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Dudová P, Boukal DS, Klecka J. 2019. Prey selectivity and the effect of diet on growth and development of a dragonfly, *Sympetrum sanguineum*. PeerJ 7:e7881 <https://doi.org/10.7717/peerj.7881>

# Prey selectivity and the effect of diet on growth and development of a dragonfly, *Sympetrum sanguineum*

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Despite a long tradition of research, our understanding of mechanisms driving prey selectivity in predatory insects is limited. According to optimal foraging theory, predators should prefer prey which provides the highest amount of energy per unit time. However, prey selectivity may also depend on previous experience and nutritional demands driven by stoichiometry. From the long-term perspective, diet composition affects predator fitness. An open question is whether short-term selectivity of predators provides a diet which is optimal in the long-term. To shed more light on these issues, we conducted laboratory experiments on prey selectivity and its long-term consequences in larvae of the dragonfly *Sympetrum sanguineum*. We conditioned the larvae to one of two prey types, the cladoceran *Daphnia magna* and *Chironomus* larvae, and then exposed them to various combinations of the two prey types. We found that dragonfly larvae conditioned to *Chironomus* larvae consumed the same amount of *D. magna*, but significantly less *Chironomus* larvae compared to dragonfly larvae conditioned to *D. magna*. However, there was no effect of experience on their success of capture and handling time, suggesting a limited role of learning in their ability to process prey. We then tested the long-term effects of diets with different proportions of both prey for survival and growth of the dragonfly larvae. Individuals fed *Chironomus*-only diet performed significantly worse than dragonflies fed *D. magna*, while larvae fed a mixed diet performed the best in terms of growth and survival until adulthood. In conclusion, we show that dragonfly larvae fed by *Chironomus* larvae performed poorly and compensated by preferring *D. magna* when both prey types were available. The superiority of the mixed diet suggests that a diverse diet may be needed to satisfy nutritional demands in *S. sanguineum* larvae. We demonstrate that merging short-term predation experiments with relevant data on predator fitness may provide better understanding of predator-prey interactions and conclude that detailed

information on the (mis)matches between prey stoichiometry and predator nutritional demands is needed for further progress.

# 1 Prey selectivity and the effect of diet on 2 growth and development of a dragonfly, 3 *Sympetrum sanguineum*

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## 14 ABSTRACT

15 Despite a long tradition of research, our understanding of mechanisms driving prey selectivity in predatory  
16 insects is limited. According to optimal foraging theory, predators should prefer prey which provides  
17 the highest amount of energy per unit time. However, prey selectivity may also depend on previous  
18 experience and nutritional demands driven by stoichiometry. From the long-term perspective, diet  
19 composition affects predator fitness. An open question is whether short-term selectivity of predators  
20 provides a diet which is optimal in the long-term. To shed more light on these issues, we conducted  
21 laboratory experiments on prey selectivity and its long-term consequences in larvae of the dragonfly  
22 *Sympetrum sanguineum*. We conditioned the larvae to one of two prey types, the cladoceran *Daphnia*  
23 *magna* and *Chironomus* larvae, and then exposed them to various combinations of the two prey types.  
24 We found that dragonfly larvae conditioned to *Chironomus* larvae consumed the same amount of *D.*  
25 *magna*, but significantly less *Chironomus* larvae compared to dragonfly larvae conditioned to *D. magna*.  
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31 that dragonfly larvae fed by *Chironomus* larvae performed poorly and compensated by preferring *D.*  
32 *magna* when both prey types were available. The superiority of the mixed diet suggests that a diverse diet  
33 may be needed to satisfy nutritional demands in *S. sanguineum* larvae. We demonstrate that merging  
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35 of predator-prey interactions and conclude that detailed information on the (mis)matches between prey  
36 stoichiometry and predator nutritional demands is needed for further progress.

37 **Key words:** predation, optimal foraging theory, prey selection, switching, ecological stoichiometry,  
38 survival, aquatic insects

## 39 INTRODUCTION

40 Predators do not attack any prey indiscriminately, but feed more or less selectively on a subset of prey they  
41 encounter (Waldbauer and Friedman, 1991; Klecka and Boukal, 2012). Research into the mechanisms  
42 and consequences of selective predation has a long tradition. Earlier research centred around the concept  
43 of optimal foraging theory (Emlen, 1966; MacArthur and Pianka, 1966; Stephens and Krebs, 1986). It  
44 focuses on the importance of energy gains on prey selectivity: it posits that consumers should maximise  
45 their energy intake by selectively consuming the most profitable resource, i.e., a resource which provides  
46 the highest energy intake per unit of time. Evidence supporting optimal foraging theory comes from

47 different consumer types including predators, herbivores, and parasitoids (Stephens and Krebs, 1986).  
48 At the same time, optimal foraging theory was criticised for being simplistic (Pyke, 1984; Pierce and  
49 Ollason, 1987). Reviews of experimental evidence have been inconclusive, because the level of support  
50 for predictions of optimal foraging theory varies widely between studies (Pyke, 1984; Stephens and Krebs,  
51 1986; Sih and Christensen, 2001). Despite that, the appeal of optimal foraging theory as a mechanistic  
52 underpinning of selective predation has been bolstered by studies demonstrating that its predictions could  
53 be used to fit the structure of empirically observed food webs (Beckerman et al., 2006; Petchey et al.,  
54 2008). However, more detailed understanding of nutritional demands of a growing number of species  
55 paints a more complex picture of mechanisms driving selective foraging (Fagan et al., 2002; Lefcheck  
56 et al., 2013) with implications for population and community dynamics (Moe et al., 2005).

57 Consumers do not only need to feed on energy-rich resources, but also on resources of the right quality  
58 to maximise fitness (Fagan et al., 2002; Mayntz et al., 2005). Insights from ecological stoichiometry  
59 (Elser et al., 2000; Sterner and Elser, 2002) suggest that animals could rarely depend on a single resource,  
60 and at least some predators can feed selectively to balance their nutritional needs (Mayntz et al., 2005).  
61 Experiments on diet-dependent growth and reproduction of animals have found that species often perform  
62 best on mixed diets (Lefcheck et al., 2013). Two hypotheses have been proposed as explanations: 1) mixed  
63 diet provides a better chance to satisfy the consumer's nutritional demands (Pulliam, 1975; Raubenheimer  
64 and Simpson, 1997) and 2) dilution of toxins helps to deal with toxic prey (Freeland and Janzen, 1974;  
65 Bernays et al., 1994). Rejecting toxic or unpalatable prey seems trivial, but in some cases predators do  
66 not learn to avoid toxic prey even when they have alternative prey to rely upon (Oelbermann and Scheu,  
67 2002). However, the empirical support of the toxin-dilution hypothesis is weak and the balanced-diet  
68 hypothesis is better supported by experimental data (Lefcheck et al., 2013).

