Running Head: Invertebrate predator-prey interactions

Title: Predator-prey interactions of terrestrial invertebrates are determined by predator body size and species identity

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**Abstract**

Predator-prey interactions shape ecosystem and can help maintain biodiversity. However, for many of the earth’s most biodiverse and abundant organisms, including terrestrial arthropods, these interactions are difficult or impossible to observe directly with traditional approaches. Based on previous theory, it is likely that predator-prey interactions for these organisms are shaped by a combination of predator traits, including body size and species-specific hunting strategies. In this study, we combined diet DNA metabarcoding data of 173 individual invertebrate predators from nine species (a total of 305 predator-prey interactions) with an extensive community body size dataset of a well-described invertebrate community to explore how predator traits and identity shape interactions. We found that 1) mean size of prey families in the field usually scaled with predator size, with species-specific variation to a general size scaling relationship (exceptions likely indicating scavenging or feeding on smaller life stages). We also found that 2) although predator hunting traits, including web and venom use, are thought to shape predator-prey interaction outcomes, predator identity more strongly influenced our indirect measure of the relative size of predators and prey (predator:prey size ratios) than either of these hunting traits. Our findings indicate that predator body size and species identity are important in shaping trophic interactions in invertebrate food webs and could help predict how anthropogenic biodiversity change will influence terrestrial invertebrates, the earth’s most diverse animal taxonomic group.

**Keywords**

Allometry, arthropod, centipede, DNA metabarcoding, hunting strategy, insect, spider

**Introduction**

Predator-prey interactions shape the structure and function of ecosystems and their responses to external influences, including anthropogenic global change (McCann 2000, Brodie et al. 2014). Species interactions are at risk of extinction following or even preceding species loss, meaning losses of interactions that shape ecosystem structure and function (Borrvall and Ebenman 2006, Valiente-Banuet et al. 2015, Donohue et al. 2017). Given these challenges, being able to understand what shapes predator-prey interactions may help understand the consequences of species loss and may help in predicting and preventing the loss of interactions and species (Brodie et al. 2014, Brose et al. 2017).

However, we have little observed interaction data for small-bodied invertebrate predator species for which empirical diet methods (e.g. gut dissections) are impossible or unfeasible to conduct (Sheppard and Harwood 2005, McLaughlin et al. 2010, Gravel et al. 2013). The lack of empirical interaction data for small-bodied invertebrate taxa is not inconsequential; these taxa represent over 50% of the earth’s animal biomass (including terrestrial and marine systems) and most animal species diversity (Mora et al. 2011, Costello et al. 2013, Bar-On et al. 2018, Stork 2018). Without these data, we cannot validate extrapolated approaches to predicting interactions based on general rules. For these consumers, species interactions are often inferred from literature reports of observed interactions from phylogenetically-related species (Simberloff and Wilson 1969, Piechnik et al. 2008, Laigle et al. 2018), based on body size feeding constraints (Digel et al. 2014, Laigle et al. 2018, Hines et al. 2019), or derived from mesocosms or feeding trials which include only pre-defined predator-prey identity pairs (Rall et al. 2011, Kalinkat et al. 2013, Digel et al. 2014, Rudolf et al. 2014, Guzman and Srivastava 2019). Thus, because these interactions are not empirically observed in natural environments, we do not know whether patterns that emerge for these interactions are real broad ecological patterns or artefacts of the rule-based diet assignment methods used to compile them.

Adding empirical data to understand how predator-prey interactions of invertebrate consumers are shaped will help to understand what general rules can predict these interactions. Traditionally, predator-prey interactions have been approached from a species-specific framework; specifically, emphasis is placed on how species identity or phylogenetic relatedness shape feeding interactions (Ings et al. 2009). However, more generalizable predictions of feeding interactions can be made using non-specific traits. Body size, for example, is a key trait that determines feeding interactions between predators and prey across ecosystems (Woodward et al. 2005). Because body size is integral to feeding interactions, both dictating the rate and range of prey a predator can consume, it is one of the primary approaches for predicting the structure of feeding interactions for biological communities, or food webs (Stouffer et al. 2005, Woodward et al. 2005, Gravel et al. 2013, Nakazawa 2017). Whereas body size alone predicts general patterns across food webs in multiple contexts, combining body size with more species-specific characteristics, including species identity, and more broadly, species traits such as locomotion or metabolic group, creates food-web models that look even more similar to empirically-observed patterns (Rudolf et al. 2014, Gray et al. 2015, Brose et al. 2019, Pomeranz et al. 2019, Potapov et al. 2019). Using general traits to describe food-web patterns across ecosystems is not only important for the development of generalizable rules describing patterns in biological communities but could also be integral to predicting and mitigating species extinctions given the rate of anthropogenic species loss (Valiente-Banuet et al. 2015).

