significant role and modify the general predator-prey body size allometries. The effect of habitat structure on predator-prey interactions was strongly dependent on behaviour of predators and prey. I argue that future studies of freshwater food web structure and dynamics should consider multiple species traits, including body size and foraging and anti-predator behaviour, to explain the processes structuring aquatic food webs.

2. Manuscript I

Predation by aquatic insects: is body size the only trait that matters?

Predation by aquatic insects: is body size the only trait that matters?

Abstract

1. Selective predation is a key process shaping the structure and dynamics of natural communities. I tested the relationship of consumption rate and prey selectivity to predator body size and behavioural traits using aquatic insects as a model group of predators in simple laboratory experiments.
2. All of the 13 predators (adult and larval Coleoptera, adult Heteroptera and larval Odonata) displayed significant prey selectivity towards some of the seven prey species offered. Preferred prey species varied among predators and ontogenetic changes of diet composition were observed in Coleoptera and larval Odonata.
3. Total prey killing rate (killed prey biomass/predator body weight) allometrically decreased with predator body weight suggesting size-specific demands on energy uptake per unit body mass in accordance with general scaling of metabolism with body weight. The mass-specific killing rate of prey was ca. three times higher in sucking predators (larval Coleoptera and Heteroptera) than chewing predators (adult Coleoptera and larval Odonata). Sucking predators thus may have more profound effect on the abundance of prey populations than equally sized chewing predators.
4. Key predator traits determining diet overlap were body weight and foraging microhabitat, whereas foraging mode (ambush/searching) was insignificant. Mean individual prey body weight consumed by a predator allometrically increased with predator body weight but chewing predators ate significantly larger prey than sucking predators.
5. Body size seems to be a key factor affecting predation in aquatic insects, but the results clearly show that other predator traits (details of foraging behaviour) also play a significant role and modify the general allometries. Future studies of food web structure and dynamics of freshwater invertebrates thus should not concentrate solely on body size but should consider also other species traits to increase their biological realism and to move towards more plausible mechanistic explanations of field data.

Introduction

Body size is considered as a key trait determining the role of species in community through size-selective predation (Warren & Lawton 1987, Cohen et al. 1993, Woodward & Hildrew 2002a, Cohen et al. 2003, Woodward et al. 2005, Woodward & Warren 2007) and allometric scaling of metabolic rate and life-history parameters with body size (Woodward et al. 2005, Brown et al. 2004, Brown et al. 2007, Atkinson & Hirst 2007). Empirical data generally show that the larger the predator, the larger the prey it consumes. For example, Claessen et al. (2000) showed that there is a clear positive relationship between the body length of cannibal and its victim in perch. Empirical data on size-dependent cannibalism led to the development of a number of theoretical models exploring the population dynamical consequences of size-dependent cannibalism (e.g. Claessen et al. 2000, 2004). Similarly, the importance of body size in predator-prey interactions in aquatic food webs was recently recognized in empirical studies (reviewed in Woodward et al. 2005, Hildrew et al. 2007). Various food web models incorporating body size of interacting species are developed. Recently, Petchey et al. (2008) build an optimal foraging model with body size allometries which successfully predicted up to 65% of food web links of real food webs, depending on the type of food web examined. At present, based on both empirical and theoretical studies, it seems that the relationship of predator and prey body size is a key factor shaping aquatic food webs.

Aquatic insects are among the most diverse groups of animals of especially small water bodies. Although diving beetles, bugs and dragonfly larvae are usually top predators and thus may greatly affect the structure and dynamics of the whole food web, their prey selectivity is poorly known. Aquatic insects have been traditionally considered as generalist predators (e.g. Cummins 1973, Peckarsky 1982). Experimental studies of their prey selectivity are scarce, but prey size selectivity of a predator was tested a few times. E.g., Pastorok (1981) found that *Chaoborus* midge larvae feed mostly on medium-sized *Daphnia* as a result of a trade-off between prey encounter rate, which rises with increasing prey size, and attack success rate, which decreases with increasing prey size. Cannibalism of large *Notonecta undulata* on smaller nymphs is shaped by the same mechanisms (Streams 1994). Experiments with stonefly larvae from temperate streams also revealed size-selective predation – large stonefly larvae prefer larger prey than small larvae (Allan et al. 1987a, Allan et al. 1987b). Foraging mode (ambush/searching) is also believed to be responsible for diet composition of predatory aquatic insects (e.g. Allan et al. 1987b, Woodward & Hildrew 2002a), but available data still do not provide solid grounds for establishing a general framework describing patterns and mechanisms of insect predation in aquatic food webs.

Despite the prevalent recognition of size-specific predation as a major process driving food web dynamics, experimental data allowing us to quantify the importance of body size for prey choice by a predator are rare irrespective of the type of habitat and organisms in question. Moreover, behavioural observations of different predators foraging on various prey types have shown that the activity and foraging behaviour of predators may also be important factors responsible for prey choice (e.g. Allan et al. 1987b, Downes 2002). The logical thought that the body size does not tell the whole story was repeatedly expressed in studies of food webs (e.g. Woodward & Hildrew 2002a, Hildrew et al. 2007, Petchey et al. 2008, Ings et al. 2009), but so far, contribution of other factors has not been quantitatively evaluated. I performed a series of laboratory experiments using aquatic insects as a model group of predators to resolve the relative importance of body size and other predator traits for the prey selectivity and consumption rates of predators.

Methods

Laboratory experiments

I performed multiple choice predation experiments with 13 species of predatory aquatic insects and seven prey species. Regionally common species dominating communities of small fishless water bodies were collected at various localities in South Bohemia (Czech Republic). Experiments were carried out in late spring in a climate room with constant temperature (20°C) and photoperiod (L:D=18:6 h). Experiments were performed in transparent plastic boxes filled with 2.5 l of ca. 8 cm deep aged tap water without any substrate on the bottom and equipped with four stripes of plastic mesh hanging in the water column to provide simple supporting structure for the animals. Experimental vessels were surrounded by sheets of brown paper to prevent disturbance of animals during experiments.

Animals collected in the field were acclimated in the climate room for two to five days prior to experiments. Predators were kept individually in small containers (0.25–0.7 l) and fed daily ad libitum with prey of a type not used in experiments (mainly larvae of Trichoptera). All predators were starved for 24 hours prior to experiments to standardize their hunger level. In the beginning of an experiment, a mixture of all prey species was released (six individuals of *Rana* tadpoles, six *Lymnaea*, 10 *Chironomus*, 10 *Cloeon*, 10 *Culex*, 10 *Asellus* and 30 *Daphnia*) and after several minutes one predator was added. After 24 hours all uneaten prey were counted. Five to nine replicates were done with each predator species, but all individual predators were used only once. Qualitative observations of predator behaviour and spatial distribution within the experimental vessels were done during experiments to collate simple classification of predator species according to their microhabitat occupation and feeding behaviour. Four control trials were performed to evaluate natural mortality of prey. To account for this mortality in predation trials, mean number of prey specimens which died during control trials was added to the number of prey individuals surviving each predation trial when calculating the number of prey individuals eaten by a predator.

After experiments, all predators and 20 randomly chosen individuals of all prey species were conserved in 80% ethanol and weighed on analytical scales to the nearest 0.001 mg after 72 h of drying at 50°C.

Data analyses

All data analyses were carried out and figures made in R 2.9.2 (R Core Development Team 2009) unless otherwise stated. The selectivity of individual predators was evaluated using Manly's α selectivity index (Manly 1974, Chesson 1983):

$$\alpha_i = \frac{\ln((n_{i0} - r_i) / n_{i0})}{\sum_{j=1}^m \ln((n_{j0} - r_j) / n_{j0})}, i=1, 2, \dots, m$$

where n_{i0} is the initial number of prey items of type i , r_i is the number of prey items of type i consumed by the predator and m is the number of prey types in the experiment.

This selectivity index (α_i) expresses the selectivity of a predator as a relative contribution of individual diet items to the predator's diet if all diet items were equally abundant in the environment. It corrects for prey depletion occurring in experiments, which lowers the density of a given prey. In several experiments with four predator species, all individuals of the most selected prey species were consumed. To calculate Manly's α in these cases, the formula was modified by adding one prey individual of the completely depleted prey type to corresponding n_{i0} and n_{j0} in the

above equation. This correction is based on the assumption that if another individual of the prey in question were present, it would survive. The corresponding estimate of α_i is slightly conservative.

Statistical properties of Manly's α are well known and hypothesis testing is straightforward (Manly 1995). For a predator individual, α_i values are not independent. Values of α_i for the set of individuals of a given predator species follow a multivariate normal distribution with each prey species representing one variable (Manly 1995). Values of α_i for individual prey species were thus compared with values expected for no selectivity using separate t-tests as recommended by Manly (1995). For better graphical presentation, the values of α_i were converted into an electivity index ranging from -1 to 1 with a value of 0 for unselective feeding, following Chesson (1983).

For each predator species, mean dry weight of prey killed during experiment per unit body weight of a predator was calculated and the killing rate among different groups of predators (Coleoptera adults/larvae, Heteroptera and Odonata) was compared using a generalized linear model (GLM) with Gamma distribution and log link function. In a separate analysis I tested similarly the effect of predator body weight and feeding mode (sucking vs. chewing) on killing rate.

I calculated the diet overlap for all pairs of predator species using Pianka's overlap index (Pianka 1973) in Ecosim 7.72 (Goteli & Entsminger 2005). This index estimates the similarity of resource use (in this case diet composition) for all pairs of (predator) species on the scale from 0 (completely different) to 1 (identical). Pianka's index was calculated using Manly's α as estimates of diet composition for individual predator species because they are independent on total consumption rates and prey depletion during experiments. I tested the effect of similarity in individual traits of predators on the diet overlap using partial Mantel test performed on dissimilarity matrices with 1000 permutations in *ecodist* 1.1.4 package for R (Goslee & Urban 2007).

I further tested which predator traits are responsible for its prey size selectivity. For each predator, I calculated mean body weight of consumed prey and associated coefficient of variation (CV) as a measure of prey size range utilized by a predator. The dependence of mean prey body weight on predator body weight and on traits describing predator's foraging behaviour was tested using GLM with Gamma distribution and log link function. The dependence of CV on predator body weight was tested using GLM with normal distribution; second-order polynomial was used to fit this clearly nonlinear relationship.

Results

Dry body weight of predators ranged from 2.74 to 528.43 mg (Table 1). Prey dry body weight ranged from 0.04 to 8.22 mg (Table 2). Predators were classified in two groups according to foraging mode (nine ambush and four searching predators), feeding mode (eight chewing and five sucking predators) and foraging microhabitat (six benthic and seven nektonic) (Table 1). Natural mortality of prey was very low in control trials and has negligible effect on the reliability of the results (Table 2).

Prey selectivity pattern

All predator species exhibited distinct selectivity (Fig. 1). Most species consumed three or four prey species at least proportionally to their relative abundance in experiments. The most selective was the diving beetle *Hydaticus seminiger*, which avoided all prey species except *Chironomus*. On the other hand, *Anax imperator* dragonfly larvae avoided only *Daphnia* and *Lymnaea* snails. I also observed ontogenetic shifts in prey selectivity in the diving beetles from the genera *Acilius* and *Dytiscus* and in *Libellula* dragonflies, where prey selectivity of two or three developmental stages were tested (Fig. 1).

