**Supplementary and Expanded Methods**

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

We individually measured the length of each predator (mm) and separated the thorax, opisthosoma, or trunk (depending on predator species, (Krehenwinkel, Kennedy, Pekár, & Gillespie, 2017; Macías-Hernández et al., 2018)) for DNA extraction following a modified CTAB extraction protocol (Fulton, Chunwongse, & Tanksley, 1995). While most individuals were run in separate samples (70%, n = 121/173), some individuals were too small to extract ample DNA from only one individual (mean size of 4.04 ± 0.12 mm in total length), and so we combined these individuals with other individuals from the same species, size range (within ± 0.5 mm in length), and sampling period. For these combined samples, we aimed for a minimum total sample weight of 5mg, and ideal sample weights of 10-20mg, a range we had previously determined to be sufficient for downstream DNA extraction and cleaning protocols. This resulted in a maxiumum of 12 individuals in one sample (SI Figure 6). Following methods in (Krehenwinkel et al., 2017), we standardized concentrations of 40uL of each sample to 20ng/ul and used Ampure XP (Agencourt, Beverly, MA, USA) beads to remove higher molecular weight predator DNA prior to PCR steps. We then amplified the CO1 gene, which is well-represented in online databases (Porter & Hajibabaei, 2018) with general metazoan primers (mlCOIintF/Fol-degen-rev; (Krehenwinkel et al., 2017; Leray et al., 2013; Yu et al., 2012)). We ran total reaction volumes per sample of 25μL, with 9μL nuclease free water, 12.5μL GoTaq Green Master Mix (Promega Corp., Madison, WI, USA), 1.25μL of each primer (at 10mM), and 1μL of DNA template (at 10ng/μL) and ran a duplicate for each sample. We followed a PCR protocol as follows: 3 minutes at 95ºC, 35 cycles of: 95ºC for 30 seconds, 46ºC for 30 seconds, 72ºC for one minute; ending with 72ºC for five minutes. We removed reaction dimer with Ampure XP beads at 0.8x bead-to-DNA ratio. We then attached Illumina index primers (Nextera XT Index Kit v2) with 5μL of PCR product per reaction and the recommended PCR protocol for these primers (Illumina, 2009). We combined and cleaned successfully amplified duplicate samples using Ampure XP beads (0.7x beads-to-DNA) and diluted each sample to 5nM in 10mM TRIS, using 1uL of each sample for sequencing.

Because of the sample size and the need for a large number of sequences per predator in order to detect rarer prey DNA ((Krehenwinkel et al., 2017), SI Figure 4), we ran samples for this study across four separate sequencing runs (SI Table 1). All individuals within a predator species were sequenced on the same run and each run contained one to five predator species. We ran 19 samples of one predator species (*H. venatoria*) across all runs to quantify run-to-run variation in sequencing (SI Figure 1). For each run, we multiplexed all samples along with one negative control and two PCR4-TOPO TA vectors (Invitrogen, Carlsbad, CA, USA) containing the internal transcribed spacer 1 region from two fungal species as positive controls (GenBank accession numbers: MG840195 and MG840196; (Apigo & Oono, 2018; Clark, Karsch-Mizrachi, Lipman, Ostell, & Sayers, 2016; Toju, Tanabe, Yamamoto, & Sato, 2012)). We submitted multiplexed samples for sequencing at the University of California, Santa Barbara Biological Nanostructures Laboratory Genetics Core. Samples were run on an Illumina MiSeq platform (v2 chemistry, 500 cycles, paired-end reads) with a 15% spike-in of PhiX. Following sequencing, samples were demultiplexed using Illumina’s bcl2fastq conversion software (v2.20) at the Core facility.