69 Studies of predator-prey interactions usually take either a behavioural approach based on short-term  
70 experiments, or focus on growth, reproduction, and population dynamics at a longer time scale. While  
71 short-term experiments of foraging behaviour help to elucidate the process of prey search, capture,  
72 and processing (Lawton et al., 1974; Sih and Christensen, 2001; Klecka and Boukal, 2014), long-  
73 term experiments are needed to examine implications of diet for fitness of consumers and evolution of  
74 interspecific interactions (Moe et al., 2005; Lefcheck et al., 2013). However, these two approaches have  
75 rarely been combined in a single study system.

76 We used larvae of the dragonfly *Sympetrum sanguineum* to investigate the links between short-term  
77 foraging decisions and long-term fitness consequences. Despite their popularity in freshwater ecology  
78 studies, mechanistic basis of prey selectivity in dragonfly larvae is little understood. As other predators,  
79 they are at least partly size-selective (Hirvonen and Ranta, 1996; Turner and Chislock, 2007; Klecka  
80 and Boukal, 2013), influenced by the behaviour and microhabitat use of their own and potential prey  
81 (Cooper et al., 1985; Johansson, 1993; Klecka and Boukal, 2012, 2013), and their predation is modulated  
82 by habitat structure (Klecka and Boukal, 2014). Evidence of learning capacity in dragonfly larvae in a  
83 foraging context suggests that they may learn to avoid unpalatable prey (Rowe, 1994) and handle prey  
84 better based on previous experience (Bergelson, 1985). Frequency-dependent food selection and prey  
85 switching (Lawton et al., 1974; Sherratt and Harvey, 1993), i.e., disproportionate preference of abundant  
86 prey and avoidance of rare prey, was also reported in larvae of a damselfly (Akre and Johnson, 1979) and  
87 a dragonfly (Bergelson, 1985). While dragonfly and damselfly larvae are commonly used in short-term  
88 predation experiments, data on long-term consequences of diet composition for their growth, survival,  
89 and reproduction are very limited (Baker, 1989; Hottenbacher and Koch, 2006).

90 We conducted two experiments to address short-term prey selectivity and long-term effects of diet on  
91 the growth and survival of *S. sanguineum* larvae. We tested whether and how their prey selectivity depends  
92 on relative abundances of two prey types, *Daphnia magna* and *Chironomus* larvae and on their previous  
93 experience with the prey. We hypothesised that conditioning the predator to one of the prey types would  
94 drive its preference in the experiment because it would learn to efficiently capture and handle the prey  
95 type it was conditioned to. Alternatively, the predator could preferentially select the other prey type if the  
96 single-prey diet during the conditioning period was not nutritionally balanced. To corroborate the findings  
97 from the short-term experiment, we conducted a long-term experiment to test how diet composition affects  
98 survival and growth of *S. sanguineum* larvae. Here, we hypothesised that *S. sanguineum* larvae would  
99 survive and grow best either when fed by *D. magna*, on which they can complete their development  
100 (Sentis et al., 2015) and which they are more likely to encounter in nature given that *Chironomus* larvae  
101 are mostly buried in sediments, or that they would grow best on a mixed diet which could more completely

102 satisfy their nutritional demands.

## 103 METHODS

### 104 Testing the role of experience on prey selectivity

105 In the first experiment, we tested feeding preferences of *S. sanguineum* larvae offered two prey types  
 106 in a wide range of ratios. We used representatives of two prey types common at sites inhabited by *S.*  
 107 *sanguineum*: the zooplankton *Daphnia magna* (mean body length 2.47 mm, SD=0.33 mm, mean dry  
 108 weight: 0.19 mg) collected in ponds near České Budějovice and *Chironomus* larvae (unidentified species,  
 109 mean body length 11.21 mm, SD=1.09 mm, mean dry weight: 1.04 mg, SD = 0.37 mg) obtained from  
 110 a local supplier of aquarium fish feed. We tested the hypothesis that preference for the two prey types  
 111 depends on previous experience of the predators. To this end we collected larvae of *S. sanguineum* of  
 112 different instars in a small pond in the south of the Czech Republic (49.1307497N, 15.0938167E) in  
 113 May-July 2011, transported them to a climatic chamber at the Institute of Entomology (22 ± 1°C, 16-h  
 114 day : 8-h night). We housed the larvae individually in 80 ml plastic cups (diameter: 57 mm, height: 54  
 115 mm) with ca. 70 ml of aged tap water. They were fed by *Tubifex* worms *ad libitum* on a daily basis until  
 116 they reached the penultimate stage.

117 After the larvae of *S. sanguineum* reached the penultimate (F-1) instar (n = 124), we relocated them  
 118 individually into larger plastic containers (15.5 x 10.5 x 10 cm, length x width x height) with cotton fabric  
 119 glued on the bottom. We assigned each individual randomly in two groups. One half of the individuals  
 120 were fed *ad libitum* by *D. magna* and the other half by *Chironomus* larvae for three days. Afterwards, we  
 121 left them to starve for 24 hours. Observation of feeding behaviour was performed in the same type of  
 122 plastic containers as in those we kept the larvae prior to the experiment and filled with 200 ml of aged  
 123 tap water. Shallow water depth (<2 cm) allowed us to record all predation events in the experimental  
 124 arena despite limited depth of field of our camera (Panasonic HDC-SD90). A single predator was placed  
 125 into the arena with one of seven different ratios of two prey types (*Chironomus* : *D. magna*; 20:0, 16:4,  
 126 13:7, 10:10, 7:13, 4:16, 0:20) and feeding was observed and filmed for 30 minutes. We carried out 7-10  
 127 replicates for each prey ratio. Each predator was used only once. The length of the observation period was  
 128 chosen based on a pilot experiment which showed that feeding rate declined afterwards due to predator's  
 129 satiation.

130 During the experiment, we recorded each predation event and replaced each prey individual killed by  
 131 the predator to keep the number of prey individuals in the experiment constant. We obtained detailed data  
 132 on each predation event from the recordings with the help of EthoLog 2.2.5 (Ottoni, 2000). We counted  
 133 the number of approaches towards the prey, attacks, successful attacks, and measured handling time. The  
 134 predators remained motionless until the initiation of the predation sequence, which prevented us from  
 135 measuring the encounter rate in a meaningful way. The first stage of the predation sequence we could  
 136 reliably identify was the approach towards the prey, which we defined as turning of the predator's body  
 137 towards the prey. Attack was defined as extending of the dragonfly's labium, and capture success as the  
 138 number of captured prey individuals divided by the number of attacks. Handling time was defined as the  
 139 time from successful attack to termination of the movement of mandibles.

