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Threats from the air: damselfly predation on diverse prey taxa

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1 **Threats from the air: damselflies exert vast predation on diverse prey taxa**

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18 **Running title: Vast predation by damselflies**

19 **Abstract**

20 **1.** To understand the distribution and strength of predation in natural communities, we need
21 to quantify which predator species are eating which prey taxa. The recent adoption of DNA-
22 based techniques has brought widespread characterization of predator diets with high
23 taxonomic resolution. To further determine impacts of predators, we should next combine
24 these DNA techniques with estimates of population size using mark-release recapture
25 methods, and accurate metrics of individual food consumption.

26 **2.** In this study, we create a novel synthesis of the scale of predation pressure exerted by
27 predatory damselfly species on their diverse prey taxa within an accurately defined area,
28 resolving who is eaten by whom, how many prey individuals are consumed per predator, and
29 to what extent the diet of individual predator species overlaps.

30 **3.** We identified the prey taxa composition of four damselfly species using DNA
31 metabarcoding, and quantified damselfly population sizes by intensive mark-release-
32 recapture. By combining both approaches with predator-specific estimates of consumption
33 rates, we estimate the summed predation pressure exerted by four damselfly species and its
34 taxonomic distribution.

35 **4.** Across a 12-hectare study area, the damselfly species collectively consumed a prey mass
36 equivalent to roughly 12,000,000 small dipterans over two months. Each damselfly individual
37 consumed 29%-66% times its own body weight in prey during its relatively short life (2-4.7
38 days) in the local population. This predation pressure was widely distributed across the local
39 insect community, including 4 classes, 19 orders, and 84 families of arthropod prey. Different
40 predator species showed extensive overlap in prey composition.

41 **5.** Our results suggest that relatively small damselflies exert a vast predation pressure on local
42 prey populations and species assemblages. Given high local population size and high

43 consumption rates per individual, the local damselfly community consumes a massive
44 amount of prey invertebrates.

45 **6.** Our synthesis of the population size, per-capita consumption rate and taxonomic
46 distribution of predation identify damselflies as a dominant predator group-harvesting insects
47 from the air. As a key prediction, we suggest that the experimental removal of damselflies
48 will reveal the same type of imprints as recently revealed for vertebrate predators including
49 birds and bats.

50 **Introduction**

51 How different trophic levels impact one another is one of the key conundrums of modern
52 ecology (Ings *et al.* 2009; Thompson *et al.* 2012). Recent studies of food web structure
53 attempt to quantify not only who eats whom from a qualitative perspective, but also how
54 frequently each type of predation event occurs (Roslin & Majaneva 2016). While such
55 targeted quantification of trophic links will elucidate the strength of direct ties between
56 predators and potential prey, there is also potential for indirect effects through shared
57 predators and prey (Schmitz & Suttle 2001; Montoya *et al.* 2009). However, studies typically
58 isolate a given module of the community from its whole biological context. To arrive at a
59 comprehensive metric of predation pressure across the full range of prey taxa is notoriously
60 difficult (Pocock, Evans & Memmott 2012). More often, the interaction of single predator
61 species with single prey species is targeted, relating consumption rates to prey size and prey
62 density, and to determine whether a single predator species can regulate its prey species or
63 even cause its local extirpation (Royama 2012).

64 The majority of current theory on predator-prey dynamics is based on the assumption of a
65 tight linkage between a single predator and prey species, resulting in Lotka-Volterra
66 dynamics and providing scope for classic population cycles (Royama 2012). Yet, even classic
67 predator-prey species pairs and population cycles have been shown to be part of more
68 complex food webs (e.g. Stenseth *et al.* 1997). This complexity calls for a re-evaluation of
69 the community-level context of predator-prey dynamics, and for a new empirical assessment
70 of the taxonomic distribution and overall predation pressure exerted by abundant predator
71 taxa within larger communities (Holt 2009; Montoya *et al.* 2009). Only by embarking on
72 such a challenging, yet essential characterization of predation in a community context, will
73 we ever acquire the satisfactory understanding of predator-prey dynamics in nature and its
74 population and community-level consequences.

75 What has hampered the community-level dissection of predator–prey relations has long been
76 the difficulties associated with assessing the dietary composition of predatory species (Roslin
77 & Majaneva 2016; Alberdi *et al.* 2018). Recently, DNA-barcoding techniques have opened
78 new ways for studying, which prey species predatory species are eating in the wild
79 (Vesterinen *et al.* 2013). Furthermore, these new DNA-based tools for diet analysis offer
80 scope for identifying the full diet of focal predators (Kaunisto *et al.* 2017; Vesterinen *et al.*
81 2018; Eitzinger 2019; Rytönen 2019). Now that we can identify the taxonomic distribution
82 of prey species, we can begin to assess which species and species assemblages are affected by
83 predation. Yet, to arrive at a satisfactory, community-level understanding of predation
84 pressure, these estimates should be combined with two other pieces of information: the
85 abundance of predators and the food consumption of each individual. For both items, recent
86 methodological developments have brought important advances. The last 30 years have seen
87 a rapid proliferation of advanced methods for making more use of Mark-Release-Recapture
88 (hereafter MRR) data, including the sensitive estimation of population size, its temporal
89 variation and local demographic rates (Lebreton *et al.* 1992; Cooch & White 2018). Also,
90 metabolic theory and parameterized models for estimating individual consumption based on
91 predator-prey body size (Yodzis & Innes 1992) or allometric (Gillooly *et al.* 2001) relations
92 have regained traction (e.g. Pettersen *et al.* 2019).