We merged, filtered (max ee = 1.0), and denoised (clustered) our sequences around amplicon sequence variants (ASVs) using the DADA2 algorithm in R (dada2 package version 1.1.14.0; Callahan et al., 2016). Prior to denoising with DADA2, we used cutadapt (version 1.18, (Martin, 2011)) to remove primers from each sequence. We compared results to a similar protocol using the UNOISE3 algorithm (unoise3 function in unoise (Edgar, 2016), but found that DADA2 gave more high-read abundance ASVs (SI Figure 2). We ran DADA2 on sequences from all sequencing runs combined but verified that this was appropriate by first ensuring that error rates per run were similar, following recommendations from the algorithm developers (SI Figure 3). We removed samples from analysis that had not been sequenced to sufficient depth using iNEXT (Hsieh, Ma, & Chao, 2016) and a lower quantile cutoff (SI Figures 4 & 5). We rarefied remaining samples (McKnight et al., 2019) based on the sample with the lowest sequencing depth which had been sequenced with 95%+ sampling completeness based on iNEXT (version 2.0.20) interpolation and extrapolation methods (Hsieh & Chao, 2017). We rarefied using the rrarefy() function in the vegan (version 2.5.6) package in R to 15,954 reads per sample.

*ASV taxonomic assignment with BLAST and BOLD*

From the output of the DADA2 algorithm, we created a list of unique ASVs which we matched to taxonomies both in the GenBank and BOLD databases. For GenBank, we used BLAST (version 2.7.1) with the blastn command for taxonomic assignment of each ASV using the computing cluster at UC Santa Barbara, comparing against the GenBank nucleotide database with an evalue of 0.01 (downloaded on November 20, 2019). We visualized and exported taxonomic alignment using MEGAN Community Edition (version 6.18.0, (Huson et al., 2016)), using default settings and selecting the subtree with all possible diet items for this species (Kingdom: Animalia, Clade: Bilateria). For BOLD taxonomic assignment, we used the BOLD IDEngine of the CO1 gene with Species Level Barcode Records (accessed May 21, 2020; 4,070,029 Sequences, 225,114 Species, and 104,607 Interim Species in database) to match each ASV list to taxonomies. We combined taxonomic assignments from both programs and discarded taxonomic assignments that were mismatched at the family level or higher (Elbrecht, Peinert, & Leese, 2017). We chose to combine prey taxonomies at the family level, similar to diet resolution in both metabarcoding and histological methods in this field (e.g. (Brose et al., 2019; Eitzinger et al., 2019; Kartzinel et al., 2015)) by summing the cumulative 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 invertebrate family corresponds to an average of 1.9 (± 0.13 SE) species, so for this system a family-level taxonomic assignment may closely mirror species-level assignments. We corrected for potential sequence jumping (‘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 (‘cross-talk’) (van der Valk, Vezzi, Ormestad, Dalén, & Guschanski, 2020). We verified ASV specificity based on positive control samples (SI Figure 8).

Prior to data analyses, we verified that samples that consisted of multiple individuals (n = 53) did not represent a disproportionate number of interaction counts by comparing the number of predator-prey interactions observed for samples based on the number of individuals comprising each sample (SI Table 5, SI Figure 6 & 7).

*Predator length-mass model*

Because we wanted to compare predator to prey mass, we had to convert the lengths taken on predators to predicted masses. We used mass data collected from predator individuals from Palmrya Atoll and from the literature (Sohlström et al., 2018; Su et al., 2020; Yaninek & Gnanvossou, 1993), Miller-ter Kuile *unpublished data*, McLaughlin et al. *unpublished data*). We fit a linear mixed effects model on log10-log10 transformed mass and length data for these predator individuals. These models included predator length as a predictor of predator mass with a random intercept and slope taking into account by-species variation in the slope and intercept of this relationship (length|species in the random effects model). We assessed model fit for this model and then predicted the values for our predator individuals based on these results. We fit models with the glmmTMB package (version 1.0.2.1) in R (version 4.0.2), assessed model fit with the MuMIn (version 1.43.12) and DHARMa (version 0.3.3.0) packages and used the predict function to predict predator masses from the model results.