The effects of predator traits on killing rate and prey selectivity

Total biomass of prey killed during the experiment expressed as a proportion of predator body weight ranged from 3.5% in adults of *Dytiscus marginalis* to 241.5% in 2nd instar larvae of *Acilius canaliculatus* and differed significantly among predator groups (Fig. 2; GLM, $F=16.99$, $P=0.0005$). The only predators killing more prey than their own body weight were 2nd and 3rd instar larvae of *Acilius canaliculatus* (Fig. 2). Biomass of prey killed per unit predator weight decreased with predator weight (Fig. 2; GLM, $F=110.74$, $P<0.0001$) and was significantly (2.9 times, $SE=0.58$) higher for sucking predators compared to chewing predators (Fig. 2; GLM, $F=27.70$, $P=0.0004$). These two predator traits explained together 92.1% of deviance of mass-specific killing rates. Total biomass of prey killed per unit predator weight declined allometrically with predator weight with exponent -0.52 ($SE=0.06$).

Analysis of diet overlaps using partial Mantel test revealed positive dependence of diet overlap on similarity in predator body weight and similarity in foraging microhabitat (Table 3).

I detected a tight positive relationship between predator weight and prey mean body weight ($F=57.43$, $P<0.0001$; Fig. 3). It was accompanied by a highly significant effect of feeding mode (sucking/chewing): sucking predators consumed 1.6 times ($SE=0.23$) larger prey than equally sized chewing predators ($F=11.78$, $P=0.0064$). These two predator traits explained together 84.55% of deviance in mean prey body weight. No other predator traits (foraging microhabitat and foraging mode) had significant effect (GLM, $P>0.05$). Coefficient of prey body weight variance (CV) significantly increased with predator weight ($F=29.27$, $P<0.0001$, explained deviance=62.4%; Fig. 3) indicating that larger predators utilize broader prey size spectre.

Discussion

How selective are predatory aquatic insects?

Predatory insects in standing and running waters (diving beetles, bugs, dragonfly larvae, damselfly larvae and stonefly larvae) have been traditionally considered as generalist predators (Cummins 1973, Peckarsky 1982) although prey selectivity experiments have been rarely carried out to verify this claim. The most thorough experiments focused on stonefly larvae inhabiting streams and rivers and concluded that stonefly larvae feed selectively (e.g. Allan et al. 1987a, Allan et al. 1987b, Tikkanen et al. 1997). The few preceding studies testing prey selectivity in predatory insects of standing waters focused on a narrow spectre of prey species – e.g., different zooplankton (Cooper et al. 1985) or different size classes of one prey species consumed by one predator species (Pastorok 1981). Gut content analyses showed interspecific diet variation in larval dragonflies (Blois 1985) but the interpretation of such field diet data is difficult.

Predatory insects of standing waters probably most often subjected to predation experiments in the past were *Notonecta* bugs, but they were used mostly in experiments focused on other aspects of predation, e.g. functional responses (Fox & Murdoch 1978) and tests of optimal diet models with one prey type (Cook & Cockrell 1978, Giller 1980). Data on diet choice among several prey species are rare; the limitations of experimental designs and range of prey tested in earlier studies was criticized by Giller (1986). Taken together, previous studies have provided little data on prey selectivity of predatory aquatic insects of standing waters, which in turn have been labelled as generalists. My results clearly show that this traditional view has to be reconsidered, given that I have tested species of all major orders of predatory insects living in standing waters. All tested predators are clearly selective (Fig. 1). Even closely related species or species with similar body size and foraging behaviour differ in their prey selectivity, e.g., adult *Acilius* diving beetles vs. adult *Hydaticus* diving beetles vs. adult *Ilyocoris* bugs.

Diving beetle larvae had the highest prey killing rates, while the lowest prey killing rate had adult diving beetles. This might reflect elevated metabolic requirements of the fast-developing beetle larvae. Moreover, interesting patterns suggesting major ontogenetic niche shifts occurred in the diving beetles from the genera *Acilius* and *Dytiscus* and in *Libellula* dragonfly larvae. This may reflect changes in body size and, in the diving beetles, foraging microhabitat or behaviour (Table 1). Change of prey selectivity during individual ontogeny could be an effective mechanism to reduce intraspecific competition.

One question left untouched in my experiments is whether the observed prey selectivity exhibited by a predator is a result of active prey choice or whether it is only passive. Detailed behavioural observations of predation by stonefly larvae have shown that their prey selection involves both passive mechanisms (different encounter rates and capture success) and active choice (Allan et al. 1987a, Allan et al. 1987b, Tikkanen et al. 1997). No comparable data are available for other orders of predatory aquatic insects (except the study of cannibalism in *Notonecta* by Streams 1994). Behavioural observations are time consuming and can be successfully accomplished only with a small number of predators – the stonefly studies mentioned above used only 1-3 predator species. Thus, I did not attempt to disentangle the mechanisms of prey selectivity in my experiments, whose goal was comparison across a broad range of predators.

Predation and predator traits

Body size clearly stands out as an important predator trait determining prey consumption and size of consumed prey. Total amount of prey killed per 24 hours and unit of predator biomass decreased with predator weight (Fig. 2). It is well established that mass-specific metabolism decreases with body mass and larger animals thus need lower energy uptake per unit biomass (e.g., Brown et al. 2004). My results are in qualitative agreement with the theoretically proposed allometry of metabolism, although the observed allometric exponent -0.52 deviates from the expected value of -0.25 . This may be an artefact caused by relatively low food availability for larger predators in the experiments or by using dead prey biomass as a proxy for energy intake. More interestingly, the amount of prey killed per unit of predator mass not only decreased with predator weight but also significantly differed between chewing predators and sucking predators; the latter killed more prey. This may be partly the consequence of sucking predators not consuming most of the indigestible prey remains unlike chewing predators or incomplete sucking of the prey, which occurs under high food supply in water bugs (Cook & Cockrell 1978, Giller 1980, Bailey 1986). Whatever the cause, sucking predators killed almost three times more prey than equally sized chewing predators. They may thus have more profound effect on prey abundance in the field.

The importance of predator's microhabitat use for diet composition even in simple environment of the experimental vessels was evident from the results of Mantel test. Body size similarity and similarity in foraging microhabitat jointly determined the diet overlap among predators (Table 3). The significant effect of predator foraging microhabitat is intriguing given that the experimental vessel had only 2.5 l of water, the water column was only 8 cm high and there was no bottom substrate (see Methods). This observation supports the findings that food webs may be highly compartmentalized (e.g. Krause et al. 2003). Such compartmentalization of aquatic food webs has been described in lakes and other large habitats, where distinct microhabitats are spatially isolated due to large distance from bottom to the surface. My experimental results suggest that such compartmentalization may hold even for very small water bodies, where the distance between bottom and water surface is small and spatial distinction between faunas of bottom and water column is much less apparent.

As expected, I detected a highly significant positive relationship between predator and prey body weight (Fig. 3, Table 4). However, body weight was again not the only significant factor. Feeding mode (sucking/chewing) had significant effect on the mean body weight of prey killed by a predator (Fig. 3, Table 4). Taken together with the results on the killing rates, feeding mode appears to have the potential to greatly modify the impact a predator has on the prey assemblage. Similarly, predators differing in their foraging mode (ambush/searching) may have different impacts on food web structure (Woodward & Hildrew 2002c).

Conclusions

These experiments demonstrate that body size is a key factor for understanding the prey selectivity of predatory aquatic insects but it is not the only trait that matters. Foraging behaviour, i.e., the way a predator uses different microhabitats and attacks and processes prey, significantly affect prey selectivity including its preferred size. In the light of these results, the use of body size as a single predictor of species' role in a food web seems oversimplified. As noted earlier by Petchey et al. (2008) and Ings et al. (2009), future studies should attempt to include additional traits; foraging behaviour seems to be an important supplement of body size which could increase the realism of predictions of food web structure and dynamics.

Tables and figures

Table 1. Predator species used in the experiments and their traits. In Coleoptera, L2 denotes 2nd instar and L3, 3rd instar larvae. In Odonata, L-2 denotes the second before the last instar larvae and L-0 denotes last instar larvae.

Species	Body weight (mg)		Foraging microhabitat	Foraging mode	Feeding mode
	Mean	SD			
Coleoptera					
<i>Acilius canaliculatus</i> adult	61.74	9.339	bottom	searching	chewing
<i>Acilius canaliculatus</i> L2	2.74	0.655	water column	ambush	sucking
<i>Acilius canaliculatus</i> L3	14.66	4.697	water column	ambush	sucking
<i>Dytiscus marginalis</i> adult	528.43	50.881	bottom	searching	chewing
<i>Dytiscus marginalis</i> L3	176.43	76.123	water column	ambush	sucking
<i>Hydaticus seminiger</i>	64.94	8.417	bottom	searching	chewing
Hemiptera					
<i>Ilyocoris cimicoides</i> adult	34.43	6.847	bottom	searching	sucking
<i>Notonecta glauca</i> adult	39.43	8.080	water column	ambush	sucking
Odonata					
<i>Anax imperator</i> L-0	267.00	54.415	water column	ambush	chewing
<i>Coenagrion puella</i> L-0	4.80	0.989	water column	ambush	chewing
<i>Libellula depressa</i> L-0	58.41	19.507	bottom	ambush	chewing
<i>Libellula depressa</i> L-2	20.94	5.573	bottom	ambush	chewing
<i>Sympetrum sanguineum</i> L-0	20.82	4.493	water column	ambush	chewing

Table 2. Prey species used in the experiments with body weight and mortality in control trials.

Species	Body weight (mg)		Mortality in control trials %	Taxon (order)
	Mean	SD		
<i>Asellus aquaticus</i>	1.77	0.523	0.0	Isopoda
<i>Cloeon dipterum</i>	0.99	0.187	3.3	Ephemeroptera
<i>Culex</i> sp.	0.56	0.144	3.3	Diptera
<i>Daphnia</i> sp.	0.04	0.032	6.1	Cladocera
<i>Chironomus</i> sp.	0.34	0.074	11.7	Diptera
<i>Lymnaea stagnalis</i> ¹	8.22	2.630	0.0	Pulmonata
<i>Rana arvalis</i> tadpoles	3.07	0.448	0.0	Anura

¹ weighed without shell

Table 3. Results of partial Mantel test of effects of similarity in individual predator traits on diet overlap. Partial effects for the two significant variables and conditional effects after their inclusion into the model for the other variables are presented.