93 Together, these three lines of research offer a novel synthesis of the community-level
94 distribution and strength of predation by different taxa on their prey taxa. Yet, to our
95 knowledge, these three developments have not been brought together to quantify the
96 predatory imprint of presumed-ecologically dominant predator taxa on their prey taxa,
97 species assemblages or focal communities.

98 As larvae and adults, odonates are largely visual predators – adults deploy various hunting
99 strategies including active foraging flights, sit-and-wait strategies coupled with sully

100 flights and the gleaning of prey from vegetation (Corbet 1999). Yet, although predatory
101 behaviour of the odonates is well known, detailed research on adult odonate foraging is
102 scarce (but see e.g. Baird & May 1997; Kaunisto *et al.* 2017). There are many large
103 information gaps in the study of odonate predation including the diets of predatory species
104 overlapping in space and time, and their combined impact on prey species, populations and
105 communities.

106 To approach such information gaps is not an easy task – their resolution requires information
107 on diets of different predator species, their survivorship and population size estimates of both
108 predators and prey. With this study, we address some of these knowledge gaps. We
109 characterize the taxonomic range and quantitative distributions of prey use by metabarcoding
110 a large sample of damselflies' faeces collected 'clean' from live individuals. To estimate the
111 population size and adult lifespan of these damselflies, we conduct a MRR study. We are able
112 to quantify predation in a community context by combining demographic information of
113 predator species with daily rates of prey consumption, weight information of focal damselfly
114 species extracted from literature and weight information on prey taxa. Overall, our study
115 reveals adult damselflies as key top predators of the insect world, exerting a vast predation
116 pressure of a wide range of aquatic and terrestrial arthropods.

117 **Materials and methods**

118 To assess the predation pressure of damselfly species on their prey, we target the four most
119 common predatory species at our study site in Southern Finland (located at ETRS-TM35FIN
120 N: 67118; E: 2460). All four species belong to family Coenagrionidae: *Coenagrion*
121 *lunulatum* (Charpentier, 1840), *C. hastulatum* (Charpentier, 1825), and *C. pulchellum*
122 (Vander Linden, 1825) and *Enallagma cyathigerum* (Charpentier, 1840). These species feed
123 mainly on dipteran prey during open foraging flights and by gleaning (Corbet 1999; Kaunisto
124 *et al.* 2017). Only sexually mature, i.e. adult-coloured and hard-winged individuals were

125 included in the study. In the following sections, methods are explained briefly with full
126 details in the electronic supplementary material (supplementary material S1, Detailed
127 material and methods).

128 *Sampling and study site*

129 To assess the population sizes and survival rates of the focal species, we conducted a MRR
130 study of the damselfly populations associated with a freshwater pond of approximately 600 m
131 x 200 m (12 ha). During the MRR study on 1 June and 2 June, we collected an additional 185
132 individuals (20–26 males and females from each species) for faecal DNA analysis.

133 *Molecular dietary analysis*

134 To establish the diets of focal species, we used established metabarcoding protocols for
135 dragonflies building on earlier optimization (Kaunisto *et al.* 2017). To amplify mitochondrial
136 COI gene, we used ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale *et al.* 2011) and for insect 16S gene,
137 we used Ins16S-1F and Ins16S-1Rshort after Clarke *et al.* (2014). The detailed protocol is
138 available in supplementary material S1 (Detailed materials and methods: Description of
139 molecular methods). After sequencing, the reads separated by each original sample were
140 uploaded on CSC servers (IT Center for Science, www.csc.fi) for bioinformatic analysis.
141 Detailed bioinformatics applied is available in the supporting material (Supplementary
142 material S1, Detailed material and methods: Bioinformatics). Labelled raw reads, ZOTUs,
143 and zotu-tables are available in the Dryad Digital Repository: <https://doi.org/XXX>.

144 *Data analysis*

145 **DAMSELFLY POPULATION PARAMETERS** – To convert MRR data into estimates of population
146 size and its temporal variation, we used the MARK software (version 9, Cooch & White
147 2018). We applied Jolly-Seber methods under the POPAN parameterization (Lebreton *et al.*
148 1992). For details, see Supplementary material S1 (Detailed material and methods: MRR

149 estimates). Estimates of time-specific population size were then integrated over time and fed
150 into downstream analyses (see INDIVIDUAL AND COMMUNITY-LEVEL CONSUMPTION RATES,
151 below).

152 **PREY IDENTITIES AND ABUNDANCES** – We identified prey to the family level, using locus-
153 specific criteria (COI and 16S). For details, see Supplementary material S1, Detailed material
154 and methods: Prey taxa). In earlier work offering proof-of-concept (Kaunisto *et al.* 2017), we
155 used prey frequencies (fraction of droppings in which read was present) rather than read
156 abundances (number of sequencing reads of this prey taxon) as measures of trophic link
157 strength. As recent work suggest that a reliance on the presence-absence data may be more
158 misleading than the use of read abundances (Deagle 2019; Lamb 2019), we used read counts,
159 using relative read abundance (RRA) calculated for each prey taxon in each sample as a
160 proxy of prey-specific biomass consumed (Appendix S1, Detailed material and methods: Eqn
161 S1 Vesterinen *et al.* 2018; Deagle 2019).