In this study, we employ novel diet DNA metabarcoding data from 173 samples of nine terrestrial invertebrate predator species to document predator-prey interactions between these predators and their prey in field conditions, which included 305 unique predator-prey interactions. We matched these data to observed data on predator body size and average body sizes of prey families from an extensive dataset of observed body sizes for the prey groups identified in predator diets. To understand how predator size, species identity, and hunting traits may drive empirical predator-prey interactions, we asked: 1) do larger predator individuals eat prey species in families with larger body sizes and does this vary by predator species identity? and 2) do predator species traits related to hunting strategy explain variations in prey size selection, or is prey size selection based on predator phylogeny?

**Materials and methods:**

*Field site and collections*

We conducted this work on Palmyra Atoll National Wildlife Refuge, Northern Line Islands (5º53’ N, 162º05’W). Palmyra Atoll has a well-characterized species list, and like many atolls, is relatively species poor, allowing for detailed characterization of potential diet items (Handler et al. 2007). Predator individuals were collected across habitat types, including different forest types and microhabitats (e.g., understory vegetation, canopy vegetation, and soil types). For each of these habitat types, we used a combination of methods, including individual collection during visual surveys for understory, and soil collections and canopy fogging with insecticide onto collection sheets for canopy individuals. All individuals were collected individually with sterilized implements (ethanol-burned forceps) in sterilized collection containers containing 95% EtOH to avoid contamination (Greenstone et al. 2011). All individuals were stored in 95% EtOH at -20ºC before DNA extraction.

We identified all predators to morphospecies using a species list for Palmyra Atoll (Handler et al. 2007) and later validated unique species by DNA metabarcoding sequence data. The predators sampled represent the most common predator species found in each habitat location and span a body size range of 0.2 – 998 mg (wet mass, Figure 1). These predators included five arachnid species (*Opopaea sp*., *Neoscona theisi*, *Heteropoda venatoria*, *Smeringopus pallidus*, and *Scytodes longipes*), one dragonfly (*Pantala flavescens*), one predatory katydid (*Phisis holdhausi*), one earwig (*Euborellia annulipes*), and one soil-dwelling centipede species (*Mecistocephalus sp*.). These predators use various hunting tools, including webs and venom and employ several different hunting strategies, including active hunting and non-active hunting (e.g., sit-and-wait or ambush, SI Table 2).

*DNA extraction, PCR amplification, library preparation, sequencing, and denoising*

Our full DNA extraction, PCR amplification, library preparation, sequencing, and denoising methods can be found in the Supplementary Information. Here we provide an abridged version.

To determine the identity of prey DNA in predator diets, we extracted and sequenced DNA from samples consisting of one or several predator individuals using high throughput sequencing methods. Multiple predator individuals were combined due to small body size (thus, inability to extract ample DNA) based on shared size (mean length difference ± 0.5 mm), species, and sampling period (70%, or 121/173 samples consisted of one predator individual, and 52/173 consisted of two or more individuals, Supplementary Methods and SI Figures 6 & 7). We extracted DNA from predator samples using a modified CTAB protocol and following methods outlined in (Krehenwinkel et al. 2017). We amplified the CO1 gene with general metazoan primers (mlCOIintf/Fol-degen-rev; (Yu et al. 2012, Leray et al. 2013, Krehenwinkel et al. 2017)) and sequenced samples on the Illumina MiSeq platform with 250 paired-end reads. We merged, filtered, and denoised our sequences to amplicon sequence variants (ASVs) using the DADA2 package in R (v1.1.14.0; (Callahan et al. 2016), SI Figures 2 & 3). We removed samples from analysis with incomplete sequencing depth using interpolation and extrapolation methods (Hsieh and Chao 2017) and then rarefied all sequencing depths to the lowest sequencing depth of remaining samples (15, 954 reads). We performed these steps in R (version 4.0.2) with the iNEXT (version 2.0.20, (Hsieh et al. 2016)) and vegan (version 2.5.6) packages.

