

1 **Dissociation between seasonal prey availability and prey consumption in a generalist**  
2 **predator**

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4 Prey selectivity in generalist predator

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26 **Abstract**

27 Generalist predators are capable of selective foraging, but are predicted to feed in close  
28 proportion to prey availability to maximize energetic intake especially when overall prey  
29 availability is low. By extension, they are also expected feed in a more frequency-dependent  
30 manner during winter compared to the more favorable foraging conditions experienced during  
31 spring, summer and fall seasons. For 18 months, we observed the foraging patterns of forest-  
32 dwelling wolf spiders from the genus *Schizocosa* (Araneae: Lycosidae) using PCR-based gut-  
33 content analysis and simultaneously monitored the activity-densities of two common prey:  
34 springtails (Collembola) and flies (Diptera). Rates of prey detection within spider guts relative to  
35 rates of prey collected in traps were estimated using Roualdes'  $c_{st}$  model and compared using  
36 various linear contrasts to make inferences pertaining to seasonal prey selectivity. Results  
37 indicated spiders foraged selectively over the course of the study, contrary to predictions derived  
38 from optimal foraging theory. Even during winter, with overall low prey densities, the relative  
39 rates of predation compared to available prey differed significantly over time and by prey group.  
40 Moreover, these spiders appeared to diversify their diets; the least abundant prey group was  
41 consistently overrepresented in the diet within a given season. We suggest that foraging in  
42 generalist predators is not necessarily restricted to frequency-dependency during winter. In fact,  
43 foraging motives other than energy maximization, such as a more nutrient-focused strategy, may  
44 also be optimal for generalist predators during prey-scarce winters.

45

46 **Keywords**

47 Molecular gut-content analysis, prey selectivity, *Schizocosa*, optimal foraging, generalist  
48 predators, predator-prey interactions

49 **Introduction**

50           Generalist predators are able to consume multiple prey types, allowing them to maintain  
51 caloric intake when prey populations vary over time. A simple extension from this fact is a core  
52 assumption of optimal foraging theory: generalist predators choose prey to best maximize capture  
53 rate (Charnov, 1976, Stephens and Krebs, 1986). They were traditionally thought to only feed in a  
54 frequency-dependent manner, consuming particular prey in proportion with which they are  
55 encountered in the wild. Energetic needs are essential to meet, but oftentimes a foraging strategy  
56 that considers more than caloric intake can be optimal (Machovsky-Capuska et al., 2016,  
57 Remonti et al., 2016). Inherent prey preferences based on size (O'Brien et al., 2005), ease of  
58 capture (Ellis et al., 2012) and/or nutritional profile (Kohl et al., 2015) can cause predators to  
59 deviate from a frequency-dependent prey selection strategy.

60           Due to elevated hunger levels, generalist predators more reliably employ a frequency-  
61 dependent foraging strategy at low prey densities (Baudrot et al., 2016). Constraints on number of  
62 prey encounters should increase the similarity between prey in the environment and prey in the  
63 diet. Generalist predators are thus assumed to forage less selectively in winters, compared to the  
64 more prey-rich spring, summer and autumn months (Perry and Pinaka, 1997). Warming  
65 temperatures can increase prey encounters and predation rates (Sentis et al., 2014), but with  
66 reduced metabolic requirements at low temperatures, it remains unclear whether the risk of  
67 starvation is significantly increased during winter (Sentis et al., 2015). Therefore, the assumption  
68 that generalist predators employ a frequency-dependent foraging strategy during prey-scarce  
69 winters warrants further investigation.

70           Characterizing seasonal shifts in selectivity of wild predators poses challenges, requiring  
71 the regular monitoring of both prey availability (a product of prey density and activity) and prey

