Threats from the air: damselfly predation on diverse prey taxa

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Running title: Vast predation by damselflies

Abstract

1. To understand the diversity and strength of predation in natural communities, researchers must quantify the total amount of prey species in the diet of predators. Metabarcoding approaches have allowed widespread characterization of predator diets with high taxonomic resolution. To determine the wider impacts of predators, researchers should combine DNA techniques with estimates of population size of predators using mark-release-recapture (MRR) methods, and with accurate metrics of food consumption by individuals.

2. Herein, we estimate the scale of predation exerted by four damselfly species on diverse prey taxa within a well-defined 12-ha study area, resolving the prey species of individual damselflies, to what extent the diets of predatory species overlap, and which fraction of the main prey populations are consumed.

3. We identify the taxonomic composition of diets using DNA metabarcoding and quantify damselfly population sizes by MRR. We also use predator-specific estimates of consumption rates, and independent data on prey emergence rates to estimate the collective predation pressure summed over all prey taxa and specific to their main prey (non-biting midges or chironomids) of the four damselfly species.

4. The four damselfly species collectively consumed a prey mass equivalent to roughly 870 (95% CL 410–1,800) grams, over two months. Each individual consumed 29%-66% (95% CL 9.4–123) of its body weight during its relatively short life span (2.1-4.7 days 95% CL 0.74–7.9) in the focal population. This predation pressure was widely distributed across the local invertebrate prey community, including 4 classes, 19 orders, and *ca.* 140 genera. Different predator species showed extensive overlap in diets, with an average of 30% of prey shared by at least two predator species.

5. Of the available prey individuals in the widely-consumed family Chironomidae, only a relatively small proportion (0.76%; 95% CL 0.35%–1.61%) were consumed.

6. Our synthesis of population sizes, per-capita consumption rates and taxonomic distribution of diets identifies damselflies as a comparatively minor predator group of aerial insects. As the next step, we should add estimates of predation by larger odonate species, and experimental removal of odonates, thereby establishing the full impact of odonate predation on prey communities.

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 links 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 selected module of the community from its broader 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 between a single predator species and a 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 acquire a satisfactory understanding of predator-prey dynamics in wild populations 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; Vesterinen *et al.* 2016). 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; Rytkönen 2019). Now that we can identify the taxonomic distribution of prey species, we can begin to assess which species and species assemblages are likely affected by predation. Yet, to arrive at even a preliminary understanding of community-level predation pressure, these estimates should be combined with two other pieces of information: the abundance of predators and the food consumption of individual predators. For both items, recent methodological developments have brought important advances. The last 30 years have seen a rapid proliferation of advanced methods for making use of Mark-Release-Recapture (hereafter MRR) data, including sensitive estimation of population size, its temporal variation and local demographic or vital rates (Lebreton *et al.* 1992; Cooch & White 2018). In addition, metabolic theory and parameterized models for estimating individual consumption based on predator-prey body size (Yodzis & Innes 1992) or allometric relations (Gillooly *et al.* 2001) have regained traction (e.g. Pettersen *et al.* 2019).

Together, these three approaches offer a novel opportunity to study community-level distribution and strength of predation by different taxa on their prey taxa. Yet, to our knowledge, these three methodological advantages have not been utilized in tandem to quantify the predatory imprint of presumed-ecologically dominant predator taxa on their prey taxa, species assemblages or focal communities.

Odonates are globally distributed predators with aquatic larvae and terrestrial adults. At both stages, they are largely visual predators – adults deploy various hunting strategies including active foraging flights, sit-and-wait strategies coupled with sallying 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). Some of the largest information gaps relate to how the diets of predatory species overlap in space and time, and the combined impact of predatory species on prey species, populations and communities. In this context, a recent pilot study by Kaunisto *et al.* (2017) resolved the prey use by adults of three odonate species, and advanced odonates as key predators of airborne arthropod prey populations.

To test the validity of this proposal is not an easy task – as its resolution requires information on diets of different predator species, their survivorship, and population size estimates of both predators and prey. With this study, we rise to this challenge. 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 population sizes and adult lifespans of these damselflies, we conduct a MRR study, and to quantify prey population sizes, we use emergence traps from standardised surfaces. 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 substantial consumption of a wide range of prey taxa by adult damselflies. Yet, when related to the massive population size of their prey, the resulting predation pressure proves rather modest.