*ASV taxonomic assignment*

To determine the identity of the sequenced DNA, we compared sequencing data to the GenBank and BOLD taxonomic databases. GenBank searches were run using the computing cluster at UC Santa Barbara. We chose to combine prey taxonomies at the family level, similar to diet resolution in both metabarcoding and histological methods in this field (Kartzinel et al. 2015, Brose et al. 2019, Eitzinger et al. 2019) summing the cumulative rarefied read abundances across the ASVs that corresponded to each diet family in each sample. Family-level data provides information comparable to previous studies; additionally, on Palmyra, each family corresponds to an average of 1.9 (± 0.13 SE) species, so a family-level taxonomic assignment may closely mirror species-level assignments. We corrected for potential sequence jumping (i.e., ‘cross-talk’) across samples by removing reads across samples that emerged in negative controls (Oono et al. 2020) and all DNA matching any predator family present on an individual sequencing run was removed as a conservative method to account for potential sequence jumping (van der Valk et al. 2020). We verified ASV specificity based on positive control samples (SI Figure 8)

*Predator and prey size determination*

We measured the length of each predator individual from the front of the head to the end of the abdomen prior to DNA extraction. We converted predator lengths to wet mass using mass-length scaling relationships for each predator species from existing datasets ((Yaninek and Gnanvossou 1993, Sohlström et al. 2018b, Su et al. 2020). Prey species masses were taken as the average mass for individuals across species within each family (SI Figures 10 & 11). Averaging prey size by family and using average prey masses in predator-prey mass scaling studies is a common method in the field (SI Figure 12), and though not being able to assign prey mass is a limitation of diet DNA metabarcoding data, compiling data in this way allows for comparisons with recent synthetic studies (Brose et al. 2019). In other words, here, we do not report the size of prey individuals that were eaten; rather, for the prey families that were eaten, we report their average body sizes observed in the field.

*Data analyses*

To determine whether individual predator size, species, or both predicted prey size, we fit a linear mixed effects model with the response variable of log10 prey mass (in mg) and predictor variables of log10 predator mass (in mg), species identity, and their interaction, with random intercepts by predator individual to account for dependence among multiple prey species observations within each individual predator. Then, to explore whether predator hunting traits or predator phylogenetic relatedness influences predator-prey size ratios, we divided predator-prey interactions based on whether or not the predator species uses webs to capture prey or uses venom to subdue prey. We determined the ratio of predator to prey size for each of these interactions (raw predator mass/prey mass) and then built a set of linear mixed models of this ratio (log transformed for data normality) as the response variable, with each type of predator trait as a predictor variable (one model with web-building and one with venom use). We compared these to two predator species relatedness models – choosing to compare the ratio of predator to prey size based on predator species and predator class, with the aim to determine whether, if hunting traits did not influence size selection, individuals within shared taxonomic groups had conserved size ratios. In each of these models, we used a nested random intercept term of predator individual within species. We considered the species model to be the model without any fixed effects and the random effects of the other models (i.e., including predator individual and predator species).

*Statistical model selection*

For the linear mixed effects models examining how predator size and species identity shape prey size, we performed model selection using the dredge() function in the MuMIn package in R (package version 1.43.17, (Barton 2020)) to compare nested models (n = 5 models) and chose the model with the lowest AICc value. To compare the predator trait and phylogeny models, we performed model selection by comparing AICc values for these models (along with a null model with no predictor variables [n = 5 total models]). For all models, we verified model assumptions using the DHARMa package in R (version 0.3.3.0, (Hartig 2020)). The color palette in our figures is from the calecopal package (version 0.1.0, (Bui et al. 2020)).