72 consumption of predators over the course of the year. Modern molecular techniques, such as  
73 PCR-based molecular gut-content analysis, overcome the practical issues of observing predation  
74 events in the field and are now commonplace in studies of trophic interactions (reviewed in  
75 Symondson, 2002, Sheppard and Harwood, 2005, Traugott et al., 2013). When applying these  
76 data to assess seasonal selectivity, however, the available statistical options are not ideal  
77 (Roualdes et al., 2016). Many older prey selectivity indices have several problems and limitations  
78 (summarized in Lechowicz, 1982, Manly et al., 2002), such as lacking statistical rigor, being  
79 limited to single prey species and time points (e.g. Ivlev, 1961, Jacobs, 1974, Strauss, 1979), or  
80 necessitating total prey densities to be known (e.g. Manly et al., 1972). Newer Monte Carlo based  
81 methods (e.g. Agustí et al., 2003) also cannot account for multiple prey over time. If applied to  
82 the unobserved count data that molecular gut-content analysis provides, to assess seasonal  
83 changes in prey selection, the available selectivity indices would either be statistically  
84 unsatisfactory or would be erroneous. In this study, we used Roualdes'  $c_{st}$  (described in detail in  
85 Roualdes et al., 2016) to test for deviations between prey availability and prey consumption in a  
86 generalist arthropod predator-prey system. This model combines the intuitive nature of selectivity  
87 indices with statistical modeling, yielding parameter estimates for presence/absence prey data,  
88 accounting for multiple prey groups over multiple time points, and allowing for statistically  
89 justified, formal hypothesis testing.

90         We used spiders in our examination of seasonal foraging selectivity, because they are  
91 abundant and widespread generalist predators able to significantly affect prey populations in  
92 terrestrial ecosystems (Finke and Denno, 2004). They are considered food-limited in nature (Wise  
93 1995), suggesting that prey consumption occurs on an opportunistic basis to maximize energetic  
94 intake (Nentwig, 1982, Riechert, 1991). Some are also winter-active (Korenko et al., 2010, Pekár

95 et al., 2015), continuing to hunt while in a supercooled state (Aitchison, 1987). Prey selectivity of  
96 predators, and more specifically spiders, is extremely difficult to infer in nature, so we used PCR-  
97 based molecular gut-content analysis to characterize the temporal consumption patterns of two  
98 common spider prey taxa: springtails (Collembola) and flies (Diptera).

99         We aimed to test the hypothesis that if generalist predators primarily feed in a frequency-  
100 dependent manner throughout the year, optimizing energy intake, then the proportion of  
101 Collembola and Diptera in the spider population's diet will closely reflect proportional  
102 Collembola and Diptera availability in the environment, as indicated by Roualdes'  $c_{st}$ . Especially  
103 in winter when low temperatures cause prey activity-densities (and thus predator-prey encounter  
104 rates) to decrease, prey consumption patterns will be expected to more closely reflect  
105 proportional prey availability. Conversely, significant deviations between the proportion of prey  
106 in the environment and the predator population's diet will occur if spiders are foraging selectively  
107 in nature, perhaps to attain certain nutrients, as laboratory studies have shown to occur in multiple  
108 taxa including mammals (Jensen et al., 2014), fish (Rubio et al., 2009) and invertebrates (Mayntz  
109 et al., 2005). The integration of ecological, molecular and mathematical approaches in this  
110 research demonstrates the use a novel and powerful toolset to assess seasonal prey selectivity  
111 within a natural system.

112

## 113 **Methods**

### 114 *Collection and monitoring*

115         We focused on two congeners, *Schizocosa saltatrix* (Hentz) and *S. stridulans* Stratton  
116 (Araneae: Lycosidae), which co-occur in deciduous leaf litter habitats throughout the Nearctic  
117 (Dondale and Redner, 1978, Stratton, 1991). All specimens were collected between October 2011

118 and March 2013 from Berea College Forest in Madison County, Kentucky, USA (37°34'22"N,  
119 64°13'11"W, elevation ~ 268 m), a mixed deciduous forest consisting of oak, maple, hickory and  
120 scattered pine. Individuals were treated as a single *Schizocosa* spp. Population due to similarity in  
121 ecology and behavior. Three HOBO Pro v2 data loggers (Onset, Cape Cod, Massachusetts, USA)  
122 were placed 1 cm above the soil surface to monitor air temperature in the vicinity of spider  
123 microhabitats throughout the collection period.