**Results**

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

Of a total of 280 samples, we successfully extracted DNA from 99% of samples (n = 278 of 280, SI Table 1). Amplification success across all samples was 80%, with 222 of 278 initially extracted samples successfully amplified and sequenced. The Illumina MiSeq runs yielded 3.9, 3.8, 3.8, and 3.3 x 107 unpaired reads that passed filtering and had Q30 quality scores of 87.82, 85.46, 79.34, and 78.02%, respectively. After quality filtering and denoising with DADA2, 3.02 x 107 paired-end reads corresponded to 1,738 ASVs. Following filtering and clustering, we determined that 40 samples had too-low sequencing depths (less than 11,211 reads total) and so they were removed from further analyses.These samples primarily came from two predator species (*Isometrus maculatus* and *Euborellia annulipes*). Of the total individual predator samples, 173 remained after removal of poorly-sequenced samples.

*ASV taxonomic assignment with BLAST and BOLD*

Sixty-seven percent (1,167 of 1,738) of ASVs matched to a taxonomic assignment at Class level or lower. Thirty percent (n = 524) of these taxonomic assignments corresponded to prey items at the family level or lower and so were used in analyses. There were two conflicting taxonomic assignments at the family level or higher between the BOLD and BLAST assignments which were removed from the final total above.

The number of individuals in a sample did not alter the number of interactions observed for that sample (SI Figure 7). Negative and positive controls were assigned to 0-11 and 1-4 ASVs, respectively (SI Figure 8). Individual ASV reads for negative control were equal or less than 366 reads (± 23 SE). ASV reads for positive controls were dominated by one ASV, suggesting high specificity in ASV assignment.

*Predator length-mass model*

The predator length-mass predictive model had a significant by-species length-mass relationship (β = 2.58, p-value < 0.001, R2m = 0.69 and R2c = 0.95, SI Figure 9).

**Literature Cited**

Apigo, A., & Oono, R. (2018). *MG840195 and MG840196*.

Brose, U., Archambault, P., Barnes, A. D., Bersier, L. F., Boy, T., Canning-Clode, J., … Iles, A. C. (2019). Predator traits determine food-web architecture across ecosystems. *Nature Ecology and Evolution*, *3*(6), 919–927. doi: 10.1038/s41559-019-0899-x

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. doi: 10.1038/nmeth.3869

Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2016). GenBank. *Nucleic Acids Research*, *44*(D1), D67–D72. doi: 10.1093/nar/gkv1276

Edgar, R. C. (2016). UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *BioRxiv*, 81257. doi: 10.1101/081257

Eitzinger, B., Abrego, N., Gravel, D., Huotari, T., Vesterinen, E. J., & Roslin, T. (2019). Assessing changes in arthropod predator–prey interactions through DNA-based gut content analysis—variable environment, stable diet. *Molecular Ecology*, *28*(2), 266–280. doi: 10.1111/mec.14872

Elbrecht, V., Peinert, B., & Leese, F. (2017). Sorting things out: Assessing effects of unequal specimen biomass on DNA metabarcoding. *Ecology and Evolution*, *7*(17), 6918–6926. doi: 10.1002/ece3.3192

Fulton, T. M., Chunwongse, J., & Tanksley, S. D. (1995). Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Molecular Biology Reporter*, *13*(3), 207–209. doi: 10.1007/BF02670897

Hsieh, T. C., & Chao, A. (2017). Rarefaction and extrapolation: Making fair comparison of abundance-sensitive phylogenetic diversity among multiple assemblages. *Systematic Biology*, *66*(1), 100–111. doi: 10.1093/sysbio/syw073

Hsieh, T. C., Ma, K. H., & Chao, A. (2016). iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*, *7*(12), 1451–1456. doi: 10.1111/2041-210X.12613

Huson, D. H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., … Tappu, R. (2016). MEGAN Community Edition - Interactive Exploration and Analysis of Large-Scale Microbiome Sequencing Data. *PLoS Computational Biology*, *12*(6), 1–12. doi: 10.1371/journal.pcbi.1004957

Illumina. (2009). Illumina adapter sequences. In *Illumina*.