162 **PREDATOR-SPECIFIC PREY USE** – To visualize the trophic interactions structures resolved by
163 the molecular data, we used package “bipartite” (Dormann 2009) implemented in program R
164 (R Core Team 2018). To illustrate the prey sharing between the four odonate species, we
165 used a Venn diagram constructed using R package ‘VennDiagram’ version 1.6.20 (Chen &
166 Boutros 2011). To test for effects of species and sex of predator on variation in prey taxon
167 composition, we used PERMANOVA (Anderson 2001). To visualize the comparison of prey
168 use among predator taxa, we performed a principal component analysis (PCA) based on
169 Bray-Curtis similarity (Bray & Curtis 1957; Legendre & Legendre 2012) of the taxonomic
170 composition of prey detected in each faecal sample.

171 **INDIVIDUAL AND COMMUNITY-LEVEL CONSUMPTION RATES** – To calculate the species-
172 specific consumption rate of predator *i* across all prey species, we used the following
173 equation:

174
$$P_i=(W\times R\times N_i\times L)$$
 (Eqn 1)

175 Here, W stands for individual predator mass, R for daily consumption rate, N_i for predator
176 population size, and L for the estimated longevity of each individual predator. To convert P_i
177 to an estimate of the number (count) of average-sized prey individuals consumed, we divide
178 P_i by b_w , i.e. average individual prey biomass. To derive overall prey consumption, we
179 summed across the four predator species i . Parameter values for W and R were extracted from
180 recent literature, whereas values of N_i were adopted from MRR estimates (see Results). As a
181 representative estimate of b_w , we made 9 and 351 weightings from randomly-selected
182 individuals, of two most common prey families, Cecidomyiidae and Chironomidae,
183 respectively.

184 **Results**

185 *Predator population size*

186 Altogether we marked and released 1,341 individuals of the four damselfly species, of which
187 we later made 32 recaptures. The longest time spans observed between mark, release and
188 recapture were 14, 15, 18, and 17 days for *C. lunulatum*, *E. cyathigerum*, *C. hastulatum* and
189 *C. pulchellum*, respectively.

190 The Jolly-Seber models suggested the smallest overall population size for *C. hastulatum*,
191 while *E. cyathigerum* was the most abundant species, with estimates varying from 5,960 to
192 22,540 (Supplementary material S1, Additional results: Table S1). Average residency time in
193 the population ranged from 2.07 days (*C. lunulatum*) to 4.68 days (*E. cyathigerum*; Table
194 S1).

195 Across individual sampling dates, estimates and temporal patterns of damselfly population
196 sizes varied widely across the four species of interest (Supplementary material S1, Additional
197 results: Fig. S1). *C. lunulatum* population estimates began near 2,000 individuals, but

198 dropped to approximately zero by the halfway point of sampling. Abundance estimates of
199 both *C. hastulatum* and *C. pulchellum* began at less than 500, quickly increased to their
200 respective maxima, and then decreased gradually throughout the rest of the season. Only *E.*
201 *cyathigerum* abundance estimates generally increased across most of the entire sampling
202 period (Fig. S1).

203 ***Prey composition***

204 The sequencing run yielded 16,188,489 quality-controlled paired-end reads. After
205 bioinformatic pipeline and subsequent filtering, the remaining (COI: 449 573 COI; 16S:
206 478 202) reads were assigned to prey families. Practically all of the filtered COI reads
207 (99.6%) could be identified to family level prey. For filtered 16S reads, ~31% were identified
208 as target prey, however, this result should still be considered good when amplifying
209 arthropod prey DNA mixed with arthropod predator DNA, see for example Pinol *et al.*
210 (2014), where only 6% of reads were assigned to prey in a study without blocking primers. In
211 this study, the most common prey order was Diptera, and the most abundant prey families
212 were Cecidomyiidae and Chironomidae (Fig. 1). The four predator species showed extensive
213 overlap in the prey use (Supplementary material S1, Additional results: Fig. S2). The most
214 common prey taxa (Cecidomyiidae and Chironomidae) were widely consumed by all odonate
215 species, and by both sexes (Supplementary material S1, Additional results: Fig. S3).
216 PERMANOVA showed significant, but weak differences between predator species
217 (ADONIS: $R^2 = 0.013$, $P = 0.005$; Supplementary material S1, Additional results: Table S2)
218 and sexes (ADONIS: $R^2 = 0.038$, $P = 0.030$; Table S2). However, pairwise PERMANOVA
219 confirmed that prey assemblage differed only between *E. cyathigerum* and *C. pulchellum*
220 (Supplementary material S1, Additional results: Table S3). Where slight differences in diet
221 were found among females and males in different species (Predator x Sex: $R^2 = 0.03$, $P =$
222 0.035 ; Table S2), samples from the same predator species or sex did not detectably cluster

223 together in the visual PCA ordinations (Supplementary material S1, Additional results: Fig.
224 S4).

225 ***Predation rates quantified***

226 Based on the information compiled in this study and from the literature, we were able to
227 parameterize Eqn 1 (above) as follows:

228 **W, INDIVIDUAL PREDATOR MASS** – All of our focal damselflies are very similar in size and
229 weight. According to Ilvonen and Suhonen (2016), the weights of our focal damselflies *C.*
230 *hastulatum*, *C. pulchellum* and *E. cyathigerum*, are 33.9 mg (s.e. 1.3, n = 22), 36.1 mg (s.e.
231 1.3, n = 22) and 35.4 (s.e. 1.3, n = 22), respectively. The fourth focal damselfly species,
232 *Coenagrion lunulatum*, was not directly weighed but since its average length matches that of
233 *C. hastulatum* (Karjalainen 2010) , we used the weight of that species, i.e. 33.9 mg, as an
234 accurate proxy.