124 Activity-density of the leaf litter invertebrate community was monitored with pitfall traps  
125 containing ethylene glycol (n = 32 arranged in two 2x8 m grids and each trap separated by ~ 10  
126 m). Traps were left open for a 6 – 12 day sample period in the middle of each month, and prey  
127 availability was inferred by calculating activity-density per day. Invertebrates were identified to  
128 the lowest taxonomic group possible and were either deemed as potential prey or non-prey for  
129 *Schizocosa* (Supplementary material Appendix 1 Table A1). Determination of potential prey was  
130 based on previous records of prey acceptability and size criteria; cursorial spiders typically  
131 consume invertebrates with a predator:prey body size ratio  $\geq 1$  (Nentwig and Wissel, 1986,  
132 Moya-Laraño and Wise, 2006) and avoid hard-bodied prey (Öberg et al., 2011). Tomocerid and  
133 entomobryid collembolans were categorized as potential prey, but isotomids, sminthurids and  
134 hypogastrurids were excluded because they were too small to be consumed by *Schizocosa*  
135 (Whitney pers. obs.).

136

### 137 *DNA extraction and PCR protocols*

138 For molecular gut-content analysis, 10 to 40 spiders were aspirated every 6-12 days from  
139 litter within the vicinity of the pitfall traps, at least 10 m away from any one trap. Specimens,  
140 which included adult and juvenile *Schizocosa*, were immediately placed in separate 1.5 mL

141 microcentrifuge tubes containing 95% EtOH and preserved at -20 °C until DNA extraction. All  
142 DNA extractions utilized Qiagen DNEasy® Tissue Extraction Kit (Qiagen Inc., Chatsworth,  
143 California, USA) following the manufacturer's animal tissue protocol. Whole bodies of juvenile  
144 spiders were used, but for larger adult spiders, leg segments below the coxae were removed to  
145 increase the prey:predator DNA ratio.

146 Predation of Collembola and Diptera, the two most abundant prey groups, was  
147 characterized using order-specific primers and PCR-based molecular gut-content analysis.  
148 Collembola-specific 18S primers, Col3F (5'-GGA CGA TYT TRT TRG TTC GT-3') and Col-  
149 gen-A246 (5'-TTT CAC CTC TAA CGT CGC AG-3') (Sint et al., 2012), produced a 228bp  
150 amplicon. PCR reactions (12.5 µL) consisted of 1x Takara buffer (Takara Bio Inc., Shiga, Japan),  
151 0.2 mM dNTPs, 0.2 µM each primer, 0.625 U Takara *Ex Taq*<sup>TM</sup> and 1.5 µL template DNA.  
152 BioRad PTC-200 and C1000 thermal cyclers (Bio-Rad Laboratories, Hercules, CA, USA) were  
153 used for PCR under the following cycling conditions: 95°C for 60 s followed by 35 cycles of  
154 94°C for 30 s, 61.2°C for 90 s, and 72°C for 60 s. Diptera-specific 18S primers, DIP-A17 (5'-  
155 TTY ATG TGA ACA GTT TCA GTY CA-3') and DIP-S16 (5'-CAC TTG CTT CTT AAA TRG  
156 ACA AAT T-3') (Eitzinger et al., 2013), produced a 198bp amplicon. PCR reactions (12.5 µL)  
157 utilized 2 µL template DNA and the same reagents described above under the following cycling  
158 conditions: 95°C for 60 s followed by 40 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 45  
159 s. Amplicons were visualized using 2% SeaKem® LE agarose (Lonza Group Ltd., Rockland,  
160 Maine, USA) stained with GelRed<sup>TM</sup> nucleic acid stain (1X; Biotium Inc., Hayward, California,  
161 USA), which revealed whether Collembola and/or Diptera DNA was present within each  
162 predator. Both primer pairs were tested for specificity against 93 non-target specimens  
163 (Supplementary material Appendix 1 Table A2).



164

165 *DNA detection time*

166 To estimate the effect of temperature on the detectability of prey DNA within *Schizocosa*,  
167 spiders were hand-collected for laboratory predation assays. We mitigated potential  
168 complications due to physiological acclimation (Sentis et al., 2015) by collecting spiders during  
169 the summer and winter for high (incubated at 25°C) and low (incubated at 5°C) temperature  
170 assays, respectively. Each spider was fed a single *Sinella curviseta* Brook (Collembola:  
171 Entomobryidae) and then starved for seven days. Afterward, high temperature spiders (n = 9) and  
172 low temperature spiders (n = 10) were fed a single *Drosophila melanogaster* (Meigen) (Diptera:  
173 Drosophilidae) and were preserved in 95% ethanol after 48h. As a control, a group of high  
174 temperature spiders (n = 3) and low temperature spiders (n = 9) were not fed after the starvation  
175 period and were immediately preserved in 95% ethanol. Diptera DNA was detected using the  
176 protocols described above.