Explanatory variable	Mantel r	P-value
Body weight	0.39	0.009
Foraging microhabitat	0.39	0.014
Foraging mode	0.16	0.202
Feeding mode	0.12	0.276
Taxon (order)	0.02	0.851

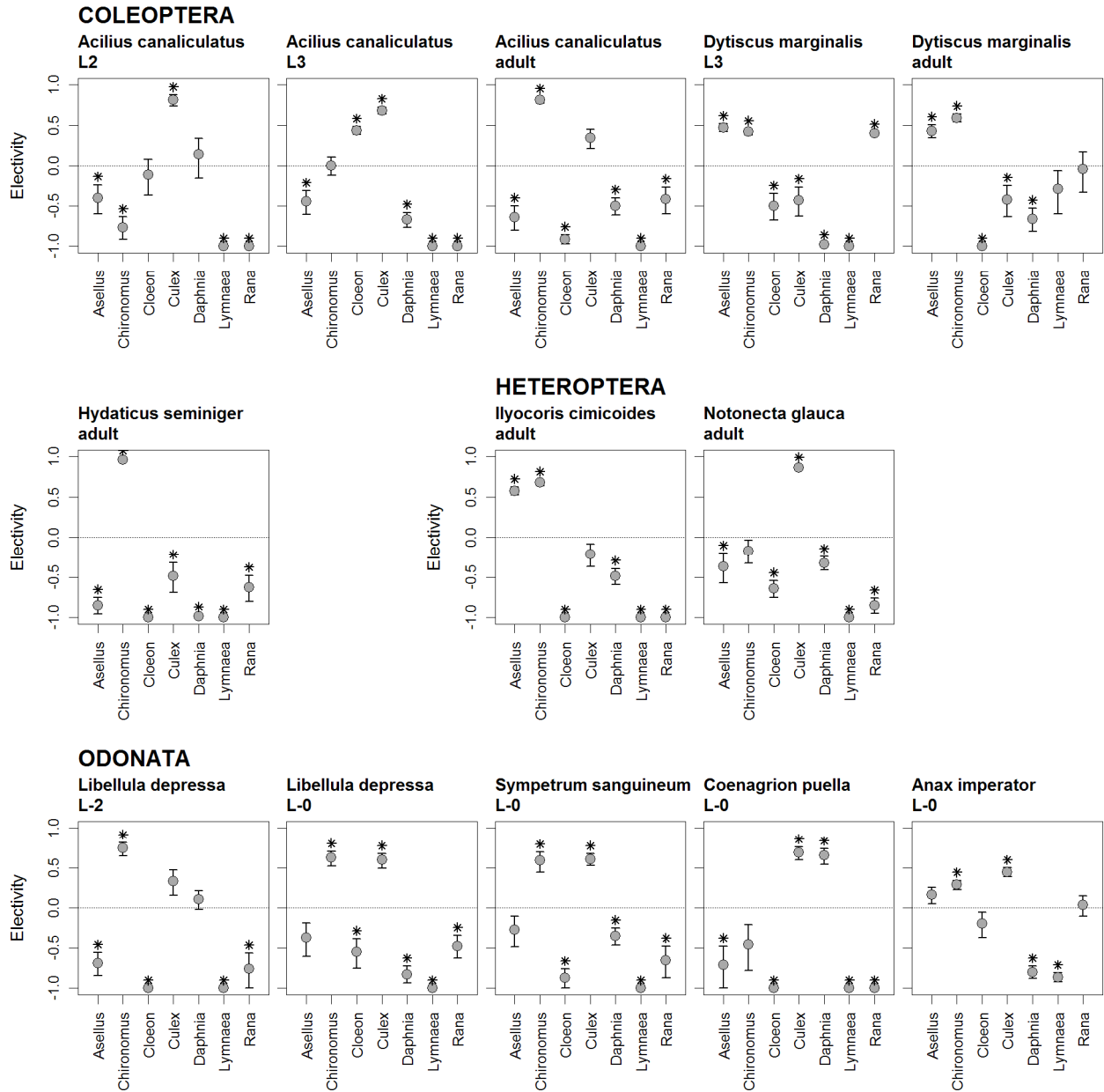


Fig. 1. Prey selectivity of predatory aquatic insects. Mean values \pm SE of electivity index are plotted. Positive values indicate preferred prey. Prey species with electivity values significantly different from zero ($P < 0.05$) are marked by an asterisk. L2 and L3 denote 2nd and 3rd instar larvae of diving beetles. L-0 and L-2 denote the last instar and the second instar before the last of dragonfly and damselfly larvae.

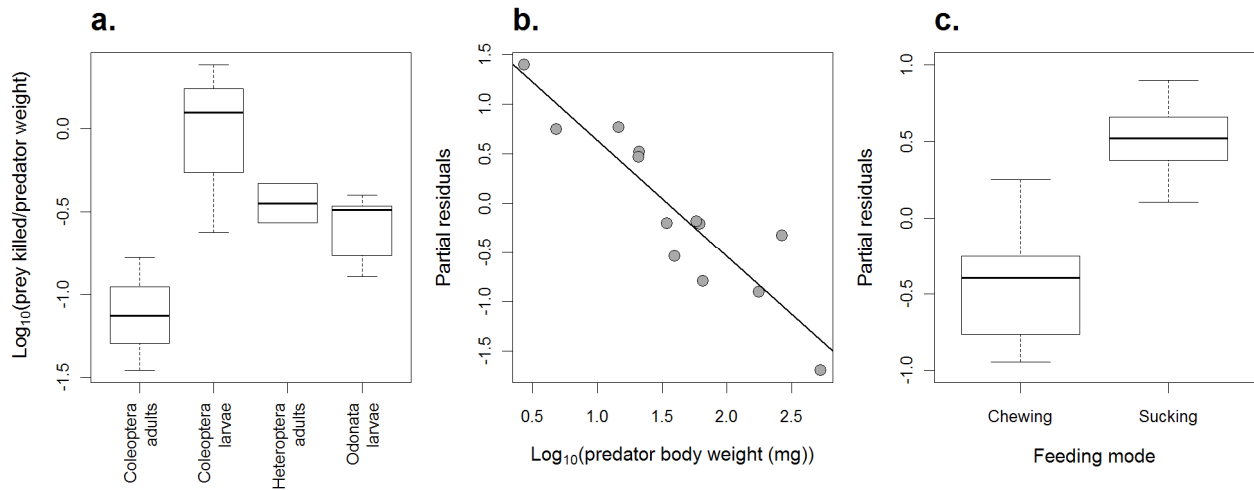


Fig. 2. Differences in total biomass of prey killed per unit of predator biomass among groups of predators (a) and its dependence on predator weight (b) and feeding mode (c). Raw data (a) and partial residuals from GLM (b and c) are plotted. Box-and-whiskers plot shows medians (horizontal lines), interquartile ranges (boxes) and complete ranges (vertical lines).

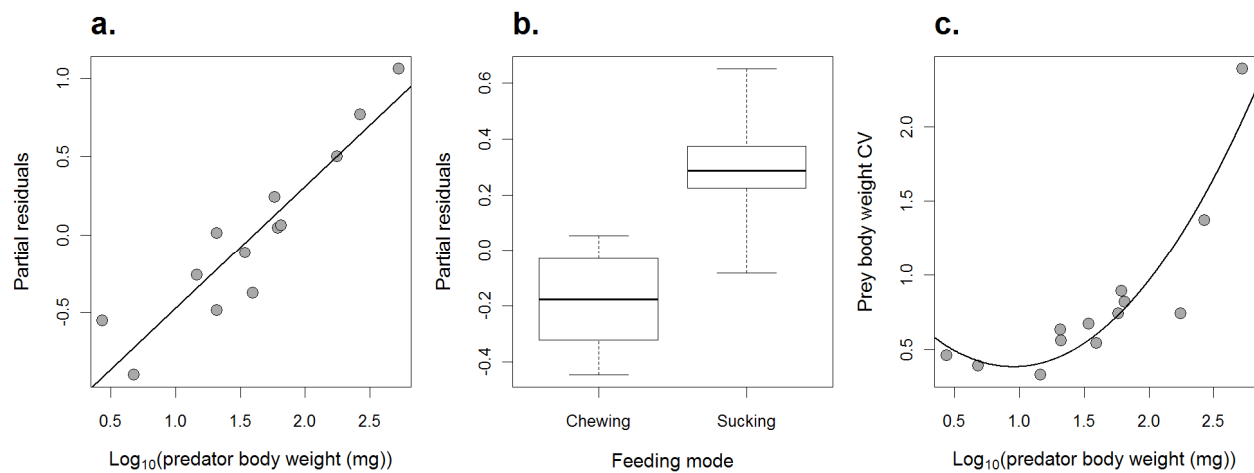


Fig. 3. Dependence of mean prey body weight and of its coefficient of variance (CV) on predator body weight and feeding mode. Partial residuals from GLM explaining prey body weight (a and b) and original values of prey weight CV (c) are plotted. Box-and-whiskers plot shows medians (horizontal lines), interquartile ranges (boxes) and complete ranges (vertical lines).

3. Manuscript II

Interactions of predator and prey traits determine prey mortality

Interactions of predator and prey traits determine prey mortality

Abstract

1. Predator-prey interactions are strongly size-dependent. However, it appears that body size is not the only trait that matters. I experimentally investigated how the mortality of prey caused by predation by aquatic insects (adult and larval Coleoptera, adult Heteroptera and larval Odonata) depends on interactions between predator and prey traits.
2. In this manuscript, I present an alternative approach to the analysis of the same data as in Manuscript I. Here, I tried to integrate the perspectives of predators and prey and focused on the analysis of prey mortality. Main conclusions support and broaden those of Manuscript I.
3. Although the mortality varied among prey species, I did not detect any prey trait that would characterize a “universal loser” suffering high overall mortality from the whole assemblage of predators.
4. The combination of predator and prey body size was an important factor responsible for prey mortality, but the interaction of predator foraging mode (ambush/searching) and prey escape ability also had profound effect on prey mortality. Prey with low ability of rapid escape suffered high mortality from both ambush and searching predators. Prey species capable of rapid escape were vulnerable to ambush predators but suffered 2.2 times lower mortality from searching predators when corrected for body size. The anti-predator effect of prey escape behaviour thus strongly depended on predator foraging mode.
5. My results show that prey mortality is driven by interactions of several predator and prey traits. Foraging behaviour of predators and anti-predator behaviour of prey explained a large amount of variability in prey mortality and should thus accompany body size in analyses aimed to achieve solid understanding of predator-prey interactions in freshwater food webs.

Introduction

Almost every animal species can become a prey of a diverse set of predators but not all predators pose the same danger for a given prey species. Many factors affect the strength of predator-prey relationships (Barbosa & Castellanos 2005). The degree of spatial and temporal co-occurrence is of major importance and escape in space (e.g. Sih 2005) or in time through life history plasticity or evolutionary changes (Stearns & Koella 1986, Day et al. 2002) is an important anti-predator defence mechanism. It seems that body size of predator and prey is a key to understanding predator-prey interactions because predator and prey body sizes are positively correlated across whole communities (e.g. Warren & Lawton 1987, Cohen et al. 1993, Woodward & Hildrew 2002a, Cohen et al. 2003, Woodward et al. 2005, Woodward & Warren 2007). Body size is, however, not the only trait that matters, because two co-occurring predators of the same size may eat similarly sized prey individuals but different prey species (see Manuscript I of this thesis).