**Results**

*DNA extraction, PCR amplification, library preparation, sequencing, denoising, and ASV taxonomy*

Complete results and QC for each step of the DNA sequencing protocol can be found in the Supplementary Information. Raw data are available on GenBank (BioProject: PRJNA715709) and our code and analyses are currently hosted in a GitHub repository (https://github.com/anamtk/DNA\_predators). Code and analyses will be published on Dryad prior to publication.

Our final analyses were performed on a total of 173 predator samples of nine species. Each predator sample contained 1–7 (average 1.76 ± 1.08 SD) prey families. Thirty percent (n = 524 of 1,738 total ASVs) of the total ASVs found in samples received taxonomic assignments from GenBank and BOLD, corresponding to prey items at the family level or lower (n = 48 prey families, 14 orders; Figure 3, SI Table 3). Although the predators used in this study represent species at the larger end of the community size spectrum (Figure 1), prey item size distribution resembled the community-wide size distribution (Figure 2).

Predator diet items varied by predator species with the widest diversity of prey items in the order Diptera and the most frequently consumed prey items in the orders Hymenoptera, Lepidoptera, and Orthoptera (Figure 3). Some predator species (including *Mecistocephalus sp.*, *E. annulipes*, *S. pallidus*)had common diet items across most individuals; for example, most *Mecistocephalus sp.* (a centipede) individuals ate collembola (Family: Isotomidae) and most *E. annulipes* (an earwig) individuals ate katydids (Family: Tettigoniidae). Conversely, some predator species (including *Opopaea sp.*, *N. theisi*, *P. flavescens,* and *S. longipes*) had more diet diversity with fewer shared diet items across individuals. Diet families by species are visualized in Figure 3 and summarized in SI Table 4.

*Prey size and predator:prey ratio predictors, and predation strategy*

The best performing model of prey size included the terms of predator mass and predator species identity, but not their interaction, with variation in by-species intercepts (Figure 4, SI Table 6) (β = 0.32, p-value = 0.001). The predator trait or species relatedness model that most explained variation in predator:prey size ratio was the model that included predator class as a predictor, followed by the predator species model. In the predator class model, there were statistically significant post-hoc differences between Arachnida and Chilopoda predators and no others (Figure 5, SI Table 7).

**Discussion**

For terrestrial invertebrate predators like the ones in our study, comprehensive field-based diet analyses have been nearly impossible or time-prohibitive without genetic methods (Polis 1991, McLaughlin et al. 2010). By combining diet DNA metabarcoding data with a well-documented species list with body size data, our study addresses such limitation and provides important empirical examination of interaction patterns for these consumers. We found that predator size and species identity are important drivers of prey size selection and resulting interaction patterns. Specifically, we 1) found that larger predator individuals do eat prey families with larger body sizes, however, individuals of some predator species eat proportionally smaller or larger prey families than would be expected by one general cross-species relationship. Then, we 2) demonstrate that predator species is a strong driver of predator:prey size ratios; no hunting strategies related to hunting tools (e.g. webs and venom) relaxed size constraints consistently across species that possessed those traits. The cross-species predator-prey size scaling relationship in our study is consistent with the combined body size and metabolic group scaling relationship from a recent synthetic study (Brose et al. 2019), though adds important empirical data to a relationship which has previously been built off inferred data for this predator group (SI Figure 12). Furthermore, our data suggest that phylogenetic similarity is important for determining predator-prey interaction outcomes. These results highlight that many food-web patterns in small, terrestrial invertebrate predator-prey interactions may be explained by a combination of predator species characteristics and that not one predator attribute alone predicts all interactions (Pomeranz et al. 2019).

Our results highlight the need for combining multiple predator attributes, including body size and species identity for explaining and predicting food web patterns (Raffaelli 2007, Rall et al. 2011, Rudolf et al. 2014). In our results, samples from predator species that may be more limited in prey sizes they can attack or handle (e.g., *Pantala flavescens* and *Mecistocephalus sp*.) ate prey from families with smaller mean body sizes compared to predators of similar or even smaller size that may be able to attack or handle larger prey (e.g. the spider predators, order Araneae). Whereas both sets of feeding interactions are still constrained by individual predator and prey size, these constraints vary depending on predator identity. Although we did not see evidence that prey family body size was specifically related to tools such as webs or venom, determining what allows predators to relax size constraints is a fruitful area of future study.