177

178 *Analysis of prey selectivity*

179 Prey selectivity was analyzed with Roualdes'  $c_{st}$  using the R (Core Team R 2014) package  
180 *spiders* (Roualdes et al., 2016). First, the model uses the binary data from molecular gut-content  
181 analysis and the count data from pitfall traps to create maximum likelihood estimates for the rate  
182 of prey capture. Second, the relative rates at which traps collect and predators test positive for  
183 certain prey are then compared using a likelihood ratio test. In brief, we let  $X_{jst}$  denote the number  
184 of prey groups  $s \in \{1, \dots, S\}$  that predator  $j \in \{1, \dots, J_t\}$  ate during time period  $t \in \{1, \dots,$   
185  $T\}$ .  $X_{jst}$  is assumed to follow a Poisson distribution with rate parameter  $\lambda_{st}$ ,  $X_{jst} \sim_{iid} P(\lambda_{st})$ , where  
186  $\lambda_{st}$  represents the rate at which predator  $j$  ate prey group  $s$  during time period  $t$ . Next, we let  $Y_{ist}$

187 represent the number of prey groups  $s$  captured within trap  $i \in \{1, \dots, I_t\}$  during time period  $t$ .  
188  $Y_{ist}$  also is assumed to follow a Poisson distribution with rate parameter  $\gamma_{st}$ , where  $\gamma_{st}$  represents  
189 the rate at which prey group  $s$  is randomly encountered in the environment during time period  $t$ ,  
190  $Y_{ist} \sim_{iid} P(\gamma_{st})$ .

191 The relative magnitudes of parameters  $\lambda_{st}$  and  $\gamma_{st}$  can be statistically interpreted to test  
192 hypotheses of predator feeding preferences. Specifically, four hypotheses on the relative  
193 magnitude of  $c_{st} = \lambda_{st}/\gamma_{st}$  can be tested. They are as follows: (1)  $c_{st} = c$  states that relative rates of  
194 detected prey within predators and sampled prey within the traps are equal for all species on all  
195 occasions (consistent with frequency-dependent foraging), (2)  $c_{st} = c_s$  states that predators  
196 consume prey groups at different rates, but those rates remain constant over time (i.e. prey  
197 preference exists, but there is no seasonal response), (3)  $c_{st} = c_t$  states that predators consume prey  
198 groups at similar rates within each time period, but rates across time vary (i.e. no prey preference,  
199 but changes in the amount of prey consumed, or trap efficiency, or both, occur over time), and (4)  
200  $c_{st} = c_{st}$  states that predation varies by time and prey group (consistent with selective foraging).

201 To more easily reference these models, we name them by the type of foraging they represent: (1)  
202 frequency-dependent, (2) species-dependent, (3) time-dependent, and (4) selective foraging.

203 These hypotheses are nested, so we used sequential likelihood ratio tests in order of most  
204 (selective foraging:  $c_{st} = c_{st}$ ) to least (frequency-dependent:  $c_{st} = c$ ) complex as in Roualdes et al.  
205 (2016).

206 To assess how prey selectivity shifts between groups and across time in greater detail, we  
207 used point estimates under the model  $c_{st} = \lambda_{st}/\gamma_{st}$  to make several linear contrasts. First, we tested  
208 the hypotheses  $c_{collembola,t} - c_{diptera,t} = 0$ , which states spiders equally prefer Collembola and Diptera  
209 during each of the 18 months throughout the study. We used the Bonferroni multiple comparisons

210 correction using a pre-correction significance level of 0.05 to determine if the prey were  
211 differentially preferred during each given month. To make comparisons in prey preference  
212 between seasons, we performed a series of linear contrasts where  $c_{st}$  estimates were averaged  
213 across certain months (“winter” = December to February, “non-winter” = March to November).  
214 Contrasts were made to (1) compare predation of prey group  $s$  in winter vs. non-winter and winter  
215 2011 vs. winter 2012 and (2) to compare predation within each season  $t$  of Collembola vs.  
216 Diptera.