Kartzinel, T. R., Chen, P. A., Coverdale, T. C., Erickson, D. L., Kress, W. J., Kuzmina, M. L., … Pringle, R. M. (2015). DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. *Proceedings of the National Academy of Sciences*, *112*(26), 8019–8024. doi: 10.1073/pnas.1503283112

Krehenwinkel, H., Kennedy, S., Pekár, S., & Gillespie, R. G. (2017). A cost‐efficient and simple protocol to enrich prey DNA from extractions of predatory arthropods for large‐scale gut content analysis by Illumina sequencing. *Methods in Ecology and Evolution*, *8*(1), 126–134. doi: 10.1111/2041-210X.12647

Leray, M., Yang, J. Y., Meyer, C. P., Mills, S. C., Agudelo, N., Ranwez, V., … Machida, R. J. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: Application for characterizing coral reef fish gut contents. *Frontiers in Zoology*, *10*(34), 1–14. doi: 10.1186/1742-9994-10-34

Macías-Hernández, N., Athey, K., Tonzo, V., Wangensteen, O. S., Arnedo, M., & Harwood, J. (2018). Molecular gut content analysis of different spider body parts. *PLoS ONE*, *13*(5), 1–16. doi: 10.1371/journal.pone.0196589

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBNet Journal*, *17*(1), 10–12. doi: doi:10.14806/ej.17.1.200

McKnight, D. T., Huerlimann, R., Bower, D. S., Schwarzkopf, L., Alford, R. A., & Zenger, K. R. (2019). Methods for normalizing microbiome data: An ecological perspective. *Methods in Ecology and Evolution*, *10*(3), 389–400. doi: 10.1111/2041-210X.13115

Oono, R., Black, D., Slessarev, E., Sickler, B., Strom, A., & Apigo, A. (2020). Species diversity of fungal endophytes across a stress gradient for plants. *New Phytologist*, *228*(1), 210–225. doi: 10.1111/nph.16709

Porter, T. M., & Hajibabaei, M. (2018). Over 2.5 million COI sequences in GenBank and growing. *PLoS ONE*, *13*(9), 1–16. doi: 10.1371/journal.pone.0200177

Sohlström, E. H., Marian, L., Barnes, A. D., Haneda, N. F., Scheu, S., Rall, B. C., … Jochum, M. (2018). Applying generalized allometric regressions to predict live body mass of tropical and temperate arthropods. *Ecology and Evolution*, *8*(24), 12737–12749. doi: 10.1002/ece3.4702

Su, G., Dudley, R., Pan, T., Zheng, M., Peng, L., & Li, Q. (2020). Maximum aerodynamic force production by the wandering glider dragonfly (Pantala flavescens, Libellulidae). *The Journal of Experimental Biology*, *223*(May). doi: 10.1242/jeb.218552

Toju, H., Tanabe, A. S., Yamamoto, S., & Sato, H. (2012). High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PLoS ONE*, *7*(7). doi: 10.1371/journal.pone.0040863

van der Valk, T., Vezzi, F., Ormestad, M., Dalén, L., & Guschanski, K. (2020). Index hopping on the Illumina HiseqX platform and its consequences for ancient DNA studies. *Molecular Ecology Resources*, *20*(5), 1171–1181. doi: 10.1111/1755-0998.13009

Yaninek, J. S., & Gnanvossou, D. (1993). Fresh and dry wei ghts of Mononychellus tanajoa (Acari: Tetranychidae): A functional description of biomass accumulation. *Experimental and Applied Acarology*, *17*(10), 775–779. doi: 10.1007/BF00051836

Yu, D. W., Ji, Y., Emerson, B. C., Wang, X., Ye, C., Yang, C., & Ding, Z. (2012). Biodiversity soup: Metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. *Methods in Ecology and Evolution*, *3*(4), 613–623. doi: 10.1111/j.2041-210X.2012.00198.x