Detailed studies on prey selectivity have usually focused on one or a few species of predators and several prey types differing in body size or mobility. Prey size selectivity (both active and passive) was detected in a wide range of predators. Aquatic invertebrates, a model group in my study, are eminent in the experimental research of predation. The classic study by Pastorok (1981) found that larvae of *Chaoborus* midges are passively size-selective predators consuming *Daphnia* of medium size. Stonefly larvae, probably the most thoroughly investigated predatory aquatic insects, are also known to be size selective (Allan et al. 1987a, Allan et al. 1987b). The narrow taxonomic focus of these detailed behavioural experiments has unfortunately prevented generalization.

On the other hand, comparative studies identifying predator-prey body size relationships across whole communities were based on gut content analyses of field-collected specimens (e.g. Warren & Lawton 1987, Cohen et al. 1993, Woodward & Hildrew 2002a, Cohen et al. 2003, Woodward et al. 2005, Woodward & Warren 2007). They included wide range of taxa but focused solely on the role of body size in predator-prey relationships ignoring other species traits. To refine these observations, I have examined the role of foraging behaviour and have shown that it can significantly affect the observed predator-prey size relationship in predatory aquatic insects (see Manuscript I).

Most studies emphasize the perspective of predators by focusing on their selectivity; the importance of prey behaviour is overlooked in empirical research on food web interactions. On the other hand, research of prey anti-predator responses usually focuses solely on prey and regard predators as passive (Lima 2002, Lima et al. 2003). Integrating predator and prey perspectives in empirical work on food web interactions is still rare and challenging but already proved to have potential to change the way we think about predator-prey behavioural interactions (Lima 2002, Lima et al. 2003). Considering predator and prey traits simultaneously thus can bring new perspectives into the research of food webs.

Here, I asked what predator and prey traits determine the mortality of different prey subjected to a diverse set of predators in an attempt to treat predators and prey as equally important participants in the predator-prey interactions. To answer this question, I carried out a series of experiments with predatory aquatic insects differing in body size, microhabitat occupation and foraging behaviour and offered them a mixture of their natural prey. I hypothesized that prey mortality is a result of complex interactions between prey traits and predator traits.

Methods

Laboratory experiments

The experiments are described in Manuscript I. I performed multiple-choice predation experiments with 13 species of predatory aquatic insects and seven prey species in a climate room with constant temperature (20°C) and photoperiod (L:D=18:6 h). Experiments were performed in transparent plastic boxes filled with 2.5 l of ca. 8 cm deep aged tap water without any substrate on the bottom and equipped with four stripes of plastic mesh suspended from water surface to the bottom to provide simple structure for attachment of animals. Predators were acclimated for two to five days in the room and starved for 24 hours prior to experiments to standardize their hunger level. In the beginning of each experiment, I released a mixture of all prey species (six individuals of *Rana* tadpoles, six *Lymnaea*, 10 *Chironomus*, 10 *Cloeon*, 10 *Culex*, 10 *Asellus* and 30 *Daphnia*) and after several minutes added one predator. After 24 hours, uneaten prey was calculated. Five to nine replicates were done with each predator species, but individual predators were used only once. I also qualitatively observed the behaviour of predators and prey and their spatial distribution within the experimental vessels during experiments to collate a simple classification of their microhabitat preferences and foraging/anti-predator behaviour. After the experiments, all predators and 20 randomly chosen individuals of all prey species were conserved in 80% ethanol and weighed on analytical scales to the nearest 0.001 mg after 72 h of drying at 50°C.

Data analyses

All data analyses were carried out and figures made in R 2.9.2 (R Core Development Team 2009) unless otherwise stated. First, I tested whether the relative mortality of individual prey species differed between predator species using a generalized linear model (GLM) with quasibinomial distribution and logit link function. Then, I tested the effect of prey traits on the overall mortality exerted by the whole set of predators on individual prey species (GLM, quasibinomial distribution and logit link function).

I further calculated the similarity of predator assemblages for all pairs of prey species using Pianka's overlap index (Pianka 1973) in Ecosim 7.72 (Goteli & Entsminger 2005). This index is routinely used for estimating the similarity of resource use among species on the scale from 0 (completely different) to 1 (identical). The similarity of predator assemblages among prey species could be easily assessed adopting this index, given that the values of mortality exerted by individual species of predators were estimated for all prey species. I tested the effect of similarity in individual traits of prey on the similarity of predator assemblages using partial Mantel test performed on dissimilarity matrices with 1000 permutations in ecodist 1.1.4 package for R (Goslee & Urban 2007).

The final analysis aimed to explain the mortality exerted by individual predators on individual prey species by interactions of predator and prey traits. Mean prey mortality was calculated for each combination of predator and prey species. Prey mortality was then related to a priori defined trait interactions (biologically meaningless interactions were not tested) using a generalized additive model (GAM) with quasibinomial distribution and logit link function. An interaction of predator and prey body weight was modelled as a smooth surface (tensor smooth); GAM analysis was performed using mgcv 1.5-5 package for R (Wood 2006).

Results

Differences in mortalities (numbers of prey individuals consumed during an experiment) imposed by the selected set of predators were highly significant in each prey species ($P < 0.0001$ in all cases; Fig. 1). The highest mortality rate was detected in medium-sized prey consumed by the largest predators. Smaller predators consumed smaller prey and imposed generally lower mortality (Fig. 2). The most vulnerable prey species were *Chironomus* and *Culex*; the least consumed was *Lymnaea*, which was eaten only several times by *Anax* larvae and *Dytiscus* adults who caused only ca. 10% mortality of *Lymnaea* (Fig. 1). Overall prey mortality averaged across the whole range of predators was not related to any of the evaluated prey traits ($P > 0.3$ in all cases, GLM).

The similarity of predation rates in different prey was significantly related to prey body weight similarity but not to similarity in any other trait (Mantel test, Table 3). More detailed GAM analysis revealed a significant effect of interaction between predator size and prey size and a significant interaction of predator foraging mode (ambush/searching) and prey ability of rapid escape (Fig. 2, Table 4). None of the remaining trait interactions was significant.

Prey with low ability of rapid escape suffered high mortality from both ambush and searching predators. Prey species capable of rapid escape were vulnerable to ambush predators but suffered 2.2 times lower mortality from searching predators after correction for body size (Fig. 2). The interaction of predator and prey body weight alone explained 38.7% of the deviance and the interaction of predator foraging mode and prey escape ability added another 12.7%; both interactions together explained 51.4% of variability in prey mortality.

Discussion

The pattern of mortality of individual prey species varied significantly. The most vulnerable prey species were *Chironomus* larvae and *Culex* larvae. High vulnerability of *Chironomus* larvae may be attributed to the absence of bottom substrate in the experiments. Under natural conditions, *Chironomus* larvae hide in the bottom substrate and may thus be well protected from most predators. Without having a chance to hide, they were easily accessible for most predators in my experiments. In this way these experiments were “unnaturally crude” but the effect of refuges on prey mortality was not of interest. Low structural complexity of experimental vessels ensured that prey mortality reflected mostly its size and behaviour, i.e., the traits I wanted to expose in the analyses. Another highly vulnerable prey was *Culex* larvae. They are capable of rapid escape movements in danger, but these were apparently not effective against most predators. It corresponds well to the fact that the larvae develop rapidly in temporary waters with low predator numbers. On the contrary, *Lymnaea* snails were not consumed by most predators at all because of their protective shell. Only adult *Dytiscus* beetles were capable of breaking the shell and *Anax* dragonfly larvae were able to drag the snail from its shell and consume it. However even these two predators caused only low mortality of *Lymnaea* snails in the experiments.

Overall prey mortality averaged across the whole range of predators was not related to any of the recorded species traits, confirming the high specificity of predation. That is, prey species not consumed by one predator may be highly vulnerable to another predator (Fig. 1).

Interacting traits of both prey and predators determined prey mortality. It is well established that predator and prey body size plays an important role in predator-prey interactions (e.g. Woodward et al. 2005, Woodward & Warren 2007) but the importance of other traits is undervalued in empirical food web studies. My experiments revealed a significant additional effect of interaction of predator foraging mode and prey ability of rapid escape on prey mortality. The form of this relationship is intriguing but logical: ambush predators can feed effectively on all prey species of suitable size irrespective of their escape behaviour, but searching predators are equally effective only in capturing prey with low escape capability. Prey capable of rapid escape suffered much lower mortality from searching predators than the other prey. This result corresponds to earlier experiments on stonefly larvae. E.g., Allan et al. (1987b) and Tikkanen et al. (1997) found that stonefly larvae, which belong to actively searching predators, feed mostly on sedentary blackfly larvae (Simuliidae) and consume low numbers of mayfly larvae (Ephemeroptera) which are able to actively escape predation. In their experiments, prey activity and escape ability were tied, i.e., sedentary prey was not capable of rapid escape and active prey was capable of rapid escape. In my experiments, prey activity and escape ability were not tied which enabled me to conclude that prey capability of rapid escape rather than its overall activity determined prey vulnerability.

The results of my experiments could have been affected by the choice of experimental species, but the prey and predator species used formed a diverse set covering (nearly) all major functional groups of macroinvertebrates in small standing waters in Central Europe. Similar comparative experiments and more detailed, trait-based analyses of field-collected diet data may boost our understanding of predator-prey interactions and help to decrease the oversimplification of current descriptions of food webs. It is unlikely that body size is the only trait describing a species' role. The prospect that we could abstract from species identity and describe food web processes based on individual body size (Woodward & Warren 2007, Ings et al. 2009) is attractive but would be justified only if body size would be the single key trait determining the role of an individual in the community.

In food web models emphasizing the role of body size in community structuring, there is already a call for truly individual-based models where each individual would be characterized by its real body size rather than its “characteristic” or maximum size (Ings et al. 2009) and where species

are described by multiple functional traits (Rossberg et al. 2009). I believe that I have shown here and in Manuscript I that predator-prey interactions of aquatic invertebrates are complex and that body size is good but not sufficient predictor of the strength of such interactions. It seems that considering foraging behaviour of predators and anti-predator behaviour of prey is a way towards more powerful explanations of predator-prey interactions forming the structure of food webs.

Tables and figures

Table 1. Predator species used in the experiments with body weight and foraging traits. Predators crawling on supporting plastic mesh are classified as foraging in the water column.

Species	Body weight (mg)		Foraging microhabitat	Foraging mode	Feeding mode
	Mean	SD			
Coleoptera					
<i>Acilius canaliculatus</i> imago	61.74	9.339	bottom	searching	chewing
<i>Acilius canaliculatus</i> L2	2.74	0.655	water column	ambush	sucking
<i>Acilius canaliculatus</i> L3	14.66	4.697	water column	ambush	sucking
<i>Dytiscus marginalis</i> imago	528.43	50.881	bottom	searching	chewing
<i>Dytiscus marginalis</i> L3	176.43	76.123	water column	ambush	sucking
<i>Hydaticus seminiger</i>	64.94	8.417	bottom	searching	chewing
Hemiptera					
<i>Ilyocoris cimicoides</i>	34.43	6.847	bottom	searching	sucking
<i>Notonecta glauca</i>	39.43	8.080	water column	ambush	sucking
Odonata					
<i>Anax imperator</i> L-0	267.00	54.415	water column	ambush	chewing
<i>Coenagrion puella</i> L-0	4.80	0.989	water column	ambush	chewing
<i>Libellula depressa</i> L-0	58.41	19.507	bottom	ambush	chewing
<i>Libellula depressa</i> L-2	20.94	5.573	bottom	ambush	chewing
<i>Sympetrum sanguineum</i> L-0	20.82	4.493	water column	ambush	chewing

Table 2. Prey species used in experiments with body weight and selected traits. Almost no prey individuals of any species used plastic mesh provided as a supporting structure. Only two microhabitats were thus recognized.