217

## 218 **Results**

### 219 *Prey availability*

220 Over the duration of the study, temperatures varied in a manner characteristic of a  
221 temperate forest (Supplementary material Appendix 1 Fig. A1). Total prey availability was  
222 positively associated with temperature ( $R^2 = 0.56$ ,  $F_{1,17} = 20.29$ ,  $P = 0.0004$ ) with the greatest  
223 number of prey captured between May and August 2012 (Fig. 1). Collembolans were the most  
224 abundant prey in almost every season, accounting for 56% of total individuals caught in traps,  
225 and their availability increased with temperature ( $R^2 = 0.40$ ,  $F_{1,17} = 10.44$ ,  $P = 0.005$ ). Dipterans  
226 were the second most abundant, representing 17% of total prey captured, and outnumbered  
227 collembolans in winter 2011, mainly due to a pulse in *Trichocera* sp. (Diptera: Trichoceridae).  
228 Unlike collembolans, the availability of dipterans was not associated with temperature ( $R^2 = 0.09$ ,  
229  $F_{1,17} = 1.56$ ,  $P = 0.23$ ).

230

### 231 *Predation*

232 A total of 1,231 spiders were collected for molecular gut-content analysis. Collembola  
233 were the most frequently detected prey, with 44% of total spiders screening positive for  
234 Collembola DNA. Monthly detection varied considerably over the study duration (Fig. 2a) but  
235 was not associated with temperature ( $R^2 = 0.09$ ,  $F_{1,17} = 2.92$ ,  $P = 0.11$ ). Diptera were detected in  
236 33% of total spiders screened and remained relatively stable throughout the study despite changes  
237 in temperature ( $R^2 = 0.27$ ,  $F_{1,17} = 1.48$ ,  $P = 0.24$ ) and proportional availability (Fig. 2b).  
238 Approximately 42% of individuals did not screen positive for either Collembola or Diptera DNA  
239 and 18% screened positive for both prey types. There was a negative correlation between  
240 individuals positive for both prey and temperature ( $R^2 = 0.24$ ,  $F_{1,17} = 5.16$ ,  $P = 0.04$ ).

241 In the DNA detectability assay at varying temperatures, 33% of *Schizocosa* tested positive  
242 for Diptera DNA after 48h at 25°C but more spiders (50%) screened positive in the 5°C treatment  
243 after 48h. No control spiders tested positive for prey DNA at either temperature.

244

#### 245 *Selectivity analyses*

246 The more parameter rich selective foraging model,  $c_{st} = \lambda_{st} / \gamma_{st}$ , fit the data better than  
247 expected by chance when tested against the following two simpler models: species-dependent ( $H_0$ :  
248  $c_s$  versus  $H_1$ :  $c_{st}$ ,  $p < 0.0001$ ) and time-dependent ( $H_0$ :  $c_t$  versus  $H_1$ :  $c_{st}$ ,  $p < 0.0001$ ). The selective  
249 foraging model states that the relative rate of prey capture between predator and traps  
250 significantly varies by time and by prey group. Since the simplest frequency-dependent model is  
251 nested within the others, it was unnecessary to test this against the selective foraging model.

252 The series of monthly linear contrasts revealed that in all but five months the relative rates  
253 of detection between the two prey groups, Collembola and Diptera, significantly differed (Fig. 3).  
254 Of those 13 months, only during January and February 2012 were relative rates of Collembola