To evaluate the selectivity of *S. sanguineum* larvae towards the two prey types, we calculated Manly's  
 $\alpha$  selectivity index (Manly, 1974; Chesson, 1983). The index compares the proportion of a prey type in  
 the diet with its proportion in the environment. We used a simple version of the index which assumes that  
 prey abundance in the environment is constant, since we replenished any consumed prey. In this case, the  
 formula is:

$$\hat{\alpha}_i = \frac{r_i/n_i}{\sum_{j=1}^m r_j/n_j}, i = 1, \dots, m \text{ (Manly, 1974)}$$

140 where  $r_i$  is the number of items of prey  $i$  consumed,  $n_i$  is the abundance of prey type  $i$  in the environment,  
 141 and  $m$  is the number of prey types. For the purpose of visualisation, we transformed Manly's  $\alpha$  into an  
 142 electivity index (Chesson, 1983), where the value of 0 corresponds to prey consumption identical to prey  
 143 availability in the environment, positive values correspond to preference for the given prey type, and  
 144 negative values correspond to avoidance of the given prey type. Since we had only two prey types, we  
 145 focused on the selectivity towards *D. magna* in these analyses; preference for *D. magna* means equally  
 146 strong avoidance of *Chironomus* larvae and vice versa.

147 We tested the effect of diet conditioning experienced by the predator prior to the experiment and  
 148 the effect of prey availability (the proportion of *D. magna*) on several measures of foraging behaviour

149 and prey choice. We used generalised linear models (GLM) in R 3.4.4 (R Core Team, 2018), where  
150 both predictors (diet conditioning and the proportion of *D. magna*) and their interaction were included  
151 without performing model selection. We fitted separate models for the following dependent variables:  
152 the number of *D. magna* and *Chironomus* larvae consumed, the number of approaches towards each  
153 prey type, the probability of attack, the probability of capture, handling time, and total prey biomass  
154 consumed. We chose the error distribution according to the properties of the response variable: we used  
155 the quasi-Poisson model for the numbers of events to account for overdispersion, quasi-binomial for ratios,  
156 and Gamma with log-link function for the biomass consumed and for handling time. In the analysis of  
157 handling time, we used generalised mixed effects models (GLMM) implemented in the lme4 package for  
158 R (Bates et al., 2015), because we had multiple observations per individual. Hence, we used predator  
159 identity as a random factor in a random intercept model. We tested the significance of the effect of diet  
160 conditioning on handling time using a likelihood-ratio test to compare a GLMM model with and without  
161 diet conditioning as a predictor.

### 162 **Testing the effect of diet on growth and mortality**

163 The second experiment aimed to test long-term effects of diet composition on survival, growth rate,  
164 developmental time and final body size in *S. sanguineum*. The experiment was carried out from May  
165 to July 2016. We collected 66 larvae of *S. sanguineum* (instars F-3 to F-1) in the same site as for the  
166 previous experiment. We reared the larvae in 200 ml plastic cups (diameter: 65 mm, height: 72 mm) filled  
167 by ca. 180 ml of aged tap water in a climatic chamber ( $20 \pm 1^\circ\text{C}$ , 16-h day : 8-h night).

168 We divided the larvae immediately after transfer from the field into three groups. One third of the  
169 individuals was fed ad libitum by *D. magna*, another third by *Chironomus* larvae, and the last third by a  
170 mixture of both prey types. Water in the cups was changed every 4-5 days and the larvae were checked for  
171 moulting and emergence daily. We measured body length and head width and weighed each individual 4  
172 days after each larval ecdysis. Growth rate was based on the change of body mass between instars F-1  
173 and F-0 under the assumption of linear growth. We used the duration of the last instar to characterize  
174 development rather than complete developmental time because the wild-caught individuals varied in the  
175 developmental stage at the beginning of the experiment. Because we knew from previous experiments that  
176 the development of the last instar at the experimental temperature takes ca. 20 days (Boukal & Peroutka,  
177 unpublished data), we put a wooden stick in each cup 10 days after the last ecdysis to enable larvae to  
178 climb out of the water before adult emergence. Subsequently, we recorded the weight, total body length,  
179 and head width in the adults measured the third day after emergence to shed excess water and clear their  
180 guts, while they were kept in 300 ml plastic boxes in the climatic chamber ( $20 \pm 1^\circ\text{C}$ , 16-h day : 8-h  
181 night).

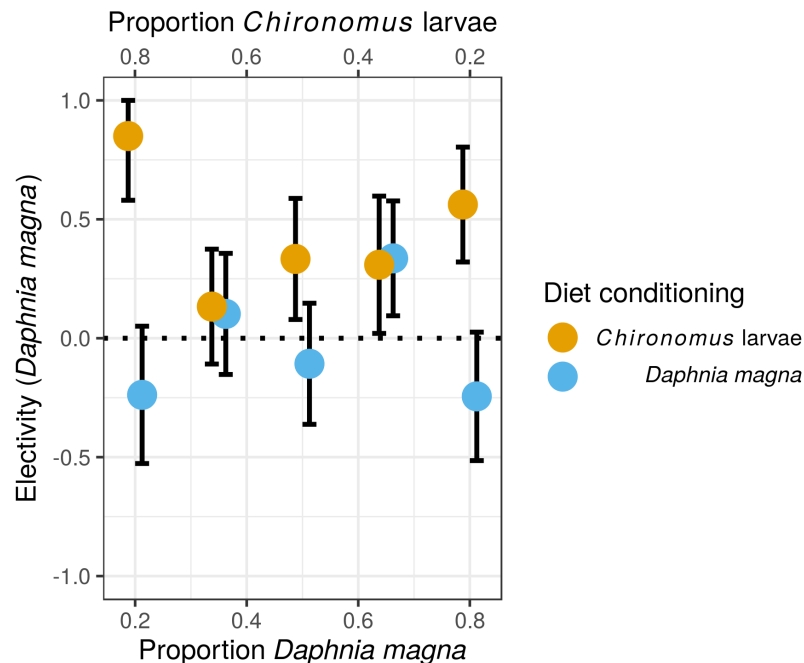
182 We tested the effect of the diet (only *D. magna*, only *Chironomus* larvae, and mixed diet) and sex on  
183 measures of growth and survival of the *S. sanguineum* larvae and traits of the adults using generalised  
184 linear models (GLM) in R 3.4.4 (R Core Team, 2018). We used a quasi-binomial model for mortality,  
185 success of the imaginal ecdysis, proportion of viable adults, and a GLM with Gamma error distribution  
186 and log link function for duration of the last instar, growth rate, and size and mass of larvae and adults.