Species	Body weight (mg)		Microhabitat occupation	Activity level	Ability of rapid escape	Taxon (order)
	Mean	SD				
<i>Asellus aquaticus</i>	1.77	0.523	bottom	high	no	Isopoda
<i>Cloeon dipterum</i>	0.99	0.187	bottom	low	yes	Ephemeroptera
<i>Culex</i> sp.	0.56	0.144	water column	high	yes	Diptera
<i>Daphnia</i> sp.	0.04	0.032	water column	high	no	Cladocera
<i>Chironomus</i> sp.	0.34	0.074	bottom	low	no	Diptera
<i>Lymnaea stagnalis</i> ¹	8.22	2.630	water column	low	no	Pulmonata
<i>Rana arvalis</i> tadpoles	3.07	0.448	bottom	high	yes	Anura

¹ weighed without shell

Table 3. Results of partial Mantel test of effects of similarity in individual prey traits on similarity of their relative vulnerability to the predators used in the experiments. Conditional effects after the inclusion of the single significant variable into the model are presented for non-significant variables.

Explanatory variable	Mantel r	P-value
Body weight	0.58	0.003
Microhabitat	0.21	0.283
Taxon (order)	0.21	0.379
Activity	-0.20	0.403
Ability of rapid escape	0.10	0.585

Table 4. The effects of a priori defined predator and prey trait interactions on prey mortality (GAM). Total deviance explained by the final model was 51.4%. The interaction of predator and prey body weight was modelled as a smooth surface (d.f.=5.80) in GAM, other terms were categorical (interactions of qualitative variables).

Interacting predator and prey traits	Prey mortality	
	F	P-value
Predator body weight x prey body weight	8.60	<0.0001
Predator foraging mode x prey ability of rapid escape	9.99	0.0022
Predator foraging mode x prey activity	2.12	0.1489
Predator feeding mode x prey ability of rapid escape	1.70	0.1968
Predator feeding mode x prey activity	0.53	0.4705
Predator microhabitat x prey microhabitat	0.01	0.9111

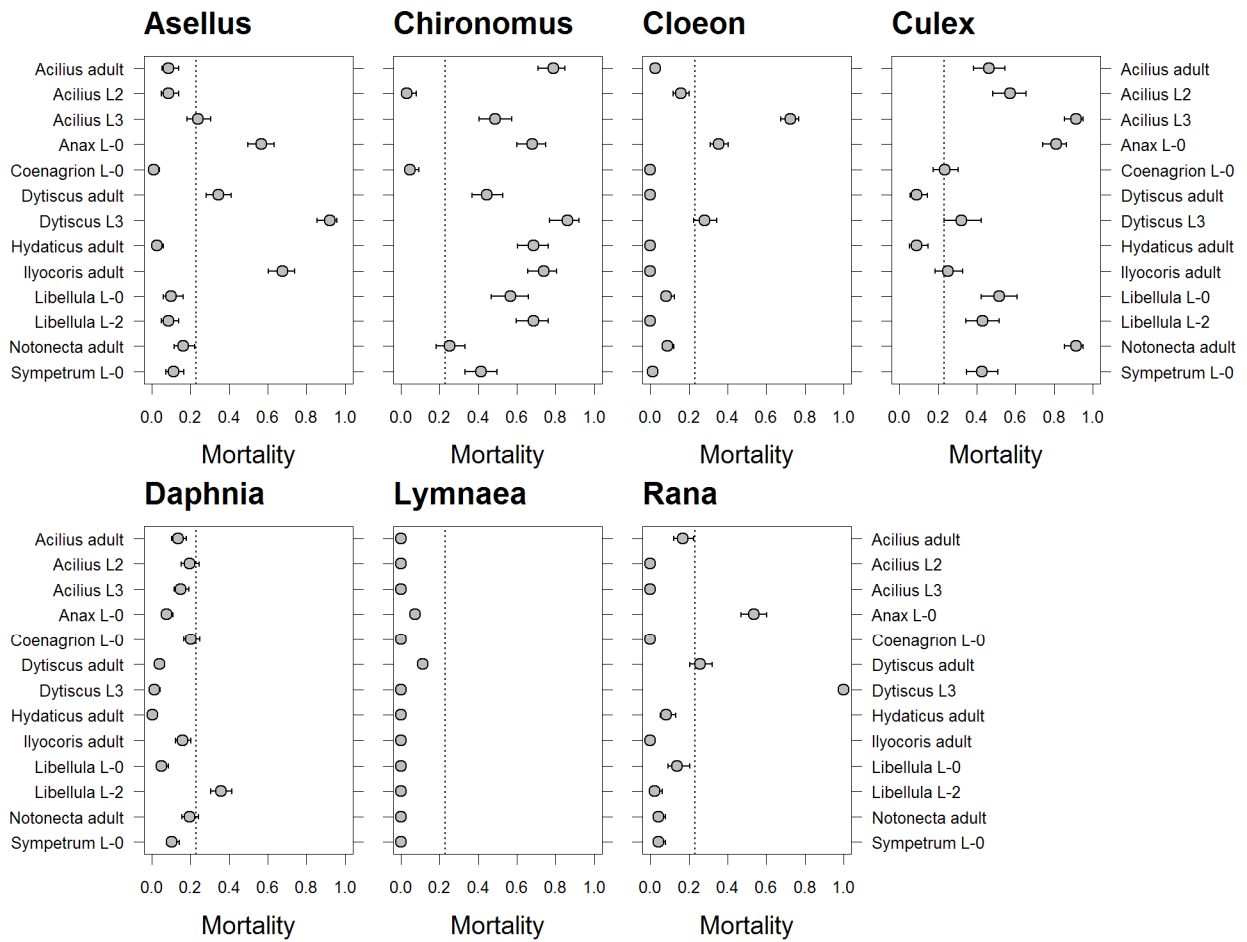


Fig. 1. Mortality of individual prey species subjected to different predators (mean \pm SE). Dotted vertical lines represent the overall mean mortality averaged across all combinations of prey and predator species to provide a benchmark for comparison.

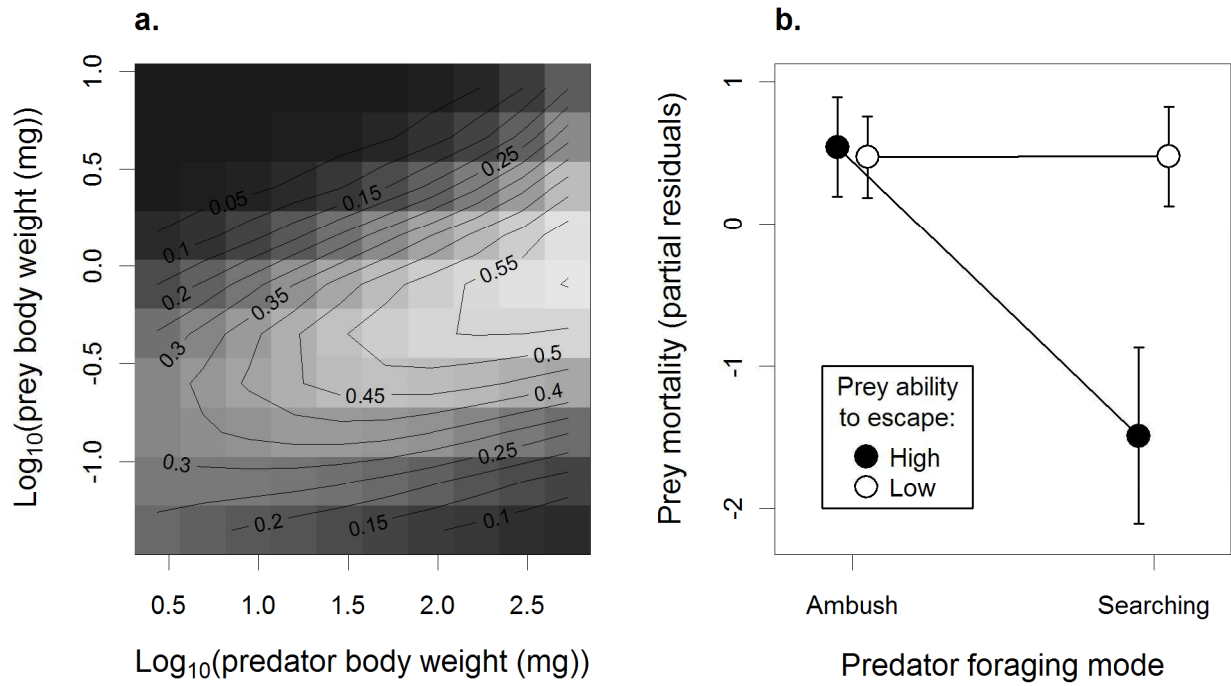


Fig. 2. The effects of predator and prey trait interactions on prey mortality (GAM). Predicted prey mortality in relation to predator and prey body weight (a) and partial residuals showing the effect of interaction between predator foraging mode and prey escape ability (b).

4. Manuscript III

Vegetation alters predator-prey interactions of freshwater invertebrates by providing perching sites for predators

Vegetation alters predator-prey interactions of freshwater invertebrates by providing perching sites for predators

Abstract

1. The structure and dynamics of food webs is driven by selective predation. I tested how is the selectivity of predatory aquatic insects affected by the level of habitat structural complexity. I performed multiple choice predation experiments under laboratory conditions in vessels with and without artificial vegetation, thereby providing two levels of habitat structural complexity.
2. The effect of habitat structure depended on microhabitat use of predators and prey. Larvae of damselflies and some species of dragonflies used the vegetation as a perching site. Only in these predators was prey selectivity affected by the presence of artificial vegetation; they consumed more *Simocephalus* (daphnid prey) compared to experiments without vegetation. Vegetation acted mostly as a perching site for these predators providing them easier access to planktonic prey. There was only a limited indication that vegetation at the same time provided refuge for *Simocephalus* from other predators. Interactions involving benthic predators or prey were virtually unaffected by the presence of vegetation.
3. It seems that habitat structural complexity may significantly alter predator-prey interactions in freshwaters. The observed effect of habitat structure was species specific and can be explained by modified behaviour of predators and prey. Microhabitat occupation of predators and related changes in foraging behaviour thus seem to mediate predator-prey interactions in freshwater food webs.
4. Habitat-mediated changes in the foraging efficiency of predators may greatly affect the structure and dynamics of food webs. I am currently testing whether the results of laboratory experiments presented here can be extrapolated to the level of natural communities in an ongoing field experiment.