255 detection significantly greater. Spiders screened positive for Diptera DNA at significantly higher  
256 relative rates during the remaining 11 months. Further linear contrasts, which are averaged across  
257 seasons, are summarized below. When comparing predation of each prey taxon individually  
258 between winter and non-winter (Fig. 4), results showed that both Collembola (95% CI: -2.24, -  
259 1.30) and Diptera (95% CI: -1.40, -0.41) were detected at a significantly higher relative rate in  
260 winter. When comparing predation of each prey taxon between the first and second winter of the  
261 study (Fig. 5), spiders were found to screen positive for relatively more Collembola in winter  
262 2011 than winter 2012 (95% CI: -3.20, -1.32), whereas spiders screened positive for relatively  
263 more Diptera in winter 2012 than winter 2011 (95% CI: 3.96, 4.95). When comparing predation  
264 between the two prey taxa within each season (Fig. 6), results showed that spiders screened  
265 positive for relatively more Collembola than Diptera in winter 2011 (95% CI: 1.61, 3.53), but  
266 relatively more Diptera than Collembola in both winter 2012 (95% CI: -4.62, -3.69) and non-  
267 winter (95% CI: -1.96, -1.36).

268

## 269 **Discussion**

270 We have demonstrated that the diet of a generalist predator does not reflect the  
271 proportional availability of potential prey, contrary to the frequency-dependent hypothesis driven  
272 by optimal foraging theory. Using Roualdes'  $c_{st}$ , we determined that the rate of predation in  
273 *Schizocosa*, relative to prey activity-densities, varied over time and by prey group. Deviations  
274 between the proportion of prey detected in spiders and the proportion of prey caught in traps also  
275 occurred in the majority of months across the study. These results suggest that *Schizocosa* feed in  
276 a selective manner and are not solely dependent on the frequency of encounters with the two  
277 focal prey groups, Collembola and Diptera.

278 Our results also show that this generalist predator did not adopt a frequency-dependent  
279 foraging strategy during winter. Low winter prey availability due to decreased temperatures is  
280 predicted to result in less selective diets (Pyke et al., 1977, Riechert, 1991), but both Collembola  
281 and Diptera were detected in predator guts at a higher rate, relative to their availability, during the  
282 winter months. Additionally, temperature was not correlated with overall Collembola predation,  
283 even though the activity-densities of this taxon were highly temperature-dependent. There was  
284 also no correlation between temperature and Diptera predation. This suggests that the capability  
285 to selectively forage is not constrained by low temperatures or low prey availability in these  
286 generalist predators. Despite slowed metabolic rates and likely fewer prey encounters, *Schizocosa*  
287 still fed discriminately during winter. These results demonstrate that energy maximization may  
288 not be the primary foraging requirement of these generalist predators, even when faced with a  
289 dearth of prey options.

290 A degree of caution was necessary when interpreting our results. First, the ability of  
291 pitfalls to trap Collembola and Diptera may have differed, possibly introducing bias into our  
292 calculations of prey availability. We contend any systematic trapping error is minute, however;  
293 both wolf spiders and pitfall traps capture actively moving, epigeal prey, and when caught,  
294 Collembola and Diptera are similarly crawling through the leaf litter. Second, there may be other  
295 mechanisms that contributed to the high rates of prey DNA detection in colder months. The  
296 effects of cold temperatures may not have affected the predatory behavior of spiders as much as  
297 the anti-predatory defenses of Collembola and Diptera (Sinclair et al., 2003, Boiteau and  
298 MacKinley, 2012, Waagner et al., 2013), or increased prey DNA detectability time due to slowed  
299 metabolic rates at colder temperatures may have caused more spiders collected during winter to  
300 test positive (Von Berg et al., 2008, Greenstone et al., 2014). The latter phenomenon likely

301 influenced our findings, but it does not explain the magnitude in which the proportion of spiders  
302 testing positive for a given prey group changed seasonally. For example, even reducing the values  
303 for spiders that consumed Collembola in winter months by 50% still suggests that spiders  
304 consume collembolans with approximately equal frequency in the winter and summer.  
305 Furthermore, if a temperature-dependent DNA detection time strongly influenced the results, then  
306 spiders captured in warmer months with relatively low prey availability (e.g., Diptera in August)  
307 would be expected to rarely test positive due to high turnover of prey DNA in predator guts.  
308 Instead, spiders frequently tested positive for this less common prey group in warm months.