235 **R, DAILY CONSUMPTION RATE** – For perching odonates, Corbet (1999) have shown
236 individuals to consume on average 14% of their own body weight per day. This estimate is
237 roughly consistent with other estimates reported in the literature: McVey: 12.5%, Higashi:
238 12.75%, Fried & May: 12.5%, Mayhew: 19% (Higashi 1973; Fried & May 1983; McVey
239 1985; Mayhew 1994). Thus, we adopt 14% as our estimate of *R*.

240 ***b_w*, AVERAGE INDIVIDUAL PREY BIOMASS** – The average fresh weight per individual of the
241 two most common prey families was for 0.063 mg (n=9, SD± 0.055 mg) Cecidomyiidae and
242 0.154 mg (n=351, SD ± 0.182 mg) for Chironomidae.

243 Across damselfly species, this yields the following species-specific estimates for the four
244 damselfly species: *C. lunulatum* (75,603 mg), *C. hastulatum* (75,775 mg), *C. pulchellum*
245 (117,685 mg), and *E. cyathigerum* (523,206 mg; Supplementary material S1, Additional
246 results: Eqns S2a–d).

247 Across all species, this sums to 792,270 mg. Dividing by the average size of prey, b_w , this
248 amounts to 12,575,727 cecidomyiids, or 5,144,616 chironomid individuals consumed per
249 season. Given an estimated average life span of ca 4 days (Table S1), each individual
250 damselfly is likely to consume prey mass equal to an average of 75 cecidomyiids per day and
251 a total of 301 cecidomyiids during its adult life.

252 **Discussion**

253 Our results suggest that damselflies exert a massive predation pressure on their prey species,
254 and will leave a key imprint on local insect populations, species assemblages and
255 communities. These findings build from the fusion of advances in molecular ecology, mark-
256 release recapture methods and metabolic theory. The synthesis of these three fields frames the
257 study of predation on arthropod prey in a community context, and suggests fruitful avenues
258 for future research. Below, we discuss these avenues in further detail.

259 *A new synthesis of new methods and classic ecological tools*

260 The insights presented in this study build on multiple methodological advances combined in a
261 new way. Recent advances in DNA-based diet analysis have allowed the qualitative
262 description of diet contents across a range of taxa, information not attainable with traditional
263 techniques (Roslin & Majaneva 2016; Roslin *et al.* 2019). We are now advancing to the stage
264 of quantification of diet contents. This approach involves not only quantifying the amount of
265 prey in the diet (Deagle 2019; Lamb 2019), but the fusion of molecular quantification with
266 more classical types of abundance estimates. In the current study, we combine quantitative
267 information on prey use gleaned from molecular data with insight from quantitative models
268 of MRR data (Lebreton *et al.* 1992; Cooch & White 2018), used to derive estimates on
269 population sizes of predatory species. Applying metabolic functions to link individual
270 damselfly weight to its consumption rate, we are able to calculate the mass and number of

271 prey items consumed by our four focal damselfly species at our focal study site. This is an
272 exciting advance, made possible by a new use of molecular data.

273 ***Predation by damselflies is quantitatively vast and taxonomically widespread***

274 The current findings show damselflies as generalist predators with a wide variety of prey
275 taxa. Dietary segregation among species and sexes proved weak. High overlap in niche space
276 has also been observed in the North American genus *Enallagma*, consistent with the
277 extensive sharing of prey by the current set of damselfly species (Siepielski *et al.* 2010).
278 Overall, the odonates feed on a very high number of prey taxa, tapping widely into the prey
279 community available at the site (Fig. 1). Besides being taxonomically widespread, we also
280 show that the overall predation rate exerted is high, and that the typical prey of damselflies
281 are small dipterans. Thus, relatively large odonates feed on relatively small dipterans with a
282 predator–prey body mass ratio of over 260–1. This finding is consistent with the observations
283 of Baird and May (1997). Using traditional techniques such as visual monitoring of predators
284 and prey, these authors reported that of the prey items captured by the dragonfly *Pachydiplax*
285 *longipennis* (Burmeister, 1839), only 3% exceeded 1.47 mg.

286 Our study is important because top predators are thought to affect the food web at lower
287 trophic levels in many ways, e.g. via trophic cascades, often resulting in dramatic changes in
288 species composition (Estes *et al.* 2011; Van Allen *et al.* 2017). The high predation rates
289 reported here suggest strong community-level impacts of damselfly on the local prey
290 communities. While our rough estimates of the number of prey items consumed in just a few
291 hectares are staggering (hundreds consumed per predator individual during its life time,
292 hundreds of thousands consumed per damselfly species during its flight period, and millions
293 consumed per season by the damselflies combined), there are several reasons why the
294 realized number of prey items is likely to be even higher than our current estimates.
295 Damselflies tend to discard less nutritious parts of their prey before ingestion (reviewed in

296 Corbet 1999). Moreover, we only included adult damselflies into this study, but newly
297 emerged individuals have also been shown to actively hunt for prey (reviewed in Corbet
298 1999). Furthermore, our focal damselfly species are among the smallest odonates, whereas
299 e.g. another rather common dragonfly of our study area, the Brown Hawker *Aeshna grandis*
300 (Linnaeus, 1758), has a body mass of nearly a gram (mean 873.1 mg, S.E. 29.5 n = 25)
301 (Ilvonen & Suhonen 2016). As a consequence of their heavier bodies, larger dragonflies will
302 consume prey in considerably higher numbers and/or mass than their smaller relatives.