Materials and methods

To assess the predation pressure of damselfly species on their prey, we target four predatory species at a site in Southern Finland (located at ETRS-TM35FIN N: 67118; E: 2460). Beyond the focal taxa, the target area features a rich odonate fauna of some 27 species, including the substantially larger (but presumably less abundant) taxa e.g. *Aeshna* (the hawkers) and *Sympetrum* (the darters). Among these, the four target species were selected as the most common predatory species based on pilot surveys (K. Kaunisto, pers. obs.).

All four focal 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. For additional details on the methods, see supplementary material S1 (Detailed material and methods).

Study site and predator sampling

To assess the population sizes and survival rates of the focal species, we conducted a Mark-Release-Recapture (MRR) study of the damselfly populations associated with a freshwater pond of approximately 600 m x 200 m (12 ha). This pond is surrounded by meadows in a suburban landscape. To compare prey use among the focal species against the background of the pool of prey available to those species, all samples of predators were collected 5–8 m from the water body along a straight path of *ca*. 85 m. This census route was sampled 14 times (all the days with good weather for damselflies to fly and forage) between 31 May - 1 August, 2016. Individuals were captured with a sweep net, identified to species and sex, and marked with dark, non-toxic permanent pen on their hind wings. After marking, individuals were released and recaptured when encountered on subsequent sampling efforts.

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. Each individual was placed into a ‘clean’ 10-ml collection tube housing a piece of dampened paper towel to avoid desiccation. To allow complete defecation, damselflies were kept in the tubes for the next 24 h (defecation time according to Kaunisto *et al.* 2017) All the faecal material was collected from the tubes, after which the faeces were frozen in 15 ml Falcon tubes at – 64° C until further 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/10.5061/dryad.zs744j4z (Vesterinen *et al.* 2020).

Prey sampling

The key prey taxa consumed by the odonates in the study area spend their larval stage in water. This assumption was confirmed by reference to the composition of prey detected (see Results). Thus, we placed emergence traps on the water surface to estimate the density of the prey population, sensu individuals developing locally during the flight period of the odonates. The density of insects emerging per m^2 during the whole season was estimated by nine traps operated at three distinct sites for the same seasonal period as the current odonate study (31 May – 1 August). These pyramid-shaped traps were constructed from plastic pipe and mesh to catch emerging species (for details, see Supplementary material S1, Detailed material and methods: Sampling and study site and Fig. S1). The traps were operated during the summer of 2009 and all insects emerging from a surface of 1m^2 were trapped, with weekly catches counted and identified to species level (Lauri Paasivirta det.; for details, see Lilley *et al.* 2012). From these data, we extracted the weeks covered by our MRR study (31 May - 1 August, 2016), assuming that the phenology of the two years (2009 vs 2016) was roughly similar. To incorporate variation between samples, we retained individual counts and based downstream estimates on resampling from these (see below).

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 CONSUMED ABUNDANCES – We identified prey to the genus 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 opted to use read counts. More specifically, we used 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).

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:

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

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 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). The exact R code and data used in the calculations are provided in Supplementary materials.

As each factor in the product forming the quantity of key interest, P_i, comes with inherent variation, these multiple sources of variation should be weighed together to arrive at an estimate (and its associated uncertainty) of total prey biomass consumed. To this end, we used parametric and semi-parametric bootstrapping (Canty & Ripley 2019). In brief, indexing species by i, we sampled from the distribution of Wi, R, Ni and L, assuming each of them to be normally distributed with a mean characterized by the observed average and a standard deviation estimated from variation between individuals (for Wi), studies (for R), or model estimates (for Ni and Li). For 100,000 independent iterations, we multiplied these sampled values together to get a single estimate of consumption, using the 2.5%, 50% and 97.5% quantiles of the resulting distribution as our estimated lower confidence limit, mean and upper confidence limits, respectively.

To derive estimates of W_i, we used estimates published by Ilvonen and Suhonen (2016), according to whom 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 exactly matches that of *C. hastulatum* (Karjalainen 2010), we used the weight (and s.e.) of that species as accurate proxies. In other words, the weight of each species was associated with an SD of 6.1.

To derive estimates of R, we used published estimates of 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 14.15% as our joint estimate of R, with an SD of 2.78 (the number of studies referenced n=5).