Almost a quarter (24% or 72/305) of the interactions in our dataset involved predators that were smaller than the mean size of prey families they consumed, seemingly violating assumptions that predators generally eat prey smaller than themselves (Nakazawa et al. 2013). Some of these interactions may, indeed, suggest that invertebrate predators possess traits that allow them to relax size-based feeding constraints. Conversely, it is important to note that these DNA diet data represent mean values for prey families as opposed to the prey individual consumed. This might explain why the earwig in our dataset (*E. annulipes*) fed on relatively large prey families (mean ± standard error of predator-prey mass ratio = 4.35 ± 1.99:1). A predator like an earwig might not be able to eat a large cricket but might easily eat its eggs or scavenge adult carcasses (Rudolf and Lafferty 2011, Wilson and Wolkovich 2011). Thus, while average prey size data may mis-represent the size of prey individuals consumed, these data revealed interaction pairs that would be deemed unlikely or impossible if using body size ratio “rules” based on average prey sizes. Thus, DNA diet data reveals predator-prey interactions that would not be included in food-web models based on mean prey size and predator traits.

Predator hunting strategies, such as web and venom use, have gained attention as important drivers of interactions in invertebrate food webs (Schmitz 2008, 2009, Laigle et al. 2018) and are often a primary way in which interactions are inferred (Digel et al. 2014, Hines et al. 2019). In our dataset, individual species deviated from a general predator-prey body size scaling relationship, and the traits that have previously gained traction for increasing relative prey size (e.g. venom or web use) do not consistently seem to do so across species; this suggests an evaluation of what other traits of predator species may shape the size constraints of predation interactions. For instance, although venom and webs might be useful traits for exploiting large-bodied prey, scavenging is an alternative way to eat large prey (Wilson and Wolkovich 2011). Or it may be that these interactions are more dictated by prey as opposed to predator traits (e.g. predator-prey matching, Gravel et al., 2013; Pomeranz et al., 2019). Phylogenetically similar spider species may have distinct ecological niches, especially on islands (Kennedy et al. 2019), and it may be that general patterns of predator-prey interactions may be as much about relative sizes as matching of other predator-prey traits (Gray et al. 2015, Pomeranz et al. 2019).

Diet DNA metabarcoding will continue to be an important tool in understanding the biology of small-bodied invertebrate consumers because it allows us to examine invertebrate diets at the individual level, with the same resolution as that of the diets of larger-bodied species (Hyslop 1980, Duffy and Jackson 1986, Baker et al. 2014). As DNA sequence databases continue to grow (Porter and Hajibabaei 2018), these analyses will likely get more specific and potentially surpass the resolution of other methods (e.g. gut dissection) even for non-invertebrate consumers (McElroy et al. 2020). For example, rather than being confined to family-level taxonomic assignments, future studies, or re-evaluations of past data could reveal a greater depth of species-level data. Although individual body size data had high resolution for the predators included in this study, we are still limited in knowing the abundance or realized size of prey items consumed by these predators because read abundance may not accurately correspond to prey biomass (Elbrecht and Leese 2015, Elbrecht et al. 2017). Data from DNA can indicate the prey families a predator eats, whereas experimental feeding trials could help to identify constraints on individual prey sizes or determine preferences for live versus dead prey (Rall et al. 2011, Wilson and Wolkovich 2011). This combination of methods is a promising next step in the field that may reveal important stage structure in invertebrate feeding interactions and even stage specialists (e.g. egg specialists) in apparent general diet assemblages based on DNA metabarcoding alone (Rudolf and Lafferty 2011, Rudolf et al. 2014). Concurrently, combining multiple genetic methods, such as the use of age-based biomarkers in RNA and DNA sequencing to determine diet age, or amino acid racemization to determine time since prey death, could help determine the age or size of prey and the degree to which predators rely on scavenged food sources, though these methods remain untested in predation interactions (Jarman et al. 2015, Macías-Hernández et al. 2018, Nielsen et al. 2018).