303 ***Predation by damselflies in a community context***

304 Our findings reveal the main predators of the airborne arthropod community to be widely
305 generalist predators. These findings are consistent with those from a pilot study (Kaunisto *et*
306 *al.* 2017), which focused on three, likewise common odonate species differing in their life
307 cycles and body size. Kaunisto *et al.* (2017) were able to demonstrate that different odonates
308 overlap extensively in their prey use – not only with each other, but also with major diurnal
309 and nocturnal airborne predators including birds and bats. The current study validates these
310 results but significantly enriches the picture. Where previous work resolved the taxonomic
311 distribution of odonate predation (Kaunisto *et al.* 2017), the current study adds important
312 detail by revealing the vastly high predation pressure dissipated through the many links.
313 While even the simplest of food webs are usually complex (Wirta *et al.* 2015), with species
314 linked by diverse connections (Williams & Martinez 2000), the predator-prey food web
315 detected for our focal damselflies and their prey is a highly connected one. Here, tight
316 coupling between individual predator–prey taxa/species pairs seems the anomaly rather than
317 the rule. In such thoroughly linked systems, an increase in the predator may result in little
318 trophic cascades, simply because the elevated predation pressure is dissipated through so
319 many channels (Visakorpi *et al.* 2015). Dissecting community-level predation patterns is thus
320 a key precondition for understanding predator-prey dynamics in real, complex food webs.

321 **Conclusions**

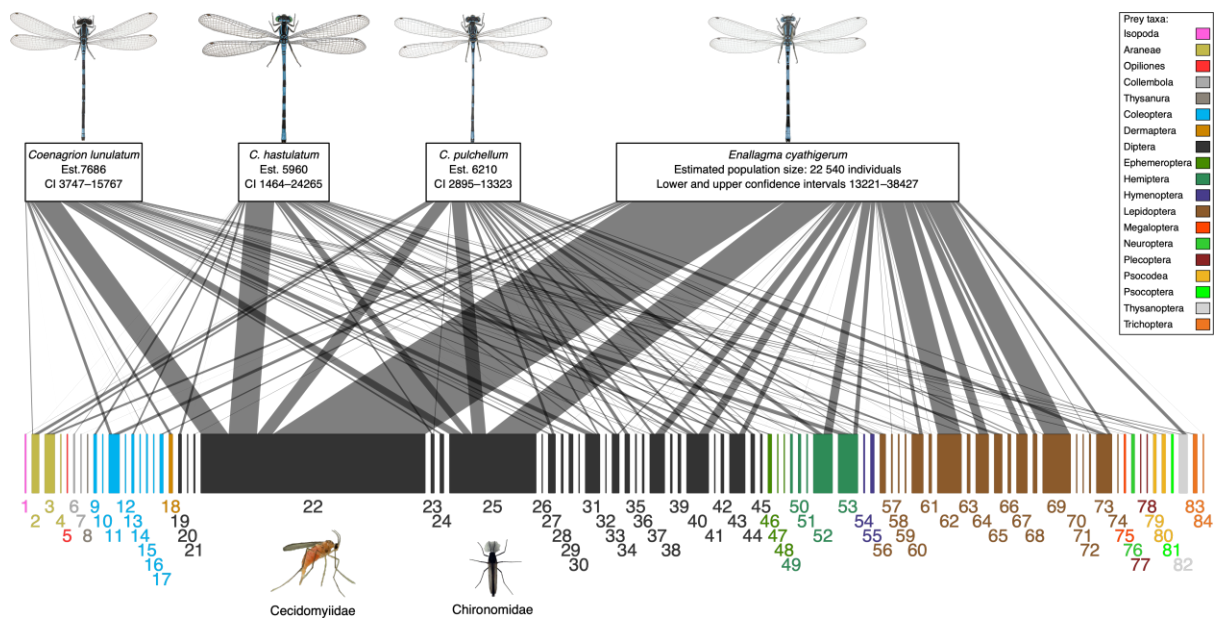
322 As the basic building block needed to understand the forces regulating animal populations,
323 and to estimate flows of energy and matter through food webs, we need sound estimates of
324 who is eating whom and in what quantities. Our results suggest that even small damselflies
325 exert a massive predation pressure on their prey species, and will leave an imprint on local
326 insect communities. What we lack are local estimates of prey populations, their reproductive
327 rates and the relative direct and indirect contributions of damselfly predation as compared to
328 other forces on dynamics of prey populations. Needless to say, such information is difficult to
329 obtain, but might eventually be reconstructed from local emergence traps, MRR studies of
330 prey species and labor-intensive fieldwork. We hope that our study stimulates the
331 quantification of key variables helping to resolve food web linages and dynamics. Our current
332 estimates take an essential first step towards understanding of top-down versus bottom-up
333 regulation of insect communities, and should next be supplemented by numerical estimates of
334 prey populations and of their demographic rates. A satisfactory understanding of predation
335 pressure can only be achieved through these steps, in which context the combination of novel,
336 DNA-based techniques with classical tools offer key catalysts. As a key prediction, we
337 suggest that the experimental removal of damselflies will reveal the same type of impact on
338 the ecosystem as recently revealed for vertebrate predators including birds and bats (Van
339 Bael, Brawn & Robinson 2003; Mooney *et al.* 2010; Mantyla, Klemola & Laaksonen 2011;
340 Maine & Boyles 2015).