To allocate the total prey biomass consumed by an individual predator species to individual Chironomidae genera, we use information on the proportion of overall sequence reads attributed to the respective genus. Altogether, we were able to gather the necessary information (prey mass and prey emergence for nine out of 11 assigned Chironomidae genera. We partitioned predator-specific consumption, P_i per odonate species, into fractions equalling the biomass of each individual prey genus consumed. To arrive at numbers of individuals consumed, we divided these weights with the mass per individual of this genus. For this purpose, we used individual-level weight data derived from the weighing of 1–36 individuals per genus (Hardwick *et al.*, unpublished). Using 100,000 bootstraps, we resampled individual prey masses from our empirical data, and divided the proportion of consumed biomass (calculated as explained above) with the prey individual mass estimate. This yielded an estimate of the number of prey individuals consumed per focal genus, with confidence limits, for each damselfly species.

To relate the numbers consumed to the number of prey individuals emerging within the study site, we bootstrapped the number of individuals separately per each chironomid genus and for all the chironomids emerging across 12 ha. By dividing the numbers consumed with the numbers emerging, we arrive at an estimate of the proportion of the prey population consumed, while propagating uncertainty across all levels. For details of the consumption rate analysis and the R-script, see the Supplements (R_script_and_data).

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 individuals (Table 1). Average residency time at our study site ranged from 2.07 days

(*C. lunulatum*) to 4.68 days (*E. cyathigerum*; Table 1). The confidence limits of these estimates were wide but did in no case overlap with zero.

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. S2). *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. S2).

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; 16S: 478 202) reads were assigned to prey families. Most of the filtered COI reads (50%) could be further identified to the genus level. For filtered 16S reads, ~31% were identified as target prey. This result should be considered relatively high for a template of prey DNA mixed with arthropod predator DNA (for an example, see Pinol *et al.* (2014), where only 6% of reads were assigned to prey in a study without blocking primers). In the current study, the most common prey order was Diptera, and the most abundant prey family was Chironomidae (Fig. 1). The four predator species showed extensive but far from complete overlap in the prey use (Fig. 2), with 30% of prey on average shared by at least two predator species. The most common prey taxa (Chironomidae) were widely consumed by all odonate species, and by both sexes (Supplementary material S1, Additional results: Fig. S3). In addition to Chironomidae, the predation pressure by the focal damselflies distributed widely across the local invertebrate prey community, including 4 classes, 19 orders, and *ca.* 140 genera (Supplementary material S1).

Predation rates quantified

Across species (Fig. 1; Supplementary material S1, Additional results: Eqns S2a–d), damselfly individuals consumed *ca.* 900 g of prey per summer (exact mean mass 872 g, 95% CL 409–1,812). Focusing on an important prey group, the non-biting midges (Diptera, Chironomidae), this amounts to 700,000 individuals (95% CL 320,000–1,470,000) 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 135 chironomids during its adult life. This figure should be related to our data from the emergence traps, which suggests the local productivity of chironomid prey to amount to 763 (95% CL 709–817) individuals per m^{-2} during season. Converted to an area of 12 ha (the surface of the water body of our study site), this equals to 91,560,000 individuals (95% CL 85,080,000–98,040,000). Thus, the four focal odonates will together consume 0.76% (95% CL 0.35–1.61; Fig 1C) of the key prey populations emerging locally.

Discussion

Our results suggest that damselflies exert taxonomically widely-distributed imprint on local insect populations, species assemblages and communities, yet remove a relatively small fraction of all prey individuals in the respective populations. 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.

Predation by damselflies is 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 larvae of 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. 1B). Despite being taxonomically widespread, we show that the overall predation rate exerted is low among the typical prey of damselflies (i.e. 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 (weight of *P. longipennis* adults 0.1 – 0.25 grams) (Baird & May 1997). This is consistent with our observations of chironomids consumed, where the average weight was 0.16mg and 95% of weights fell within the interval 0.13–0.19 (Supplement Fig. S5).

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 predation rates reported here point to limited community-level impacts of damselfly on the local prey communities, since our rough estimates of the proportion of prey individuals consumed ranged from 0.3% to 1.6%. While impacts on prey populations depend on what relative fraction of individuals is consumed, there is also an absolute dimension to this. We note that just the four damselflies observed here will consume roughly 0.9 kg of prey insects and 5.4 million prey individuals. If *not* consumed, this mass would offer a substantial amount of food for other predators, including birds and bats.