Small-bodied invertebrate predators (both terrestrial and marine) are the most diverse and abundant predators on earth (Mora et al. 2011, Costello et al. 2013, Bar-On et al. 2018) and until now, the predation interactions of these consumers in the wild have been largely unknown. Like other predators in multiple other ecosystem contexts (Brose et al. 2019), the predation interactions of small-bodied predators are driven by a combination of measurable and generalizable predator attributes, including body size and species identity. Using empirical datasets, such as those built by diet DNA metabarcoding data, will be key to determining which traits shape and mediate species interactions. Not only will this information build a deeper understanding of the generality of feeding interactions and food webs across environmental contexts and consumer groups, but could be key to predicting and mitigating ongoing biodiversity loss (Borrvall and Ebenman 2006, Valiente-Banuet et al. 2015, Donohue et al. 2017). Given the growing evidence of global terrestrial invertebrate declines (Desquilbet et al. 2020, van Klink et al. 2020) and the importance of these organisms to broader ecosystem functions, empirical information such as that provided in the present study is critical to develop models and generalizable rules that will aid in understanding and predicting the effects of global change on Earth’s ecosystems.

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**Author Contributions**

AM-tK, AA, and HY conceived the ideas of this study. AMtK, AB, EF, DO, ML, TB, JC, RD, MK, KL, JM, CM, KP, and DW conducted field work for this study. AM-tK, AA, AB, RB, TB, MK, ML, MM, JM, KP, and RY conducted laboratory work for this study. AMtK led the writing of this manuscript with conceptual contributions from AA, AB, BD, EF, ML, DO, DP, RD, KL, and HY and all authors provided editorial and intellectual feedback on aspects of the manuscript. All authors have read and agree to the content of this manuscript.

**Data Availability**

Raw DNA sequencing data for this project can be found on GenBank (BioProject: PRJNA715709). Processed data, code, and analyses can be found on GitHub (https://github.com/anamtk/DNA\_predators) and will be published on Dryad upon article acceptance. Additional data were drawn from Sohlström, Lucas, et al., 2018; Sohlström, Marian, et al., 2018; Su et al., 2020; and Yaninek & Gnanvossou, 1993.

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**Figures**

**Diagram

Description automatically generated**

Figure 1: Predator size distributions of predator individuals across the nine predator species. The x-axis scale depicts absolute values but has been log10 transformed. Predator individuals span from 2x10-1 mg (*Opopaea sp*.) to 9.9x102 mg (*H. venatoria*) in wet weight. The facets in this figure have been ordered by increasing predator species mean size.

Chart, histogram

Description automatically generated

Figure 2: While the predator species in this study skew toward the larger side of the size spectrum of the Palmyra community (dark grey: predator species, light grey: community), the prey families detected in DNA data (medium grey) represent much of the range of the community size spectrum.