Introduction

It has been recognized for a long time that prey can gain refuge from predation in complex environment. During last decades a lot of evidence accumulated that foraging efficiency of predators is usually impaired by the habitat structure (reviewed in Denno et al. 2005). The research of effects of habitat structural complexity on the strength of predator-prey interactions is traditionally intense in terrestrial ecosystems. Many studies test how vegetation density or structural complexity affects top-down control of herbivorous invertebrates by their predators (reviewed in Langelloto & Denno 2004, Denno et al. 2005, Sanders et al. 2008). Most of these studies support the hypothesis that complex habitat structure provides refuges for prey and lowers the searching efficiency of predators. The effect of habitat structure on intraguild predation seems to be similar; i.e. intraguild predation is less intense in habitats with higher structural complexity (reviewed in Janssen et al. 2007).

However not all studies comply with the rule that higher complexity leads to lower predator efficiency. One of the rare counterexamples is the study of Denno et al. (2002) who manipulated vegetation structure in mesocosm and field experiments to test the effects of vegetation structure on suppression of herbivorous planthoppers by wolf-spiders in intertidal marshes. In a mesocosm experiment, in which dispersal was limited, they found that spiders foraged more efficiently on planthoppers in complex structured environment.

Vegetation density is an important factor affecting the composition of freshwater invertebrate communities; species richness and total abundance is usually positively correlated with vegetation density (e.g. Crowder & Cooper 1982) or structural complexity (Warfe & Barmuta 2006). The effect of vegetation is likely to be very complex. It affects water chemistry through physiological processes. Plant tissues, periphyton growing on plants and plant decaying matter are used as food by a number of aquatic animals. One of the little understood roles of aquatic vegetation is its contribution to habitat structure. There are some well-known examples of predator-prey interactions where aquatic vegetation serves as a refuge for prey. It seems that in complex habitats, the effect of predators on prey communities is weaker than in simple habitats (e.g. Crowder & Cooper 1982, Gilinsky 1984, Swisher et al. 1998). However, our knowledge is still limited and some results suggest that weakening predator-prey interactions in dense vegetation may not be general but rather species-specific. For example, Lombardo (1997) found no effect of vegetation architectural complexity (simple leaves vs. complex leaves) on the predation rate of *Enallagma* damselfly larvae on its prey. Warfe & Barmuta (2006) found only limited effect of vegetation complexity on prey depletion by a fish preying on aquatic invertebrates. Recently, Yee (2010) showed that intraguild predation in larvae of diving beetles in some species combinations decreased in the presence of vegetation but in other cases remained unaltered or increased. The author speculated that this may reflect contrasting modes of foraging behaviour exhibited by different species.

I hypothesized that aquatic vegetation may serve not only as a refuge for prey but may also provide perching sites for predatory aquatic insects because many predatory aquatic insects crawl on vegetation. They may search refuge from fish predation or predation by larger invertebrates, but they may also use vegetation as a supporting structure to gain easier access to planktonic food. The aim of my study was to test in laboratory experiments whether this “perching site hypothesis” is more suitable for explaining the predation rates of aquatic insects on several types of prey under two levels of habitat structural complexity than the traditional “refuge hypothesis”.

Methods

Laboratory experiments

To test the effect of habitat structural complexity on predator-prey interactions in aquatic invertebrates, I performed multiple choice predation experiments in two series – in the first decade of May 2009 (hereafter referred to as spring series) and in the first decade of July 2009 (summer series). I used nine species of predatory aquatic insects in both series of experiments (Table 1). They were offered four species of prey in spring series and five in summer series (Table 2). Predators and prey were collected in small pools in a reclaimed sandpit near Suchdol nad Lužnicí (Třeboňsko PLA, South Bohemia, Czech Republic) and represented dominant species at the locality. Experiments were performed in a climate room with constant temperature (20°C) and photoperiod (L:D=18:6 h) in transparent plastic boxes filled with 4 l of ca. 11 cm deep aged tap water and equipped with 0.5 cm deep layer of sand at the bottom. The selectivity of predators was tested in experiments with and without artificial vegetation. The vegetation was made of 10 stripes of green plastic mesh (20 cm long, 5 cm wide) joined into a bunch placed in the middle of the experimental vessel. The “leaves” of the artificial plants intersected the whole water column and reached all sides of the experimental vessel.

Animals collected in the field were acclimated in the room for two to three days prior to experiments. Predators were kept individually in small containers (0.25–0.7 l) and fed daily ad libitum with a mixture of natural prey. Predators were starved for 24 hours prior to experiments to standardize their hunger level. In the beginning of an experiment a mixture of all prey species was released (six individuals of *Rana* tadpoles, 12 *Cloeon* larvae, 12 *Chironomus* larvae and 30 *Simocephalus* in spring experiments and 10 small and 10 large *Chironomus* larvae, 10 *Sigara* larvae, 20 small *Cloeon* larvae and 30 *Simocephalus* in July) and after several minutes one predator was added. After 24 hours, uneaten prey was counted. Six replicates with vegetation and six without vegetation were done with each predator species, but individual predators were never reused. Spatial distribution of predators and prey within experimental vessels was recorded at the end of each experiment to estimate the proportion of individuals present or time spent at the bottom, at the vegetation, in the water column and near water surface. Eight control trials (four with and four without vegetation) were performed to evaluate natural mortality of prey. To account for this mortality in predation trials, mean number of prey specimens which died during control trials was added to the number of prey individuals surviving each predation trial when calculating the number of prey individuals eaten by a predator.

Data analyses

Data analyses were carried out and figures made in R 2.9.2 (R Core Development Team 2009). The selectivity of individual predators was evaluated using Manly's α (Manly 1974, Chesson 1983). This selectivity index expresses the selectivity of a predator as a relative contribution of individual diet items to the predator's diet if all diet items would be equally abundant in the environment. I compared the selectivity of each predator species between experiments with and without artificial vegetation. I tested the difference in predator's α for each prey species in experiments with and without vegetation by a t-test. Separate testing for each prey is necessary because the values of α for different prey species are not independent for a given predator individual (see Manly 1995 for information on statistical properties of α). For better graphical presentation, the values of α were converted into an electivity index ranging from -1 to 1 with a value of 0 for unselective feeding, following Chesson (1983). However, the selectivity index, Manly's α , measures only the relative diet composition. I further tested the difference in prey mortality among predator species and between experiments with and without vegetation for each

prey species using a generalized linear model (GLM, quasibinomial distribution, logit link function).

Results

Natural prey mortality in control trials was negligible (< 3% in spring and summer trials in most prey species) and thus had no effect on the results. Only *Simocephalus* had higher mortality in the summer experiments (4.6%). It corresponded to one individual dying for other causes than predation per experiment; this was accounted for in subsequent analyses (see Methods).

All predators seemed to feed selectively (Fig. 1). The most preferred prey was *Simocephalus*, which was selected for by all species of predators. Other prey preferred by most predators was small *Chironomus* larvae. On the other hand, *Rana* tadpoles in spring experiments and large *Chironomus* larvae in summer experiments were avoided by most predators. Other feeding links were more specific, e.g. adult *Notonecta* and larvae of *Sympetrum* selected for larvae of *Sigara* (apart of *Simocephalus*) in summer experiments.

The presence of artificial vegetation caused only very limited changes in the selectivity of predators. The larvae of damselflies (*Coenagrion* and *Platycnemis*) and of the dragonfly *Sympetrum* selected more strongly for *Simocephalus* and less for *Chironomus* larvae in the presence of vegetation in spring experiments, but this trend was not so clear in summer experiments (Fig. 1). In *Simocephalus*, highly significant interaction of predator species and vegetation presence on its mortality was detected (Fig. 2, GLM, $F=6.58$ and $P<0.0001$ for spring experiments and $F=3.88$ and $P=0.0006$ for summer experiments). No effect of vegetation on prey mortality was apparent in other prey species ($P>0.05$ in all cases). Predators causing higher mortality of *Simocephalus* in experiments with vegetation were mostly those who crawled on the vegetation (Fig. 2). The three odonate larvae which spent most of their time sitting on the vegetation caused together ca. 1.7 times higher *Simocephalus* mortality in experiments with vegetation (ca. 59%) compared to experiments without vegetation (ca. 36%) in the spring and 1.6 times in the summer (ca. 54% in experiments with vegetation and ca. 35% in experiments without vegetation). On the contrary, the predators not sitting on vegetation caused ca. 1.3 times higher *Simocephalus* mortality in experiments without vegetation (ca. 47%) than with vegetation in spring experiments (ca. 36%). However, in the summer, overall *Simocephalus* mortality caused by these predators was virtually the same in both treatments (ca. 44%).

Discussion

The presence of artificial vegetation significantly affected the selectivity of some predators and their predation pressure on prey. The effect of habitat structure (artificial vegetation) was, however, species specific. Habitat structural complexity is usually viewed as a source of refuges for prey and it was many times reported that complex habitat structure including dense vegetation diminishes the foraging efficiency of predators (reviewed in Denno et al. 2005). However, not all studies support this hypothesis. In freshwater habitats, Warfe & Barmuta (2006) found a species specific effect of vegetation complexity on survival rates of aquatic invertebrate prey exposed to a fish predator as did Yee (2010) in the study of intraguild predation in diving beetle larvae.

In my experiments, artificial vegetation provided perching sites for damselfly and some dragonfly larvae, which gain easier access to planktonic prey, *Simocephalus*. The presence of vegetation altered to some degree the relative composition of their diet (Fig. 1) and increased their predation pressure on *Simocephalus* (Fig. 2).

On the other hand, the presence of vegetation had very little effect on the selectivity of the other predators, which did not crawl on the vegetation. Their prey selectivity remained unaltered in all cases (Fig. 1). There was an indication of vegetation acting as a refuge for *Simocephalus* against these predators in the spring experiments, but it was not supported by summer experiments (Fig. 2). Most prey species in my experiments were dwelling at the bottom and made little use of vegetation (Table 2), which can explain why the vegetation did not serve as a refuge for them. *Simocephalus* was the only planktonic prey moving through the water column and one could suppose that it would be more difficult to detect and capture among the leaves of artificial plants. However, the evidence that vegetation hinders predation on *Simocephalus* was very limited.

My results suggest that the effect of habitat structure on predator-prey interactions is mediated by microhabitat occupation of both predators and prey. This corresponds well to the findings of Sanders et al. (2008) who found in a field experiment taxon specific changes in the density of herbivorous insects after manipulations of habitat structure which could be attributed to differences in prey refuge use and predator foraging behaviour. Similarly, Yee et al. (2010) argued that different effect of vegetation complexity on the intensity of intraguild predation in different combination of species of diving beetle larvae can be explained by differences in foraging behaviour of interacting species.

The laboratory experiments presented here are a part of a larger project which involves field manipulation of small pools in a reclaimed sandpit. The data from these laboratory experiments will be used to generate hypotheses concerning the structure of natural communities of outdoor experimental pools. It will enable me to test whether results of simple laboratory experiments on predator-prey interactions in aquatic invertebrates can be successfully extrapolated to the level of whole communities of small water bodies.