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349



351

352 Figure 1. Visual representation of the taxonomic distribution and quantified strength of

353 trophic links from each of our four focal odonate species to their prey. The blocks in the

354 upper row represent predators, with their size scaled to represent the estimated population

355 size. The blocks in the lower row represent prey families, with two most common prey

356 families highlighted. A line connecting a predator with a prey represents detected predation

357 events, and the thickness of the line represents the relative read abundance (RRA) of each

358 predation record (Supplementary material S1, Detailed material and methods: Eqn S1). See

359 the “Data analysis” in the main text for details on the RRA. The numbers below the lower

360 blocks correspond to the prey family numbers (Supplementary material S1: Table S4). Photo

361 credits: Göran Liljeberg & Hallvard Elven, Artdatabanken.no (CC BY-SA 3.0, Odonata),

362 Hallvard Elven, Artdatabanken.no (CC BY-SA 4.0, Cecidomyiidae, modified); František

363 Šaržík, biolibs.cz (CC-BY, Chironomidae, modified).

364

365 **References**

- 366 Alberdi, A., Aizpurua, O., Bohmann, K., Gopalakrishnan, S., Lynggaard, C., Nielsen, M. &
 367 Gilbert, M.T.P. (2018) Promises and pitfalls of using high - throughput
 368 sequencing for diet analysis. *Molecular Ecology Resources*, Early view.
- 369 Anderson, M.J. (2001) A New Method for Non-Parametric Multivariate Analysis of
 370 Variance: Non-Parametric MANOVA for Ecology. *Austral Ecology*, **26**, 32-46.
- 371 Baird, J.M. & May, M.L. (1997) Foraging behavior of *Pachydiplax longipennis* (Odonata:
 372 Libellulidae). *Journal of Insect Behavior*, **10**, 655-678.
- 373 Bray, J.R. & Curtis, J.T. (1957) An ordination of the upland forest communities of
 374 southern wisconsin. *Ecological Monographs*, **27**, 326-349.
- 375 Chen, H. & Boutros, P.C. (2011) VennDiagram: a package for the generation of highly-
 376 customizable Venn and Euler diagrams in R. *BMC Bioinformatics*, **12**.
- 377 Clarke, L.J., Czechowski, P., Soubrier, J., Stevens, M.I. & Cooper, A. (2014) Modular
 378 tagging of amplicons using a single PCR for high-throughput sequencing.
 379 *Molecular Ecology Resources*, **14**, 117-121.
- 380 Cooch, E. & White, G. (2018) Program MARK: A gentle introduction. Available from:
 381 <http://www.phidot.org/software/mark/docs/book/>.
- 382 Corbet, P.S. (1999) *Dragonflies: behaviour and ecology of Odonata. Revised edition*.
 383 Harley Books, Colchester.
- 384 Core, T.R. (2018) *A Language and Environment for Statistical Computing*. Vienna,
 385 Austria.
- 386 Deagle, B.E., Austen C. Thomas, Julie C. McInnes, Laurence J. Clarke, Eero J. Vesterinen,
 387 Elizabeth L. Clare, Tyler R. Kartzinel, and J. Paige Eveson (2019) Counting with
 388 DNA in Metabarcoding Studies: How Should We Convert Sequence Reads to
 389 Dietary Data? *Molecular Ecology*, **28**, 391-406.
- 390 Dormann, C.F., J. Frund, N. Bluthgen, and B. Gruber (2009) Indices, Graphs and Null
 391 Models: Analyzing Bipartite Ecological Networks. *The Open Ecology Journal*, **2**, 7-
 392 24.
- 393 Eitzinger, B., N. Abrego, D. Gravel, T. Huotari, E. J. Vesterinen, and T. Roslin (2019)
 394 Assessing changes in arthropod predator-prey interactions through DNA -
 395 based gut content analysis—variable environment, stable diet. *Molecular Ecology*,
 396 **28**, 266–280.
- 397 Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R.,
 398 Essington, T.E., Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Oksanen, T.,
 399 Paine, R.T., Pickett, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W.,
 400 Shurin, J.B., Sinclair, A.R.E., Soule, M.E., Virtanen, R. & Wardle, D.A. (2011)
 401 Trophic Downgrading of Planet Earth. *Science*, **333**, 301-306.
- 402 Fried, C.S. & May, M.L. (1983) Energy expenditure and food intake of territorial male
 403 *Pachydiplax longipennis* (odonata, libellulidae). *Ecological Entomology*, **8**, 283-
 404 292.
- 405 Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M. & Charnov, E.L. (2001) Effects of size
 406 and temperature on metabolic rate. *Science*, **293**, 2248-2251.
- 407 Higashi, K. (1973) Estimation of the food consumption for some species of dragonflies.I.
 408 Estimation by observation for the frequency of feeding flights of dragonflies. *Rep.*
 409 *Ebino.Biol. Lab.*, **1**, 119-129.
- 410 Holt, R.D. (2009) *Toward a trophic island biogeography. The theory of island*
 411 *biogeography revisited*. Princeton University Press, Princeton, New Jersey, USA.

412 Ilvonen, J.J. & Suhonen, J. (2016) Phylogeny affects host's weight, immune response and
413 parasitism in damselflies and dragonflies. *R. Soc. open sci*, **3**.

414 Ings, T.C., Montoya, J.M., Bascompte, J., Bluthgen, N., Brown, L., Dormann, C.F., Edwards,
415 F., Figueroa, D., Jacob, U., Jones, J.I., Lauridsen, R.B., Ledger, M.E., Lewis, H.M.,
416 Olesen, J.M., van Veen, F.J.F., Warren, P.H. & Woodward, G. (2009) Ecological
417 networks - beyond food webs. *Journal of Animal Ecology*, **78**, 253-269.

418 Karjalainen, S. (2010) *Suomen sudenkorennot*. Tammi, Helsinki.

419 Kaunisto, K.M., Roslin, T., Saaksjarvi, I.E. & Vesterinen, E.J. (2017) Pellets of proof: First
420 glimpse of the dietary composition of adult odonates as revealed by
421 metabarcoding of feces. *Ecology and Evolution*, **7**, 8588-8598.