## 187 **RESULTS**

### 188 **Prey selectivity**

189 Although we observed frequency-dependent food selection, i.e., the number of each prey type eaten  
190 increased with their increasing availability in the environment, there was no evidence for switching. The  
191 preference of *S. sanguineum* larvae for *D. magna* vs. *Chironomus* larvae based on Manly's  $\alpha$  selectivity  
192 index (Fig. 1) did not depend on the relative availability of the two prey types ( $F_{4,82} = 0.35$ ,  $P = 0.844$ ,  
193 prey availability used as a categorical variable), but was significantly affected by diet conditioning  
194 ( $F_{1,82} = 4.34$ ,  $P = 0.008$ ). The interaction of the two predictors was not statistically significant ( $F_{4,7} =$   
195  $1.65$ ,  $P = 0.170$ ). Larvae of *S. sanguineum* conditioned to *D. magna* consumed both prey types in  
196 agreement with their relative availability (no difference of the selectivity index from random expectation;  
197  $t_{42}$ ,  $P = 0.954$ ), while those conditioned to *Chironomus* larvae consumed significantly more *D. magna*  
198 and less *Chironomus* larvae than expected based on prey availability ( $t_{43}$ ,  $P = 0.0002$ ), see Fig. 1.

199 The number of *D. magna* consumed by *S. sanguineum* did not depend on diet conditioning (Fig. 2A,  
200  $F_{1,102} = 0.0046$ ,  $P = 0.946$ ), but *S. sanguineum* conditioned to *Chironomus* larvae consumed significantly  
201 less *Chironomus* in the experiment across all combinations of prey availability (Fig. 2B,  $F_{1,104} = 5.25$ ,  $P =$



**Figure 1.** Selectivity of larvae of *Sympetrum sanguineum* towards *Daphnia magna*. Values of electivity index  $>0$  indicate preference for *D. magna*, while values  $<0$  indicate avoidance of *D. magna*.

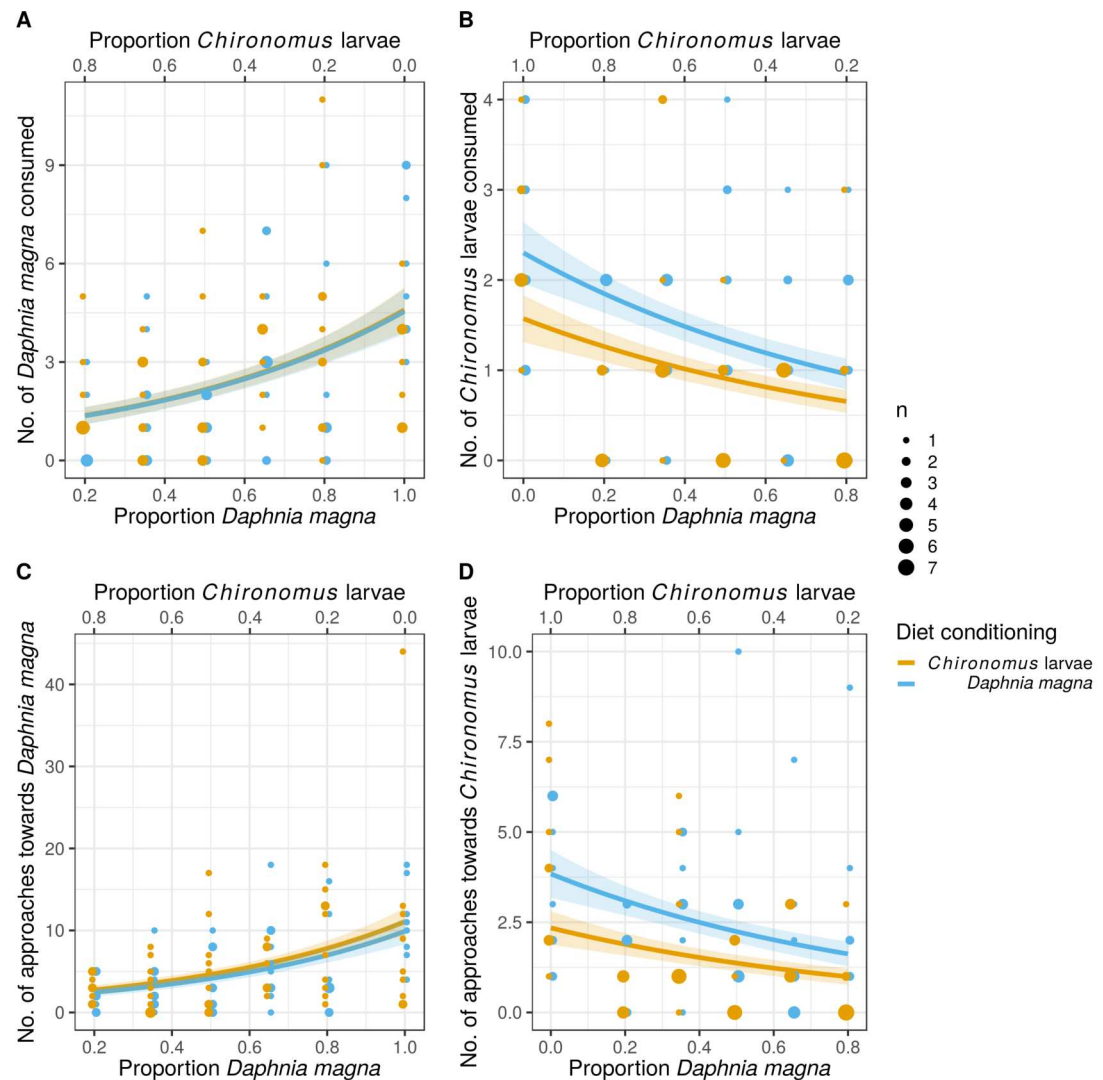
0.024). Analyses of individual steps of the predation sequence showed that this difference arose when the larvae approached towards prey. The number of approaches towards *D. magna* was independent of diet conditioning (Fig. 2C,  $F_{1,102} = 0.43$ ,  $P = 0.513$ ), while the number of approaches towards *Chironomus* larvae was significantly lower in *S. sanguineum* conditioned to *Chironomus* (Fig. 2D,  $F_{1,104} = 6.31$ ,  $P = 0.014$ ). The following steps of the predation sequence were not significantly affected by diet conditioning: probability of attacking *D. magna* ( $F_{1,89} = 2.04$ ,  $P = 0.157$ ) and *Chironomus* larvae ( $F_{1,77} = 0.03$ ,  $P = 0.855$ ), capture probability of *D. magna* ( $F_{1,87} = 2.67$ ,  $P = 0.106$ ) and *Chironomus* larvae ( $F_{1,74} = 2.14$ ,  $P = 0.148$ ), and handling time of *D. magna* ( $\chi^2_1 = 0.24$ ,  $P = 0.623$ ) and *Chironomus* larvae ( $\chi^2_1 = 1.15$ ,  $P = 0.285$ ).