Our current figures may well be underestimates, since several considerations suggest that the realized number of prey items may be higher than observed. Damselflies tend to discard less nutritious parts of their prey before ingestion (reviewed in Corbet 1999), thus reducing their representation in the faecal DNA contents. 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 most likely consume prey in considerably higher numbers and/or mass than their smaller relatives. For example, assuming a similar relation between body mass and prey consumed (14.15% of body mass consumed per day; see above), a single *Aeshna grandis* individual would consume 30 times more prey per day than an individual of our target damselfly species. Thus, for a full appreciation of the community-wide predation pressure exerted by odonates – not only damselfly – we should next derive MRR estimates for multiple species in the local community. The current study shows that and how it can be done.

Predation by damselflies in a community context

Our findings reveal odonates 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 strength of the 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 abundance may not result in dramatic 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.

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.

Uncertainties, limitations and future directions

In the current study, we have made every effort to propagate known sources of variation into our overall estimates. Needless to say, each quantity involved comes with chance variation, and our estimates are thus associated with wide confidence limits (see Fig. 1). We should also stress that the prey insects involved have high fecundity (e.g. Neems, Lazarus & McLachlan 1998), and that mortality of adult individuals to predation may have little impact on actual population determination or regulation. While both factors limit the scope for conclusive inference regarding the final impact of odonate predation on prey demography and community dynamics between years, these are aspects which are best addressed through manipulative experiments, e.g. exclusion by mist nets (Maine & Boyles 2015). In this context, our study generates a clear, testable hypothesis: the exclusion of odonate predation should increase densities of a wide range of prey by a relatively low amount, and this rebound should be taxonomically widespread, with no particular prey group disproportionately benefitting from predator release (se Fig. 1B). In other words, the experimental removal of damselflies should come with an effect very different from that 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).

As the basic building block needed to understand the forces regulating animal populations, and to estimate flow of energy and matter through food webs, we need sound estimates of who is eating whom and in what quantities. We hope that our study stimulates the quantification of key variables helping to resolve food web linkages and dynamics. Our current estimates take the essential first step towards understanding top-down versus bottom-up regulation of insect communities, and should next be supplemented by even wider community-level MRR studies, numerical estimates of prey demographics and manipulative field experiments.

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Table 1. Best-fitting Jolly-Seber models (POPAN parameterization) and associated demographic estimates for each damselfly species (estimated total population size and average residency estimate during the sampling period). The difference between each listed model's *AICc* score and that of the species' next best competing model was >2. Both survival (ϕ) and probability of entry (*pent*) for *C. hastulatum* were dependent on time following a polynomial. *pent* for all three other species was time-dependent as a factorial ($pent_{(t)}$). Survival was linearly time-dependent for *C. lunulatum* and *E. cyathigerum* ($\phi_{(T)}$), and time-independent (constant; $\phi_{(.)}$) among *C. pulchellum* individuals during the sampling period.

Species	Model	*AICc*	Parameters	Overall population size (95% CL)	Average residency estimate, in days (95 % CL)
Coenagrion lunulatum	$\phi_{(T)}p_{(.)}pent_{(t)}$	168.87	16	7686.09 (3746.73 - 15767.37)	2.07 (0.74 - 7.89)
Coenagrion hastulatum	$\phi_{(T2)}p_{(.)}pent_{(T2)}$	94.02	8	5960.10 (1463.93 - 24265.39)	2.68 (1.18 - 7.02)
Coenagrion pulchellum	$\phi_{(.)}p_{(.)}pent_{(t)}$	156.24	15	6210.76 (2895.25 - 13323.06)	3.75 (2.77 - 5.19)
Enallagma cyathigerum	$\phi_{(T)}p_{(.)}pent_{(t)}$	314.79	16	22540.08 (13221.14 - 38427.49)	4.68 (2.88 - 7.90)