Tables and figures

Table 1. Predator species used in the experiments and their spatial distribution within the experimental vessels – the proportion of individuals within each microhabitat at the end of the experiment. In Odonata, L-0 denotes last instar and L-2, second before the last instar larvae, in *Laccophilus*, L3 denotes 3rd (last) instar larvae and in *Chaoborus*, L4 denotes 4th (last) instar larvae.

Species	With vegetation				Without vegetation		
	Bottom	Vegetation	Water column	Water surface	Bottom	Water column	Water surface
SPRING							
Odonata							
<i>Coenagrion puella</i> L-0	0.00	1.00	0.00	0.00	1.00	0.00	0.00
<i>Libellula depressa</i> L-0	0.83	0.17	0.00	0.00	1.00	0.00	0.00
<i>Libellula depressa</i> L-2	0.83	0.17	0.00	0.00	1.00	0.00	0.00
<i>Platycnemis pennipes</i> L-2	0.00	0.83	0.17	0.00	1.00	0.00	0.00
<i>Sympetrum sanguineum</i> L-2	0.00	0.83	0.17	0.00	1.00	0.00	0.00
Heteroptera							
<i>Notonecta glauca</i> L2	0.00	0.33	0.00	0.67	0.17	0.00	0.83
Coleoptera							
<i>Hydroglyphus geminus</i> adult	0.66	0.17	0.17	0.00	0.66	0.17	0.17
<i>Laccophilus minutus</i> adult	0.66	0.17	0.17	0.00	1.00	0.00	0.00
Diptera							
<i>Chaoborus</i> sp. L4	0.00	0.00	1.00	0.00	0.00	1.00	0.00
SUMMER							
Odonata							
<i>Coenagrion puella</i> L-0	0.00	1.00	0.00	0.00	1.00	0.00	0.00
<i>Libellula depressa</i> L-0	0.67	0.33	0.00	0.00	1.00	0.00	0.00
<i>Platycnemis pennipes</i> L-2	0.00	1.00	0.00	0.00	1.00	0.00	0.00
<i>Sympetrum sanguineum</i> L-0	0.17	0.83	0.00	0.00	1.00	0.00	0.00
Heteroptera							
<i>Notonecta glauca</i> adult	0.00	0.33	0.00	0.67	0.33	0.00	0.67
Coleoptera							
<i>Laccophilus minutus</i> adult	0.83	0.17	0.00	0.00	0.83	0.00	0.17
<i>Laccophilus minutus</i> L3	0.83	0.17	0.00	0.00	0.83	0.17	0.00
<i>Potamonectes canaliculatus</i> adult	1.00	0.00	0.00	0.00	1.00	0.00	0.00
Diptera							
<i>Chaoborus</i> sp. L4	0.00	0.00	1.00	0.00	0.00	1.00	0.00

Table 2. Prey species used in the experiments and their spatial distribution within the experimental vessels – mean proportion of individuals within each microhabitat at the end of experiments. In *Sigara*, L2 denotes 2nd instar larvae.

Species (order)	With vegetation				Without vegetation		
	Bottom	Vegetation	Water column	Water surface	Bottom	Water column	Water surface
SPRING							
<i>Chironomus</i> sp. small (Diptera)	1.00	0.00	0.00	0.00	1.00	0.00	0.00
<i>Cloeon dipterum</i> (Ephemeroptera)	0.77	0.23	0.00	0.00	1.00	0.00	0.00
<i>Rana arvalis</i> tadpoles (Anura)	0.95	0.05	0.00	0.00	1.00	0.00	0.00
<i>Simocephalus</i> sp. (Cladocera)	0.00	0.00	0.99	0.01	0.00	0.92	0.08
SUMMER							
<i>Chironomus</i> sp. large (Diptera)	0.98	0.02	0.00	0.00	1.00	0.00	0.00
<i>Chironomus</i> sp. small (Diptera)	1.00	0.00	0.00	0.00	1.00	0.00	0.00
<i>Cloeon dipterum</i> (Ephemeroptera)	0.94	0.06	0.00	0.00	1.00	0.00	0.00
<i>Sigara</i> sp. L2 (Heteroptera)	0.90	0.10	0.00	0.00	1.00	0.00	0.00
<i>Simocephalus</i> sp. (Cladocera)	0.00	0.00	0.98	0.02	0.00	0.98	0.02

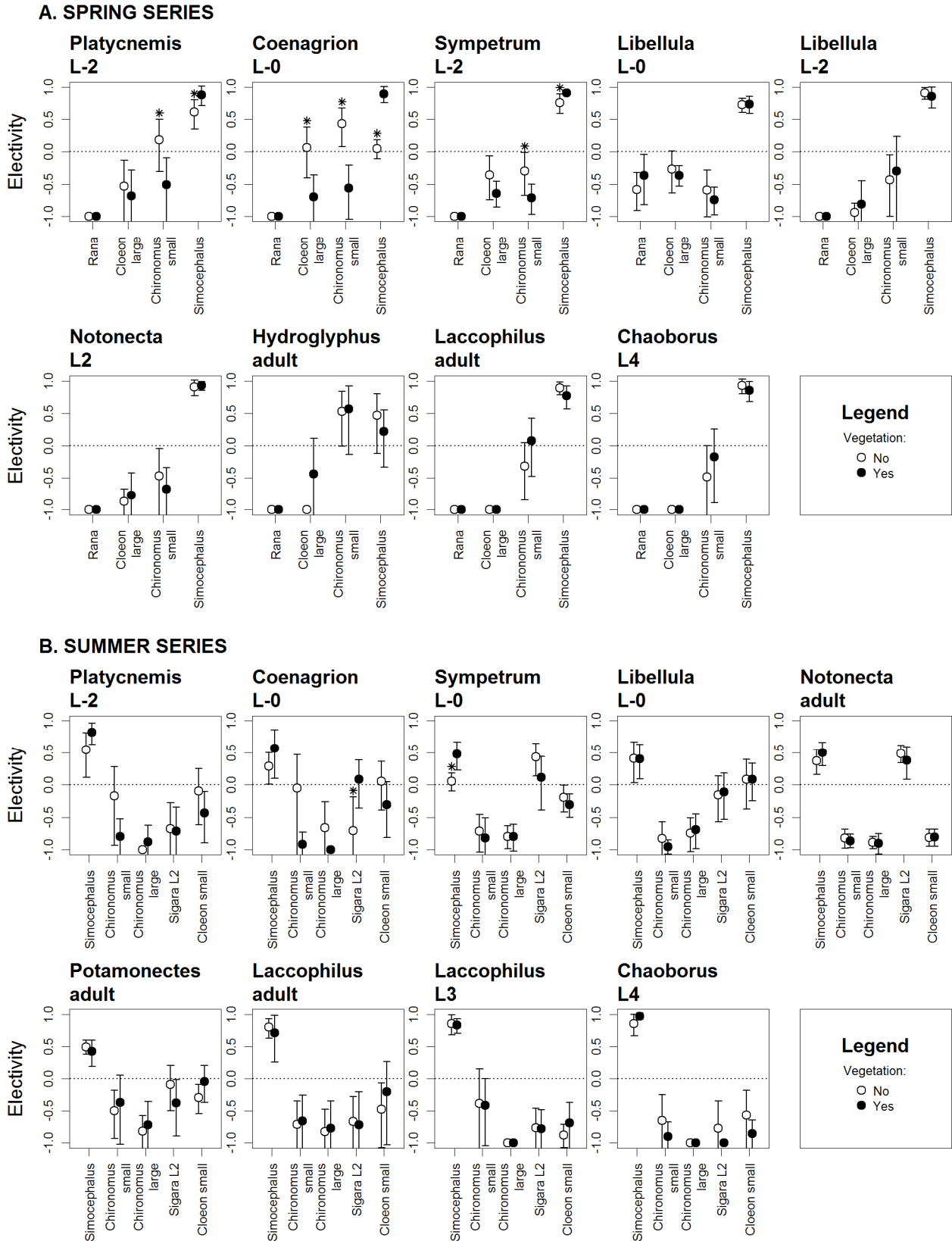


Fig. 1. Prey selectivity of individual species of predators in spring (A) and summer (B) series of experiments. Mean electivity values \pm SE are displayed. Asterisks denote significant difference of electivity values between experiments with and without artificial vegetation for a particular prey species (two sample t-test, $P < 0.05$).

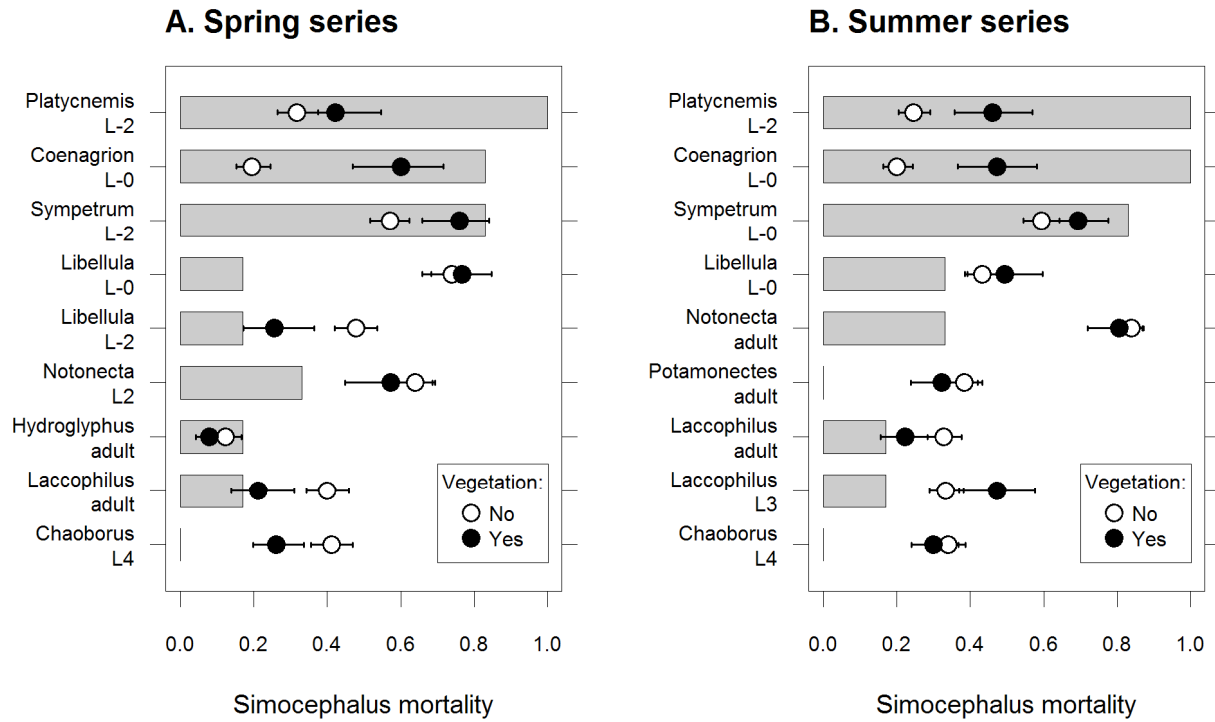


Fig. 2. The mortality of *Simocephalus* depends on the interaction between predator species and vegetation presence in both spring (A) and summer (B) series of experiments (GLM). Mean mortality \pm SE is plotted. Grey stripes display the estimate of the proportion of time spent by individual predators sitting on the vegetation (the same scale as for *Simocephalus* mortality).