### 211 The effects of diet on mortality and larval development of *Sympetrum sanguineum*

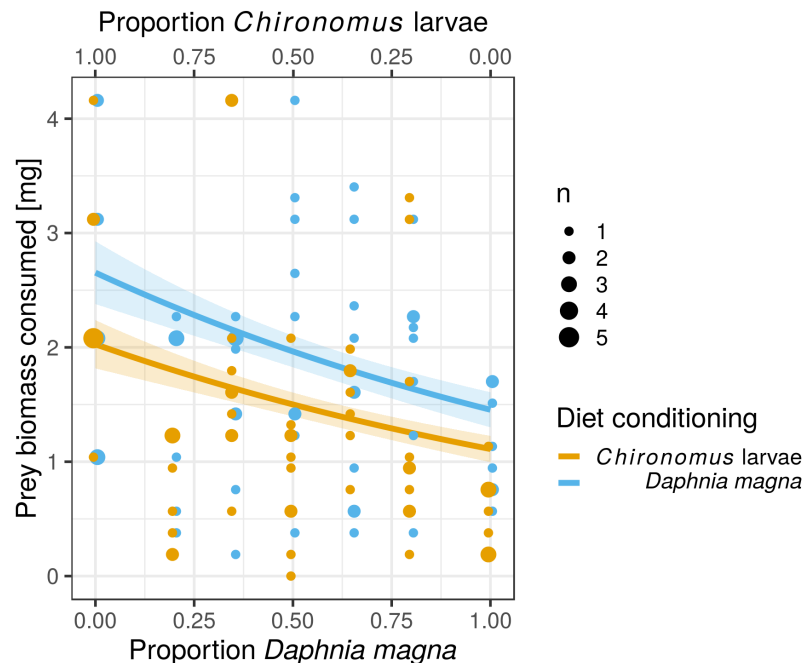
212 Out of the 66 individuals which entered the experiment (20-23 in each treatment), 53 individuals survived  
 213 until the imaginal ecdysis. Nine individuals died during the imaginal ecdysis, leaving 44 live adults.  
 214 However, only 38 individuals had no obvious morphological defects (remaining stuck in the larval exuviae,  
 215 creased wings, deformed legs). Mortality differed significantly between individuals reared on different  
 216 prey types (Fig. 4A,  $F_{2,63} = 3.33$ ,  $P = 0.042$ , with *Chironomus*-only diet leading to the highest mortality  
 217 (55%). The lowest mortality (17.4%) was found in individuals reared on the mixed diet (Fig. 4A). Data  
 218 on the probability of successful imaginal ecdysis (defined as the ratio of adults without visible defects and  
 219 capable of flight to the larvae entering the imaginal ecdysis) mirrored these results, with the lowest success  
 220 in individuals reared on *Chironomus*-only diet, and highest success in individuals reared on the mixed diet  
 221 (Fig. 4B,  $F_{2,50} = 5.74$ ,  $P = 0.006$ ). When larvae of *S. sanguineum* were reared on *Chironomus* larvae  
 222 only, 12 of the initial 20 larvae reached the imaginal ecdysis, during which 3 died and 4 other individuals  
 223 suffered severe defects, leaving only 5 viable adults capable of flight (i.e., only 25% of the initial number  
 224 of larvae). Survival to viable adult stage of larvae reared on *D. magna*-only diet was 2.4-times better,  
 225 resulting in 14 viable adults out of 23 larvae (i.e., 61%). Individuals reared on the mixed diet performed  
 226 best, with 19 viable adults out of the initial 23 larvae (i.e. 83%). These differences in the proportions of  
 227 viable adults were highly significant (Fig. 4C,  $F_{2,63} = 7.37$ ,  $P = 0.0013$ ).

228 We also observed differences between diets in the duration of the last instar (Fig. 4D,  $F_{2,49} =$   
 229 31.98,  $P < 0.0001$ ), which lasted on average 19 days in larvae fed the mixed diet, one day longer in larvae





**Figure 2. Predation on *Daphnia magna* and *Chironomus* larvae by larvae of *Sympetrum sanguineum*.** Number of *D. magna* in the diet increased (A) and number of *Chironomus* larvae decreased (B) with increasing proportion of *D. magna* in the environment. Number of *Chironomus* larvae consumed was significantly lower in the larvae of *S. sanguineum* conditioned to *Chironomus* larvae prior to the experiment compared to those conditioned to *D. magna* (B). The number of approaches towards *D. magna* was independent of diet conditioning (C), while the number of approaches towards *Chironomus* larvae was lower in predators conditioned to them. (D). The size of the points is proportional to the number of observations with the same x- and y- values. The coloured bands denote the standard error of the predicted values.