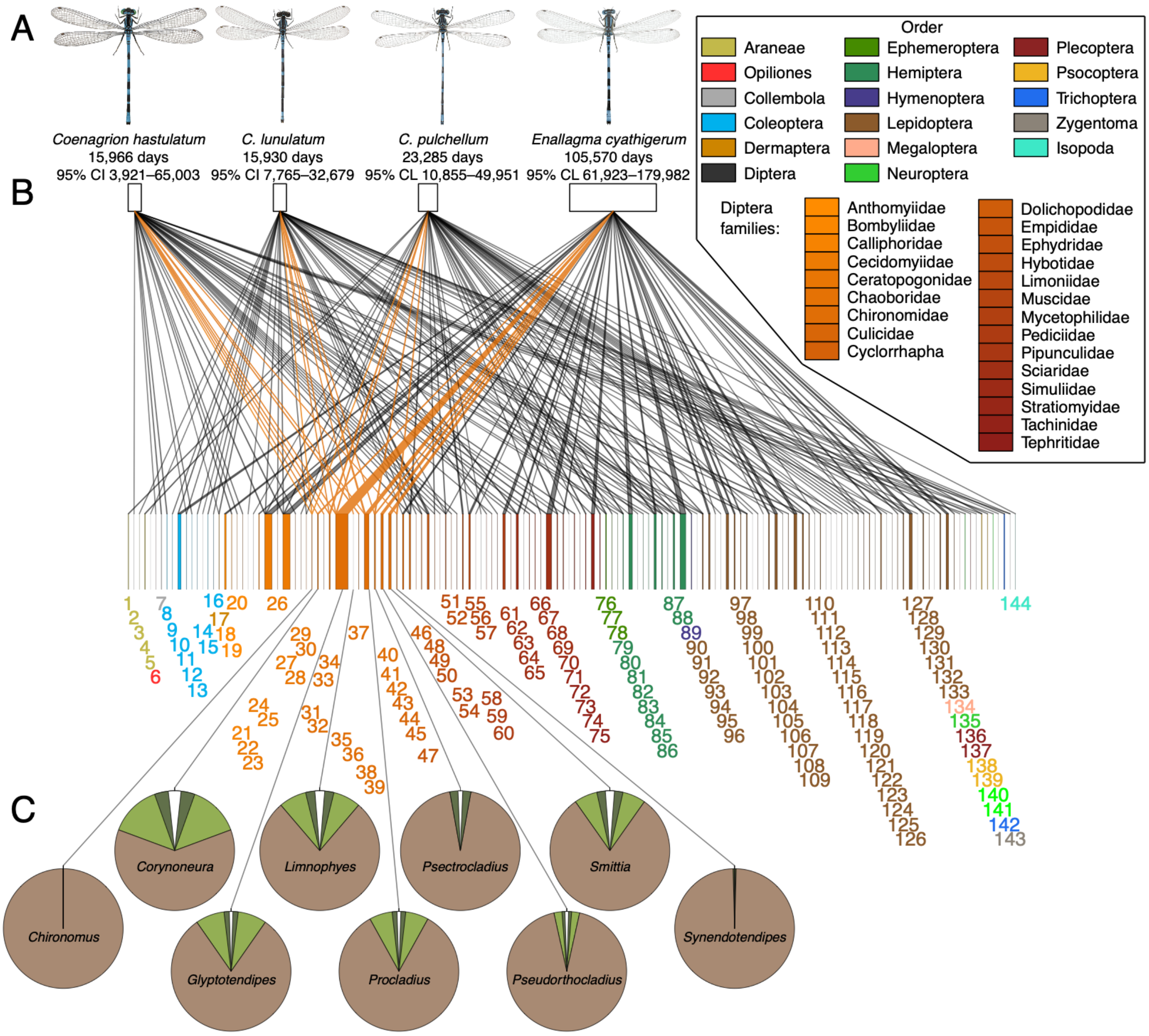

Figure 1. A) The four damselfly species studied here, and their estimated total number of individual-predation days with 95% confidence limits. B) Visual representation of the taxonomic distribution and quantified strength of trophic links from each of our four focal odonate species to their prey. The blocks in the upper row represent predators, with their size scaled to represent the estimated damselfly abundance and residency. The blocks in the lower row represent prey genera. A line connecting a predator with a prey represents predation events detected by molecular methods (Chironomidae highlighted in orange), 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). C) The pie charts show the proportion of available prey consumed by the four focal species, during their flight period. The dark green sector shows the median estimate, the thin middle white slice the lower 95% confidence limit, and the wide light green slice the upper 95% confidence limits. See supplement R_script_and_data for exact methods and script. Numbers below prey taxa refer to taxonomic names given (Supplementary material S1: Table S4)