309         The results from this study provide evidence that *Schizocosa* were selectively feeding to  
310 diversify their diets. This is best exemplified by the result that spiders consumed the least  
311 proportionally available prey group at a higher relative rate (supporting the hypothesis in  
312 Harwood et al. 2004) during each winter. Diptera outnumbered Collembola in winter 2011 (Dec  
313 2011 to Feb 2012), but spiders were found to prefer Collembola, whereas spiders preferred  
314 Diptera in winter 2012 (Dec 2012 to Feb 2013) despite higher densities of Collembola. This trend  
315 also extended beyond winter; less abundant dipterans were over-represented in spider diets  
316 throughout the non-winter months compared to collembolans. Due to constraints on predator  
317 functional responses to high prey availability (e.g. handling time), exceptionally high numbers of  
318 Collembola (e.g. during spring and summer) would be expected to dampen the signal used to  
319 detect any inherent preference for this prey. Although the presence/absence data from molecular  
320 gut-content analysis cannot quantify amount consumed, we would expect nearly all collected  
321 spiders to test positive for Collembola DNA in this scenario. Instead, with Collembola being the  
322 most plentiful prey resource but Diptera being consistently consumed at a higher relative rate, we  
323 contend that spiders sought out Diptera throughout most of the study for the purpose of diet

324 diversification. In winter 2011, the only season where dipterans were most abundant, spiders  
325 preferred Collembola, likely and similarly to equalize the proportion of each prey group in their  
326 diets.

327         Nutrient balance in spider diets supports growth and development, and can lead spiders to  
328 employ a nutrient-specific foraging strategy (Mayntz and Toft, 2001, 2006, Wilder, 2011). This  
329 may explain why spiders preferentially consumed the less available prey and appeared to balance  
330 intake of the two focal groups. Collembola, especially large-bodied members of the Tomoceridae  
331 and Entomobryidae, are known to be of exceptional prey quality for arthropod predators (Bilde et  
332 al., 2000), including wolf spiders (Rickers et al., 2006). Conversely, Diptera are typically  
333 considered to be lower in nutritional value (Toft and Wise, 1999), but can still be an important  
334 dietary component (e.g. Bardwell and Averill, 1997, Morse, 1997, Ishijima et al., 2006). In fact,  
335 there is an additive effect of pairing the two prey in spider diets. Toft and Wise (1999) displayed  
336 improvements in *Schizocosa ocreata* survival, weight gain, and development when fed both  
337 dipterans (*D. melanogaster*) and collembolans (*Tomcerus bidentatus* Folsom). In our study, 32%  
338 of the *Schizocosa* individuals to test positive for target prey DNA did so for both groups, and  
339 more than half of those positive for dipterans were also positive for collembolans. Spiders have  
340 shown to be capable of nutrient-based prey selection (Jackson et al., 2005, Wilder and Rypstra,  
341 2010), so it stands to reason that nutrient-specific foraging may be a mechanism driving  
342 *Schizocosa* to prefer the less abundant prey group. The proportion of spiders screening positive  
343 for both Collembola and Diptera increased as temperatures decreased, suggesting that the fitness  
344 benefits from a diverse diet may be especially important during winter (Whitney et al., 2014).  
345 However, further investigation into the mechanism at play in this system is required.



346 Molecular gut-content analysis, coupled with mathematical modeling of selectivity was  
347 used here to infer how generalist predators respond to seasonal changes in prey availability. Our  
348 results challenge the traditional notion that generalist predators feed solely opportunistically to  
349 maximize prey capture rate (Stephen and Krebs, 1986). We found that throughout the study  
350 duration, and unexpectedly during winter in particular, *Schizocosa* were not limited to consuming  
351 prey strictly according to proportional prey availability. They consistently consumed the least  
352 abundant of the two prey groups at a higher relative rate within a given season, which may  
353 indicate diet diversification is occurring. The monitoring of prey size and quality (i.e. nutrient  
354 composition) in parallel to prey availability would be necessary to better understand if nutrient-  
355 specific foraging is the cause for shifts in predator foraging decisions (Wilder, 2011). The  
356 molecular and statistical methods demonstrated here may prove useful in further investigations of  
357 seasonal shifts in trophic interactions. Extending knowledge about predator selectivity for  
358 different prey groups to ecosystem functioning (e.g. changes in primary productivity and  
359 decomposition) can provide insights into how ecosystems will respond to future perturbations.