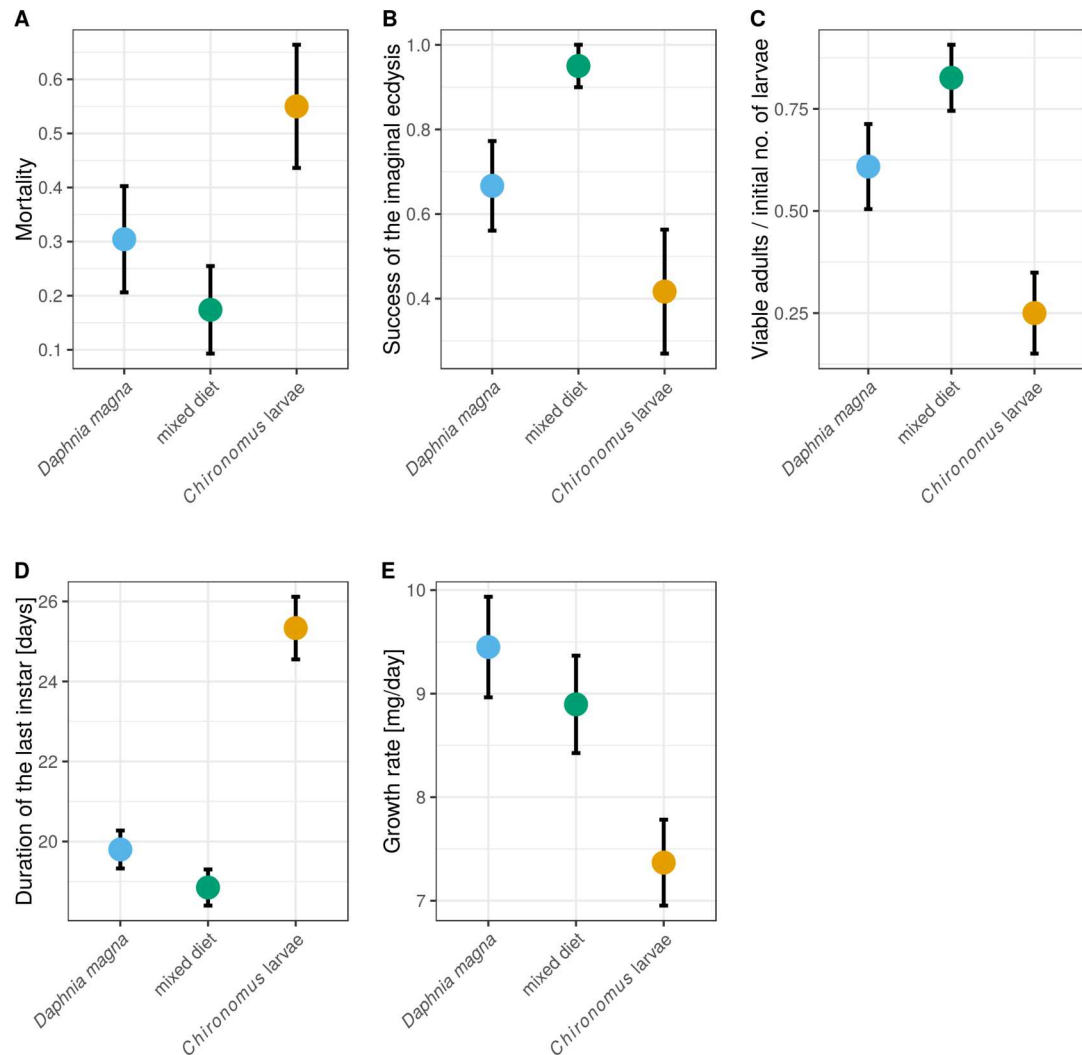
**Figure 3.** Total biomass of prey consumed was lower in larvae of *Sympetrum sanguineum* conditioned to *Chironomus* larvae prior to the experiment. The estimate of the total biomass consumed is based on dry body mass of *D. magna* and *Chironomus* larvae with the assumption that prey was completely consumed. Legend as in Fig. 2.

230 fed *D. magna*, and four more days longer in larvae fed *Chironomus* larvae. Growth rate was also affected  
 231 by diet (Fig. 4E,  $F_{2,47} = 5.52$ ,  $p = 0.007$ ) and was lowest in *S. sanguineum* larvae fed only *Chironomus*.

232 Larval diet affected adult size (Fig. 5) although it did not significantly affect body size of F-0 larvae  
 233 despite those fed a mixed diet being slightly heavier (Fig. 5A,  $F_{2,62} = 2.18$ ,  $P = 0.122$ ). Similarly,  
 234 differences in larval body length and head width between the three groups of F-0 larvae were not  
 235 significant (Fig. 5B-C, body length:  $F_{2,62} = 0.99$ ,  $P = 0.378$ , head width:  $F_{2,62} = 0.99$ ,  $P = 0.377$ ).  
 236 Adult weight depended on larval diet (Fig. 5D,  $F_{2,37} = 3.40$ ,  $P = 0.044$ ) but did not differ between  
 237 sexes ( $F_{1,35} = 0.58$ ,  $P = 0.451$ ). Adults from larvae fed only *D. magna* or only *Chironomus* had very  
 238 similar weights, while adults from the mixed diet treatment were heavier. Body length and head width was  
 239 also affected by larval diet (length:  $F_{2,35} = 20.63$ ,  $P < 0.0001$ , head width:  $F_{2,34} = 12.32$ ,  $P < 0.0001$ )  
 240 but did not differ between sexes (length:  $F_{1,33} = 2.15$ ,  $P = 0.152$ , head width:  $F_{1,32} = 0.82$ ,  $P = 0.373$ ).  
 241 Body lengths and head widths of adults from *D. magna*-only and mixed diet treatments were similar,  
 242 while adults emerging from larvae fed only *Chironomus* were ca. 15% shorter and had slightly smaller  
 243 head widths (Fig. 5E-F).