5. References

- Allan, J.D., Flecker, A.S. & McClintock, N.L. (1987a) Prey size selection by carnivorous stoneflies. *Limnology and Oceanography*, 32, 864-872.
- Allan, J.D., Flecker, A.S. & McClintock, N.L. (1987b) Prey preference of stoneflies: sedentary vs. mobile prey. *Oikos*, 49, 323-331.
- Atkinson, D. & Hirst, A.G. (2007) Life histories and body size. *Body Size: The Structure and Function of Aquatic Ecosystems* (eds. Hildrew, A.G., Raffaelli, D.G. & Edmonds-Brown, R.), pp. 33-54. Cambridge University Press, Cambridge, UK.
- Bailey, P.C.E. (1986) The feeding behaviour of a sit-and-wait predator, *Ranatra dispar* (Heteroptera: Nepidae): optimal foraging and feeding dynamics. *Oecologia*, 68, 291-297.
- Barbosa, P. & Castellanos, I. (2005) *Ecology of predator-prey interactions*. Oxford University Press, Oxford, UK, 394 pp.
- Blois, C. (1985) The larval diet of three anisopteran (Odonata) species. *Freshwater Biology*, 15, 505-514.
- Brown, J.H., Allen, A.P. & Gillooly, J.F. (2007) The metabolic theory of ecology and the role of body size in marine and freshwater ecosystems. *Body Size: The Structure and Function of Aquatic Ecosystems* (eds. Hildrew, A.G., Raffaelli, D.G. & Edmonds-Brown, R.), pp. 1-15. Cambridge University Press, Cambridge, UK.
- Brown, J.H., Gillooly, J.F., Allen, P.A., Savage, V.M. & West G.B. (2004) Toward a metabolic theory of ecology. *Ecology*, 85, 1771-1789.
- Chesson, J. (1983) The estimation and analysis of preference and its relationship to foraging models. *Ecology*, 64, 1297-1304.
- Claessen, D., de Roos, A.M. & Persson, L. (2000) Dwarfs and giants: cannibalism and competition in size-structured populations. *American Naturalist*, 155, 219-237.
- Claessen, D., de Roos, A.M. & Persson, L. (2004) Population dynamic theory of size-dependent cannibalism. *Proceedings of the Royal Society of London B: Biological Sciences*, 271, 333-340.
- Cohen, J.E., Jonsson, T. & Carpenter, S.R. (2003) Ecological community description using the food web, species abundance, and body size. *Proceedings of the National Academy of Sciences*, 100, 1781-1786.
- Cohen, J.E., Pimm, S.L., Yodzis, P. & Saldaña, J. (1993) Body sizes of animal predators and animal prey in food webs. *Journal of Animal Ecology*, 62, 67-78.
- Cook, R.M. & Cockrell, B.J. (1978) Predator ingestion rate and its bearing on feeding time and the theory of optimal diets. *Journal of Animal Ecology*, 47, 529-547.
- Cooper, S.D., Smith, D.W. & Bence, J.R. (1985) Prey selection by freshwater predators with different foraging strategies. *Canadian Journal of Fisheries and Aquatic Sciences*, 42, 1720-1732.
- Crowder, L.B. & Cooper, W.E. (1982) Habitat structural complexity and the interaction between bluegills and their prey. *Ecology*, 63, 1802-1813.
- Cummins, K.W. (1973) Trophic relations of aquatic insects. *Annual Review of Entomology*, 18, 183-206.
- Day, T., Abrams, P.A. & Chase, J.M. (2002) The role of size-specific predation in the evolution and diversification of prey life histories. *Evolution*, 56, 877-887.
- Denno, R.F., Finke, D.L. & Langelloto, G.A. (2005) Direct and indirect effects of vegetation structure and habitat complexity on predator-prey and predator-predator interactions. *Ecology of predator-prey interactions* (eds. Barbosa, P. & Castellanos, I.), pp. 211-239. Oxford University Press, Oxford, UK.

- Denno, R.F., Gratton, C., Peterson, R.A., Langelloto, G.A., Finke, D.L. & Huberty, A.F. (2002) Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. *Ecology*, 83, 1443-1458.
- Downes, S.J. (2002) Size-dependent predation by snakes: selective foraging or differential prey vulnerability? *Behavioral Ecology*, 13, 551-560.
- Fox, L.R. & Murdoch, W.W. (1978) Effects of feeding history on short-term and long-term functional responses in *Notonecta hoffmanni*. *Journal of Animal Ecology*, 47, 945-959.
- Gilinsky, E. (1984) The role of fish predation and spatial heterogeneity in determining benthic community structure. *Ecology*, 65, 455-468.
- Giller, P.S. (1980) The control of handling time and its effects on the foraging strategy of a heteropteran predator, *Notonecta*. *Journal of Animal Ecology*, 49, 699-712.
- Giller, P.S. (1986) The natural diet of the Notonectidae: field trials using electrophoresis. *Ecological Entomology*, 11, 163-172.
- Goslee, S.C. & Urban, D.L. (2007) The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, 22, 1-19.
- Gotelli, N.J. & Entsminger, G.L. (2005) *EcoSim: Null models software for ecology. Version 7*. Acquired Intelligence Inc. & Kesey-Bear, Jericho. URL: <http://garyentsminger.com/ecosim.htm>.
- Hildrew, A.G., Raffaelli, D.G. & Edmonds-Brown, R. (eds.) (2007) *Body Size: The Structure and Function of Aquatic Ecosystems*. Cambridge University Press, Cambridge, UK, 234 pp.
- Ings, T.C., Montoya, J.M., Bascompte, J., Blüthgen, N., Brown, L., Dormann, C.F., Edwards, F. et al. (2009) Ecological networks – beyond food webs. *Journal of Animal Ecology*, 78, 253-269.
- Janssen, A., Sabelis, M.W., Magalhães, S., Montserrat, M. & Van der Hammen, T. (2007) Habitat structure affects intraguild predation. *Ecology*, 88, 2713-2719.
- Krause, A.E., Frank, K.A., Mason, D.M., Ulanowicz, R.E. & Taylor, W.V. (2003) Compartments revealed in food-web structure. *Nature*, 426, 282-285.
- Langelloto, G.A. & Denno, R.F. (2004) Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. *Oecologia*, 139, 1-10.
- Lima, S.L., Mitchell, W.A. & Roth, T.C. (2003) Predators feeding on behaviourally responsive prey: some implications for classical models of optimal diet choice. *Evolutionary Ecology Research*, 5, 1083-1102.
- Lima, S.L. (2002) Putting predators back into behavioral predator-prey interactions. *Trends in Ecology and Evolution*, 17, 70-75.
- Lombardo, P. (1997) Predation by *Enallagma* nymphs (Odonata, Zygoptera) under different conditions of spatial heterogeneity. *Hydrobiologia*, 356, 1-9.
- Manly, B.F.J. (1974) A model for certain types of selection experiments. *Biometrics*, 30, 281-294.
- Manly, B.F.J. (1995) Measuring selectivity from multiple choice feeding-preference experiments. *Biometrics*, 51, 709-715.
- Pastorok, R.A. (1981) Prey vulnerability and size selection by Chaoborus larvae. *Ecology*, 62, 1311-1324.
- Peckarsky, B.L. (1982) Aquatic insect predator-prey relations. *BioScience*, 32, 261-266.
- Petchey, O.L., Beckerman, A.P., Riede, J.O. & Warren, P.H. (2008) Size, foraging, and food web structure. *Proceedings of the National Academy of Sciences*, 105, 4191-4196.
- Pianka, E.R. (1973) The structure of lizard communities. *Annual Review of Ecology and Systematics*, 4, 53-74.
- R Core Development Team (2009) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL: <http://www.R-project.org>.
- Rossberg A.G., Brännström Å. & Dieckmann U. (2009) How trophic interaction strength depends on traits. *Theoretical Ecology*, 3, 13-24.

- Sanders, D., Nickel, H., Grützner, T. & Platner, C. (2008) Habitat structure mediates top-down effects of spider and ants on herbivores. *Basic and Applied Ecology*, 9, 152-160.
- Sih, A. (2005) Predator-prey space use as an emergent outcome of a behavioral response race. *Ecology of predator-prey interactions* (eds. Barbosa, P. & Castellanos, I.), pp. 240-255. Oxford University Press, Oxford, UK.
- Stearns, S.C. & Koella, J.C. (1986) The evolution of phenotypic plasticity in life-history traits – predictions of reaction norms for age and size at maturity. *Evolution*, 40, 893-913.
- Streams, F.A. (1994) Effect of prey size on attack components of the functional response by *Notonecta undulata*. *Oecologia*, 98, 57-63.
- Swisher, B.J., Soluk, D.A. & Wahl, D.H. (1998) Non-additive predation in littoral habitats: influences of habitat complexity. *Oikos*, 81, 30-37.
- Tikkanen, P., Muotka, T., Huhta, A. & Juntunen, A. (1997) The roles of active predator choice and prey vulnerability in determining the diet of predatory stonefly (Plecoptera) nymphs. *Journal of Animal Ecology*, 66, 36-48.
- Warfe, D.M. & Barmuta, L.A. (2006) Habitat structural complexity mediates food web dynamics in a freshwater macrophyte community. *Oecologia*, 150, 141-154.
- Warren P.H. & Lawton J.H. (1987) Invertebrate predator-prey body size relationships: an explanation for upper triangular food webs and patterns in food web structure? *Oecologia*, 74, 231-235.
- Wood S.N. (2006) *Generalized additive models: An introduction with R*. Chapman & Hall/CRC, Boca Raton, USA, 392 pp.
- Woodward, G. & Hildrew, A.G. (2002a) Body-size determinants of niche overlap and intraguild predation within a complex food web. *Journal of Animal Ecology*, 71, 1063-1074.
- Woodward, G. & Hildrew, A.G. (2002b) Differential vulnerability of prey to an invading top predator: integrating field surveys and laboratory experiments. *Ecological Entomology*, 27, 732-744.
- Woodward, G. & Hildrew, A.G. (2002c) The impact of a sit-and-wait predator: separating consumption and prey emigration. *Oikos*, 99, 409-418.
- Woodward, G. & Warren, P. (2007) Body size and predatory interactions in freshwaters: scaling from individuals to communities. *Body Size: The Structure and Function of Aquatic Ecosystems* (eds. Hildrew, A.G., Raffaelli, D.G. & Edmonds-Brown, R.), pp. 98-117. Cambridge University Press, Cambridge, UK.
- Woodward, G., Ebenman, B., Emmerson, M., Montoya, J.M., Olesen, J.M., Valido, A. & Warren, P.H. (2005) Body size in ecological networks. *Trends in Ecology and Evolution*, 20, 402-409.
- Yee, D.A. (2010) Behavior and aquatic plants as factors affecting predation by three species of larval predaceous diving beetles (Coleoptera: Dytiscidae). *Hydrobiologia*, 637, 33-43.