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

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Running title: Vast predation by damselflies

Abstract

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

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

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

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

5. Our results suggest that relatively small damselflies exert a vast predation pressure on local 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.

Introduction

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

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

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

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

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

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

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

Materials and methods

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

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

Sampling and study site

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

Molecular dietary analysis

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

Data analysis

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

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

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

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

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

$$P_i = (W \times R \times N_i \times L) \quad (\text{Eqn 1})$$

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

Results

Predator population size

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

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

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

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

Prey composition

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

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

Predation rates quantified

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

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

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

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

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

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

Discussion

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

A new synthesis of new methods and classic ecological tools

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

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

Predation by damselflies is quantitatively vast and taxonomically widespread

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

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

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

Predation by damselflies in a community context

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

Conclusions

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

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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, biolib.cz (CC-BY, Chironomidae, modified).

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