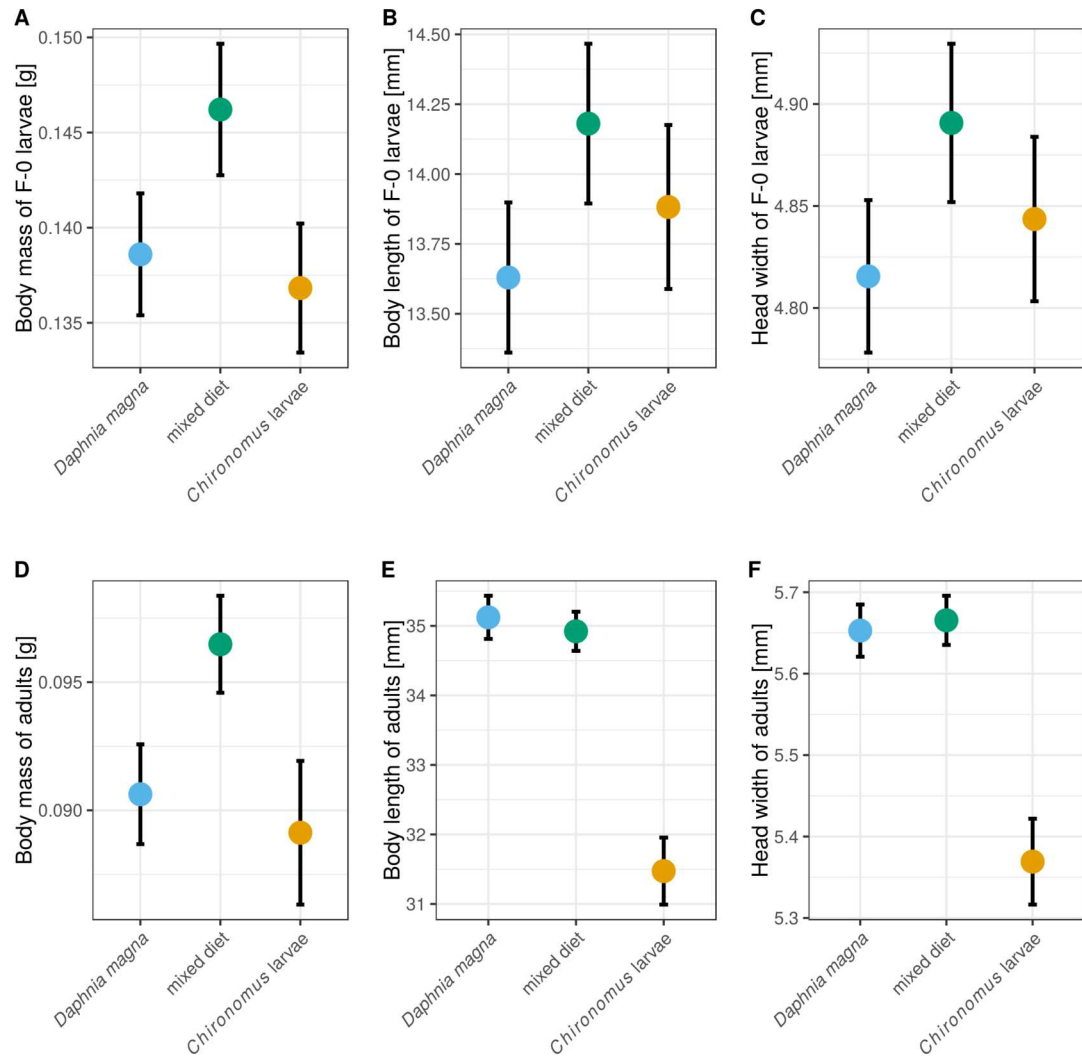
## 244 DISCUSSION

245 Our experiments showed that prey identity has important consequences on the effects of previous  
 246 diet on predator's feeding preferences, its growth, and survival. We found no evidence for selective  
 247 feeding in *S. sanguineum* dragonfly larvae conditioned to *D. magna* as the larvae consumed *D. magna*  
 248 and *Chironomus* larvae proportionally to their availability in the experiment, while *S. sanguineum*  
 249 conditioned to *Chironomus* consistently preferred *D. magna*. Our previous evidence for *S. sanguineum*  
 250 feeding preferences were equivocal: the larvae preferred cladocerans over *Chironomus* larvae and  
 251 other alternative prey in one experiment (Klecka and Boukal, 2014), while they fed preferentially on  
 252 mosquito and *Chironomus* larvae in a different multiple-choice experiment (Klecka and Boukal, 2012),  
 253 which demonstrates that prey selectivity is context-dependent. The avoidance of *Chironomus* larvae by  
 254 dragonflies conditioned to them is in line with the poor long-term performance of dragonflies reared on  
 255 *Chironomus*-only diet. However, *S. sanguineum* reared on mixed diet performed the best in terms of



**Figure 4. Mortality and larval development of *Sympetrum sanguineum* depended on diet.**

Mortality refers to the entire period the larvae spent in the experiment until reaching adulthood. The success of the imaginal ecdysis means that the larvae entering the imaginal ecdysis emerged as a viable adult capable of flight with no apparent morphological defects. Mean and standard error of the fitted values are plotted.



**Figure 5.** The effect of larval diet on the size of last-instar larvae and adults of *Sympetrum sanguineum*. Mean and standard error of the fitted values is plotted.

256 growth and survival until adulthood in the long-term experiment. Broader diet thus apparently benefits  
257 this predator, as reported in multiple other species of consumers (Lefcheck et al., 2013).

### 258 **What determines predator diet and prey selectivity?**

259 Although prey size is an important predictor of the diet of predators (Woodward and Hildrew, 2002;  
260 Brose et al., 2006; Riede et al., 2011; Klecka and Boukal, 2013; Boukal, 2014), it does not explain all  
261 variation in prey choice. Under a purely size-dependent optimal foraging perspective, one would predict  
262 that *S. sanguineum* dragonfly larvae should prefer *Chironomus* larvae that were ca. 5.5-times heavier  
263 but had only 2.3-times longer handling time than *D. magna*. This shows that other factors affecting the  
264 energetic value of a prey, such as the proportion of digestible tissue relative to total body mass or prey  
265 defence mechanisms (Woodward and Warren, 2007), affect prey choice in *S. sanguineum* larvae. Prey  
266 selectivity may also depend on the interplay between predator's foraging mode and prey mobility (Allan  
267 et al., 1987; Sih and Christensen, 2001; Woodward and Hildrew, 2002; Klecka and Boukal, 2012, 2013)  
268 and microhabitat use of both prey and predators (Woodward and Hildrew, 2002; Klecka and Boukal, 2012,  
269 2014). However, we performed our experiments in a very simple environment with a limited possibility  
270 for these factors to affect the outcomes, although they may be important in natural habitats.

271 We performed the experiments on prey selectivity with seven different abundance ratios of the two  
272 prey types to also evaluate the effect of relative prey availability on prey selectivity of *S. sanguineum*  
273 larvae. Switching between different prey types based on their relative abundances, specifically preference  
274 of the prey type which happens to be more abundant, was observed in some studies (Lawton et al., 1974;  
275 Sherratt and Harvey, 1993). However, we found no evidence of prey switching in our experiment as the  
276 strength of prey preference did not change significantly with relative abundance of the two prey types  
277 (Fig. 1).

278 We expected that predators conditioned to one prey type would either preferentially consume the  
279 same prey in the experiment because of an increased detection or capture efficiency (Bergelson, 1985),  
280 or that they would prefer the other prey type to compensate for potential nutritional imbalance caused  
281 by a prolonged consumption of a single prey type (Karimi and Folt, 2006). Little is known about the  
282 ability of dragonfly larvae to learn to capture specific prey or form a search image (Tinbergen, 1960).  
283 Bergelson (1985) performed experiments on learning in the larvae of *Anax junius* (Odonata: Aeschnidae).  
284 They found that conditioning to a single prey type led to increased probability of successful capture and  
285 decreased handling time, and successful capture reinforced the probability of later attacks on the same  
286 prey type. However, there was no indication of a search image formation, i.e., no effect of previous  
287 experience on the probability of orientation towards prey (Bergelson, 1985).