422 Lamb, P.D., Ewan Hunter, John K. Pinnegar, Simon Creer, Richard G. Davies, and Martin I.
423 Taylor (2019) How Quantitative Is Metabarcoding: A Meta - analytical Approach.
424 *Molecular Ecology*, **28**, 420-430.

425 Lebreton, J.D., Burnham, K.P., Clobert, J. & Anderson, D.R. (1992) Modeling survival and
426 testing biological hypotheses using marked animals - a unified approach with
427 case-studies. *Ecological Monographs*, **62**, 67-118.

428 Legendre, P. & Legendre, L. (2012) *Numerical Ecology*. Elsevier, Amsterdam.

429 Maine, J.J. & Boyles, J.G. (2015) Bats initiate vital agroecological interactions in corn.
430 *Proceedings of the National Academy of Sciences of the United States of America*,
431 **112**, 12438-12443.

432 Mantyla, E., Klemola, T. & Laaksonen, T. (2011) Birds help plants: a meta-analysis of top-
433 down trophic cascades caused by avian predators. *Oecologia*, **165**, 143-151.

434 Mayhew, P.J. (1994) Food intake and adult feeding behaviour in *Calopteryx splendens*
435 (Harris) and *Erythromma najas* (Hansemann) (Zygoptera: Calopterygidae,
436 Coenagrionidae). *Odonatologica*, **2**, 115-124.

437 McVey, M.E. (1985) Rates of color maturation in relation to age, diet, and temperature in
438 male *Erythemis simplicicollis* (Say) (Anisoptera: Libellulidae). *Odonatologica*, **14**,
439 101-114.

440 Montoya, J.M., Woodward, G., Emmerson, M.C. & Sole, R.V. (2009) Press perturbations
441 and indirect effects in real food webs. *Ecology*, **90**, 2426-2433.

442 Mooney, K.A., Gruner, D.S., Barber, N.A., Van Bael, S.A., Philpott, S.M. & Greenberg, R.
443 (2010) Interactions among predators and the cascading effects of vertebrate
444 insectivores on arthropod communities and plants. *Proceedings of the National*
445 *Academy of Sciences of the United States of America*, **107**, 7335-7340.

446 Pettersen, A.K., White, C.R., Bryson-Richardson, R.J. & Marshall, D.J. (2019) Linking life-
447 history theory and metabolic theory explains the offspring size-temperature
448 relationship. *Ecology Letters*, **22**, 518-526.

449 Pinol, J., San Andres, V., Clare, E.L., Mir, G. & Symondson, W.O.C. (2014) A pragmatic
450 approach to the analysis of diets of generalist predators: the use of next-
451 generation sequencing with no blocking probes. *Molecular Ecology Resources*, **14**,
452 18-26.

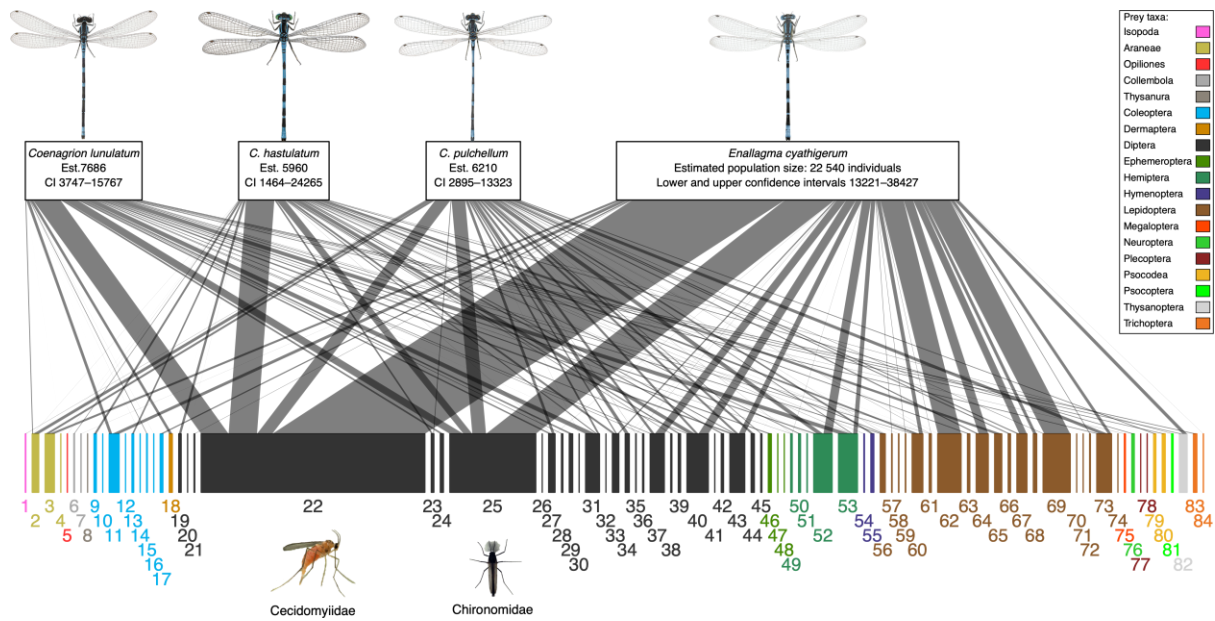
453 Pocock, M.J.O., Evans, D.M. & Memmott, J. (2012) The Robustness and Restoration of a
454 Network of Ecological Networks. *Science*, **335**, 973-977.