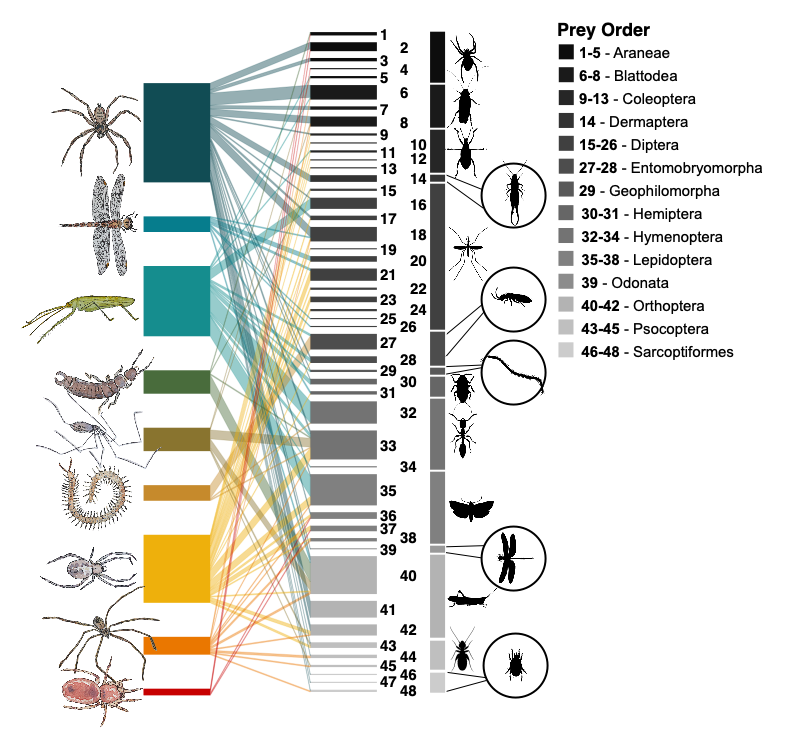


Figure 3: Prey families (right bars) detected in the DNA of predator samples (left bars). The width of the predator bars vary due to sample size, the width of the line (interaction) connecting each predator to each prey represents the frequency of that prey family in that predator species sample, and the width of the prey bar corresponds to the number of times that prey family occurs in any predator’s diet. Prey families correspond to 48 families of 14 orders of arthropods, including arachnids, collembola, and insects.



Figure 4: A log10-log10 transformed relationship shows that larger predators eat larger prey families (panel (a), slope = 0.32), though the effect is mediated by predator species identity (b). The dashed line in panel (a) represents the 1:1 relationship between predator and prey size. Continuous axis labels represent absolute values but the scale between them has been log10 transformed. In panel (b), “+” and “-“ symbols indicate species that either have significantly higher (“+”) or lower (“-“) prey family sizes relative to predator body size and the general predator-prey body size patterns.

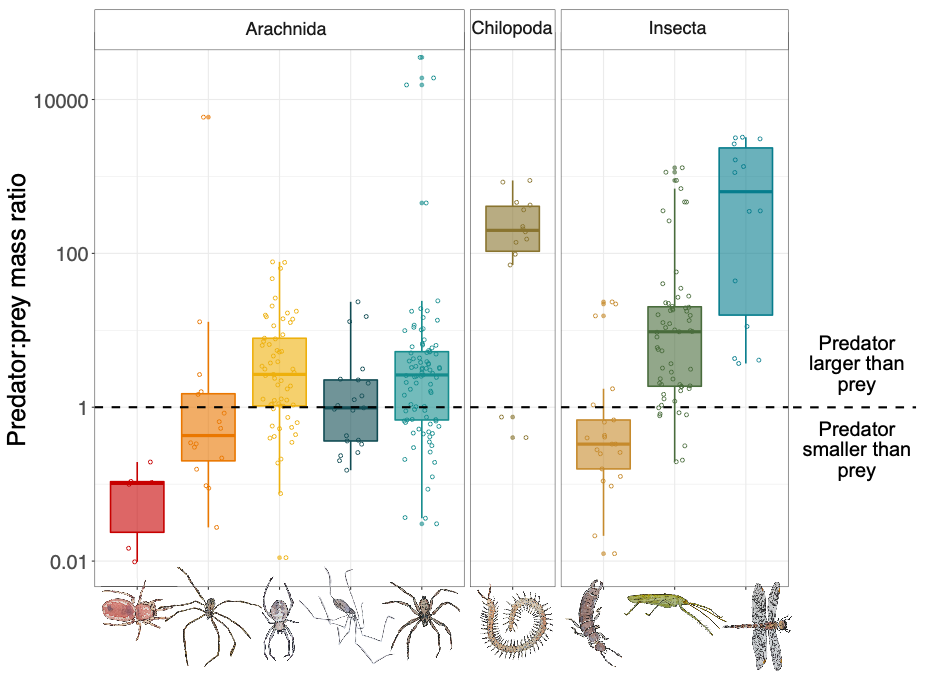


Figure 5: Predator class identity was a stronger predictor of predator:prey size ratios than specific hunting traits (e.g. web or venom use). In this figure, the dashed line indicates interactions where predators are the same size as prey families they consumed. The y-axis is presented with absolute values but displayed on a log10-transformed scale to demonstrate the spread in the data. Twenty-four percent (72/305) of the interactions in our dataset corresponded to predators eating prey families with average sizes larger than themselves (interactions below the dashed line), contrary to assumptions about size-based predation interactions.