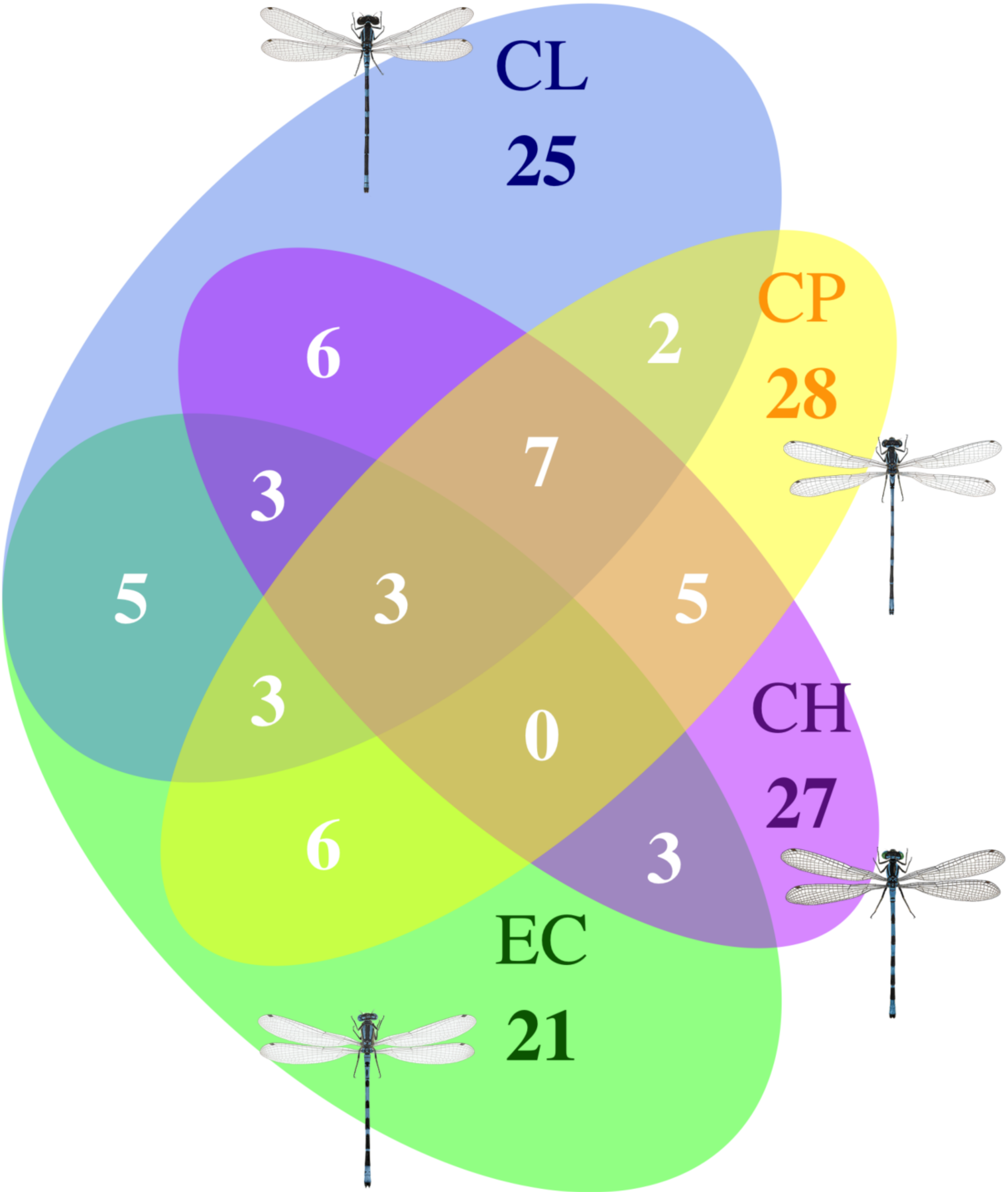

Figure 2. Overlap in prey use between the four odonate species (CL = *Coenagrion lunulatum*, CP = *C. pulchellum*, CH = *C. hastulatum* and EC = *Enallagma cyathigerum*). The Venn diagram shows the number of prey genera used exclusively by single damselfly species

(number on pure colours), and by multiple species (numbers on overlapping sections). Altogether, three taxa where shared by all four damselflies (number in the middle).

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