455 Roslin, T. & Majaneva, S. (2016) The use of DNA barcodes in food web construction-
456 terrestrial and aquatic ecologists unite! *Genome*, **59**, 603-628.

457 Roslin, T., Traugott, M., Jonsson, M., Stone, G.N., Creer, S. & Symondson, W.O.C. (2019)
458 Introduction: Special issue on species interactions, ecological networks and
459 community dynamics – Untangling the entangled bank using molecular
460 techniques. *Molecular Ecology*, **28**, 157-164.

461 Royama, T. (2012) *Analytical population dynamics*. Springer Science & Business Media.
 462 Rytönen, S., E. J. Vesterinen, C. Westerduin, T. Leviäkangas, E. Votka, M. Mutanen, P.
 463 Välimäki, M. Hukkanen, M. Suokas, M. Orell (2019) From feces to data: A
 464 metabarcoding method for analyzing consumed and available prey in a bird-
 465 insect food web. *Ecology and Evolution*, **9**, 631–639.
 466 Schmitz, O.J. & Suttle, K.B. (2001) Effects of top predator species on direct and indirect
 467 interactions in a food web. *Ecology*, **82**, 2072-2081.
 468 Siepielski, A.M., Hung, K.L., Bein, E.E.B. & McPeck, M.A. (2010) Experimental evidence
 469 for neutral community dynamics governing an insect assemblage. *Ecology*, **91**,
 470 847-857.
 471 Stenseth, N.C., Falck, W., Bjornstad, O.N. & Krebs, C.J. (1997) Population regulation in
 472 snowshoe hare and Canadian lynx: Asymmetric food web configurations
 473 between hare and lynx. *Proceedings of the National Academy of Sciences of the*
 474 *United States of America*, **94**, 5147-5152.
 475 Thompson, R.M., Brose, U., Dunne, J.A., Hall, R.O., Hladyz, S., Kitching, R.L., Martinez, N.D.,
 476 Rantala, H., Romanuk, T.N., Stouffer, D.B. & Tylianakis, J.M. (2012) Food webs:
 477 reconciling the structure and function of biodiversity. *Trends in Ecology &*
 478 *Evolution*, **27**, 689-697.
 479 Van Allen, B.G., Rasmussen, N.L., Dibble, C.J., Clay, P.A. & Rudolf, V.H.W. (2017) Top
 480 predators determine how biodiversity is partitioned across time and space.
 481 *Ecology Letters*, **20**, 1004-1013.
 482 Van Bael, S.A., Brawn, J.D. & Robinson, S.K. (2003) Birds defend trees from herbivores in
 483 a Neotropical forest canopy. *Proceedings of the National Academy of Sciences of*
 484 *the United States of America*, **100**, 8304-8307.
 485 Vesterinen, E.J., Lilley, T., Laine, V.N. & Wahlberg, N. (2013) Next Generation Sequencing
 486 of Fecal DNA Reveals the Dietary Diversity of the Widespread Insectivorous
 487 Predator Daubenton's Bat (*Myotis daubentonii*) in Southwestern Finland. *Plos*
 488 *One*, **8**.
 489 Vesterinen, E.J., Puisto, A.I.E., Blomberg, A.S. & Lilley, T.M. (2018) Table for five, please:
 490 Dietary partitioning in boreal bats. *Ecology and Evolution*, **8**, 10914-10937.
 491 Visakorpi, K., Wirta, H.K., Ek, M., Schmidt, N.M. & Roslin, T. (2015) No detectable trophic
 492 cascade in a high-Arctic arthropod food web. *Basic and Applied Ecology*, **16**, 652-
 493 660.
 494 Williams, R.J. & Martinez, N.D. (2000) Simple rules yield complex food webs. *Nature*,
 495 **404**, 180-183.
 496 Wirta, H.K., Vesterinen, E.J., Hamback, P.A., Weingartner, E., Rasmussen, C., Reneerkens,
 497 J., Schmidt, N.M., Gilg, O. & Roslin, T. (2015) Exposing the structure of an Arctic
 498 food web. *Ecology and Evolution*, **5**, 3842-3856.
 499 Yodzis, P. & Innes, S. (1992) Body size and consumer-resource dynamics. *American*
 500 *Naturalist*, **139**, 1151-1175.
 501 Zeale, M.R.K., Butlin, R.K., Barker, G.L.A., Lees, D.C. & Jones, G. (2011) Taxon-specific PCR
 502 for DNA barcoding arthropod prey in bat faeces. *Molecular Ecology Resources*, **11**,
 503 236-244.
 504

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3 Figure 1. Visual representation of the taxonomic distribution and quantified strength of
4 trophic links from each of our four focal odonate species to their prey. The blocks in the
5 upper row represent predators, with their size scaled to represent the estimated population
6 size. The blocks in the lower row represent prey families, with two most common prey
7 families highlighted. A line connecting a predator with a prey represents detected predation
8 events, and the thickness of the line represents the relative read abundance (RRA) of each
9 predation record (Supplementary material S1, Detailed material and methods: Eqn S1). See
10 the “Data analysis” in the main text for details on the RRA. The numbers below the lower
11 blocks correspond to the prey family numbers (Supplementary material S1: Table S4). Photo
12 credits: Göran Liljeberg & Hallvard Elven, Artdatabanken.no (CC BY-SA 3.0, Odonata),
13 Hallvard Elven, Artdatabanken.no (CC BY-SA 4.0, Cecidomyiidae, modified); František
14 Šaržík, biolib.cz (CC-BY, Chironomidae, modified).