288 In our experiment, diet conditioning did not affect the attack and capture probabilities or the handling  
289 time, and we found no evidence for the effect of learning on foraging efficiency except an avoidance of  
290 *Chironomus* larvae, manifested as lower number of approaches towards this prey and lower number of  
291 *Chironomus* larvae consumed by *S. sanguineum* conditioned to this prey. This, together with the results of  
292 the second long-term rearing experiment, hints at possible compensation for nutritional imbalance caused  
293 by *Chironomus*-only diet during the three-day conditioning period (Elser et al., 2000; Fagan et al., 2002;  
294 Mayntz et al., 2005) and is comparable to earlier findings of unpalatable prey avoidance in larvae of the  
295 coenagrionid damselfly *Xanthocnemis zealandica* (Rowe, 1994). One limitation of our data is that we  
296 cannot estimate the encounter rate, because the predators generally remained motionless until the prey  
297 came very close and the first indication that the predator detected the prey was that it moved towards  
298 the prey, which we interpret as an approach towards the prey rather than an encounter. We thus rely on  
299 comparing the number of approaches towards different prey types rather than on estimating the probability  
300 of an approach upon encounter, but consider the conclusions valid.

### 301 **Does prey selectivity feed back on individual fitness?**

302 Our short-term selectivity experiment and long-term rearing experiment together indicate that long-term  
303 fitness consequences rather than short-term energy gains may underlie prey selectivity in larval dragonflies.  
304 We observed pronounced long-term effects of diet composition on mortality, growth, and adult size in *S.*  
305 *sanguineum*. The results are broadly in agreement with the prey selectivity experiment that a *Chironomus*-  
306 only diet may not be suitable for *S. sanguineum*. Multiple fitness components were affected by the diet.  
307 Larvae of *S. sanguineum* fed only *Chironomus* had lower survival and growth rate, and lower success  
308 of the imaginal ecdysis than those fed only *D. magna* or a mixed diet. Differences in the initial size  
309 of the last-instar larvae were subtle, but the morphology of the adults emerging from larvae reared on

310 *Chironomus*-only diet was significantly altered: they were shorter and had smaller head width compared  
311 to individuals reared on *D. magna* or mixed diet, although the difference in body masses were small.  
312 Interestingly, Hottenbacher and Koch (2006) also reported that larvae of the congeneric *Sympetrum*  
313 *striolatum* reared on *Chironomus* larvae reached smaller size, measured as head width, compared to  
314 larvae reared on *Artemia salina*, which is not their natural prey. This implies that our results may be valid  
315 also for other zooplankton groups and that substantial effects of the diet may be accrued within a single  
316 developmental stage.

317 The most likely explanation of the multiple negative effects of *Chironomus*-only diet on the growth  
318 and development of *S. sanguineum* larvae is based on stoichiometry, or prey quality in a broader sense,  
319 given that the predators were fed *ad libitum* and had high capture success rate and no apparent difficulties  
320 in handling either prey type. Although both Chironomidae and Daphniidae are widely used to feed  
321 predators in the laboratory, little is known about their exact impact on growth and development of insects  
322 predators. *Daphnia* have a high phosphorus content compared to other aquatic invertebrates, which  
323 may be significant since phosphorus is often a limiting element for animal growth. Elser et al. (2000)  
324 reported an average C:N:P ratio of 93:16:1 in *D. magna*, which is almost twice as rich in phosphorus than  
325 *Chironomus* larvae with an average C:N:P ratio of 166:28:1 (Liess and Hillebrand, 2005), which could  
326 explain our results (Sterner, 1997). We lack comparable data for the dragonflies and do not know their  
327 nutritional demands, but predators generally have different elemental composition than herbivores, such  
328 that prey stoichiometry can affect the predator performance (Fagan et al., 2002).

329 We also found small but consistent evidence that individuals of *S. sanguineum* performed best on a  
330 mixed diet rather than *D. magna*-only diet. This is line with our expectations as dragonfly larvae tend  
331 to have broad diets (Klecka and Boukal, 2012). In general, very few predators are strictly specialised,  
332 and most benefit from nutritional diversity in their diet (Lefcheck et al., 2013). Many studies, albeit  
333 not on odonates, showed positive effects of prey diversity on growth, survival, and reproduction of  
334 other predatory invertebrates such as spiders, beetles and mites (Oelbermann and Scheu, 2002; Harwood  
335 et al., 2009; Muñoz-Cárdenas et al., 2014; Marques et al., 2015). The positive effect of diet diversity  
336 may, however, be reversed when the prey mixture contains toxic or very low-quality prey (Oelbermann  
337 and Scheu, 2002; Lefcheck et al., 2013). The superiority of the mixed diet in our study suggests that  
338 *Chironomus* larvae are not directly toxic for *S. sanguineum* larvae or that any costs of potential toxicity  
339 are outweighed by the benefits of mixed diet.

## 340 Conclusions

341 In conclusion, we found that larval diet can significantly affect foraging behaviour, survival and growth of  
342 dragonfly larvae and body size of the emerged adults. The combined evidence from our two experiments  
343 shows that *Chironomus* larvae are lower-quality prey for *S. sanguineum* than *D. magna*. Surprisingly, the  
344 effects of diet conditioning on dragonfly foraging behaviour were limited to the avoidance of the inferior  
345 prey after previous exposure to it, which suggests some but limited role of learning in their foraging.  
346 Detailed nutritional demands and the underlying role of stoichiometry in predator-prey interactions in  
347 aquatic invertebrates are very little understood, especially in contrast to the many detailed studies on  
348 herbivorous zooplankton (Sterner and Schulz, 1998; Elser et al., 2000; Hessen, 1990; DeMott, 1998;  
349 Vrede et al., 1999). Our study shows the merit of combining short-term studies on prey selectivity  
350 with long-term rearing experiments. We suggest that more detailed insights into nutritional demands  
351 of predators and stoichiometry of their prey could provide a better mechanistic understanding of prey  
352 selectivity in predatory invertebrates.

## 353 ACKNOWLEDGEMENTS

354 The work of DSB was supported by University of South Bohemia, Faculty of Science, Department  
355 of Ecosystem Biology & Soil and Water Research Infrastructure (MEYS; projects LM2015075 and  
356 EF16\_013/0001782 – SoWa Ecosystems Research).

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