360

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512  
513

514 **FIGURE LEGENDS**

515  
516 **Figure 1.** Mean ( $\pm$  SE) number of Collembola, Diptera and other potential prey captured in pitfall  
517 traps separated by month.

518  
519 **Figure 2.** Comparison between temporal changes in prey availability and predation frequencies  
520 on (a) Collembola and (b) Diptera. Relative prey activity-densities of each prey (left axis, bars)  
521 were surveyed using pitfall traps and the proportion of *Schizocosa* spiders testing positive for  
522 DNA of each target prey group (right axis, lines) was determined using PCR-based molecular  
523 gut-content analysis.

524  
525 **Figure 3.** Linear contrasts testing the null hypothesis  $c_{collembola,t} + c_{diptera,t} = 0$ , which states spiders  
526 equally prefer Collembola and Diptera in a given month. Positive values indicate spiders ate  
527 Diptera at a higher relative rate and negative values indicate spiders ate Collembola at a higher  
528 relative rate. Confidence intervals were derived from point estimates under the model  $c_{st} = \lambda_{st}/\gamma_{st}$   
529 and using Bonferroni multiple comparisons at a significance level of 0.05. Results are significant  
530 if confidence interval does not overlap zero, with the exception of November 2011.

531  
532 **Figure 4.** Linear contrasts testing the null hypothesis  $c_{s,winter} - c_{s,non-winter} = 0$ , which states spiders  
533 equally prefer a given prey group in winter and non-winter. Positive values indicate spiders ate a  
534 prey group at a higher relative rate during non-winter and negative values indicate spiders ate a  
535 prey group at a higher relative rate during winter. Confidence intervals were derived from point  
536 estimates under the model  $c_{st} = \lambda_{st}/\gamma_{st}$  and using Bonferroni multiple comparisons at a  
537 significance level of 0.05. Results for both Collembola (95% CI: -2.24, -1.30) and Diptera (95%  
538 CI: -1.40, -0.41) are significant, since confidence intervals do not overlap zero.

539  
540 **Figure 5.** Linear contrasts testing the null hypothesis  $c_{s,winter'11} - c_{s,winter'12} = 0$ , which states  
541 spiders equally prefer a given prey group in winter 2011 (Dec '11, Jan '12, and Feb '12) and  
542 winter 2012 (Dec '12, Jan '13, and Feb '13). Positive values indicate spiders ate a prey group at a  
543 higher relative rate during winter 2012 and negative values indicate spiders ate a prey group at a  
544 higher relative rate during winter 2011. Confidence intervals were derived from point estimates  
545 under the model  $c_{st} = \lambda_{st}/\gamma_{st}$  and using Bonferroni multiple comparisons at a significance level of  
546 0.05. Results for both Collembola (95% CI: -3.20, -1.32) and Diptera (95% CI: 3.96, 4.95) are  
547 significant, since confidence intervals do not overlap zero.

548  
549 **Figure 6.** Linear contrasts testing the null hypothesis  $c_{col,t} - c_{dip,t} = 0$ , which states spiders equally  
550 prefer Collembola and Diptera during the following seasons: winter 2011, winter 2012, and non-  
551 winter. Positive values indicate spiders ate Diptera at a higher relative rate and negative values  
552 indicate spiders ate Collembola at a higher relative rate. Confidence intervals were derived from  
553 point estimates under the model  $c_{st} = \lambda_{st}/\gamma_{st}$  and using Bonferroni multiple comparisons at a  
554 significance level of 0.05. Results for winter 2011 (95% CI: 1.61, 3.53), winter 2012 (95% CI: -  
555 4.62, -3.69), and non-winter (95% CI: -1.96, -1.36) are significant, since confidence intervals do  
556 not overlap zero.

557 **SUPPLEMENTARY MATERIAL**

558 **Figure S1.** Monthly mean temperature at soil level in Berea College Forest. The shaded region  
559 denotes range between the mean maximum and minimum monthly temperatures.

560  
561 **Table S1.** Invertebrates collected from pitfall traps considered as potential prey or not for  
562 *Schizocosa*.

563  
564 **Table S2.** List of non-target taxa used to verify specificity of primers for Collembola and Diptera.  
565 The number tested is provided whenever more than one specimen for a particular group was used.