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Working Title: Finding the source of food chain lengthening: intermediate predators but not top predators expand their prey base in more productive environments

**Introduction:**

Baby:

Ecosystem productivity can shift community composition and interaction dynamics across trophic groups. This shapes food web structures where more productive habitats can support more organisms (within and across species) that drive changes in food chain lengths (e.g. Young et al. 2013, other food chain length studies). Understanding the mechanisms that shift community structure through changing interactions can help us understand how ecosystems are structured and build ecological theory. Furthermore, understanding how trophic dynamics respond to shifting habitats and ecological due to habitat loss, invasive species, and climate change, will be important for predicting and mitigating biodiversity loss.

Zoomed in Baby: Talk about some of the mechanisms of food chain lengthening/trophic shifts in more depth – maybe talking about omnivory in more detail. Outline different mechanisms from Young paper and others. Switching to new prey, eating bigger prey,

Werewolf: Despite almost a century of study, we still do not know the many mechanisms by which trophic dynamics shift with shifting resources. What is needed is an understanding of this because… While it has been established that habitat productivity can increase food chain length, we don’t know what the mechanism(s) is/are.

Silver Bullet: In this study, we combine multiple sources of diet information, including novel diet DNA metabarcoding data and stable isotope data (δ15N and δ13C) to explore how shifting environmental productivity can alter trophic dynamics. Specifically, we explore whether a) top predators and/or b) intermediate predators alter their trophic interactions in response to increased resource availability. We ask 1) Do a) top predator and/or b) intermediate predator isotopic trophic positions increase, and 2) is this due to changes in diet DNA prey composition for either a) top or b) intermediate predators. Combining stable isotope-based trophic data with diet DNA data provides a unique opportunity to evaluate the mechanisms by which trophic shifts occur. Specifically, do top predators increase their trophic positions by switching prey sources, or are increased trophic positions of top predators due to diet shifts among intermediate predators?

**Methods:**

*Study Site:*

We conducted this study on Palmyra Atoll, Central Line Islands (GPS COORDS). The atoll consists of ~20 islets that vary in primary productivity due to nutrient additions from seabird guano; seabirds prefer to nest on islets of the atoll with native vegetation and avoid nesting in forests dominated by an invasive palm species, *Cocos nucifera* (Young et al. 2010 PNAS). Palmyra Atoll has a well-categorized species list (Handler et al.) of which the animals are primarily invertebrate organisms (~400 species,), with top and intermediate predator species including several spider species (Arachnida:Araneae, Miller-ter Kuile et al. 2022, Young et al. 2013). In this study, we examined trophic patterns for a spider top predator *Heteropoda venatoria* and for spider intermediate predators *Keija mneon, Scytodes longipes,* and *Neoscona theisi*, which are all common predator species on the island and *H. venatoria* predates all or some of these intermediate predator species (Miller-ter Kuile et al. 2021, 2022).

*Predator collection:*

We collected predator individuals for isotope samples across islets that span the “low” and “high” ranges of the productivity gradient. For diet DNA data, we collected predators across islets spanning the “high” and “low” ranges of productivity. We collected intermediate predator diet DNA data from predators in “intermediate” to “high” ranges of productivity; however, we used the vegetation we collected these individuals in as a “proxy” for productivity: individuals collected in forest tree canopies indicative of high productivity levels (*Pisonia grandis*, *Heliotropium argentea*, and *Pandanus tectorius*) were assigned to the “high productivity” category, and individuals collected in forest tree canopies indicative of low productivity levels (*Cocos nucifera*) were assigned to the “low productivity” category. While this is an imperfect categorization that may not capture across-productivity variation in diet, because we collected these individuals on the largest and most habitat-diverse islets on the atoll, their diets on these islets likely represent a broad set of their available trophic interactions (CITE SOMETHING?). Futhermore, because we collected them in large and specific microhabitats (tree canopies of specific trees), we assume they remain in these microhabitats throughout their lives.

*Isotope data:*

For isotope samples, all intermediate and some top predator isotope data was originally used for analyses in Young et al. 2013. For additional top predator isotope sample collection, we followed procedures for isotope sample processing in Young et al. 2013, including freezing tissues and then drying them before grinding them [give isotope methods here]. All isotope data are reported for predator individuals.

*Diet DNA data:*

All diet DNA data was originally used in Miller-ter Kuile et al. (2021, 2022) and our full sample processing procedures can be found in these papers. In summary, all individuals were collected in individual sterilized containers with sterilized implements and kept frozen until eventual DNA extraction. We extracted diet DNA from full organisms using a modified CTAB procedure and followed the methods outlined in Krehenwinkel et al. {YEAR} for isolating consumed DNA from taxonomically-similar consumers using AmpureXP beads and an optimized PCR primer pair (GIVE PAIR). We multiplexed and processed all samples on an Illumina (look at other paper for methods). We merged and cleaned sequences with \_\_\_\_. We assigned taxonomies to prey DNA using the GenBank and BOLD taxonomic databases. We grouped all prey to Order because we were interested in broad shifts in diet suggesting shifts in degrees of omnivory. All diet DNA data are from individuals or sets of \_-\_ individuals from the same collection period (see Miller-ter Kuile et al. 2022).

*Data analysis:*

To examine how stable isotope-based trophic position of top and intermediate predators shifts with habitat productivity, we developed a linear mixed effects model with crossed fixed effects of islet productivity (“high” and “low”) and predator trophic category (“intermediate” and “top”). This structure is similar to a two-way ANOVA, in which the relationship between productivity level can vary depending on predator trophic category. We used a Gaussian error distribution and random effects of islet and year to account for spatial and temporal non-independence among data points.

To examine how diet DNA shifts with habitat productivity for both top and intermediate predators, we ran two separate PERMANOVA analyses comparing individual predator diet composition between “high” and “low” productivity sites. We ran one model for each predator category and used the Jaccard dissimilarity index based on the presence-absence nature of our diet composition data. In the event of dissimilarity in diet composition between “high” and “low” productivity categories, we determined whether dissimilarity (beta diversity) was based on turnover (shifting to new diet sources) or nestedness (the diet source of one community is a subset of another environment’s prey community).

We ran all statistical analyses in R (CITE, version) and cleaned data with the here (cite, version) and tidyverse packages (CITE VERSION). We ran mixed effects models in the glmmTMB package (cite version) and ran model diagnostics using the DHARMa (cite version) and the effects (cite, version) packages. We used the adonis() function in vegan (cite version) to run PERMANOVA analyses and determined the contributions of beta diversity using the vegan package and the betapart package (cite version). All data and code used to generate all steps of the analyses in this manuscript can be found on GitHub (link) and will be available on Dryad and Zenodo following acceptance.

**Results**

How many isotope samples for each predator category and productivity category?

How many DNA samples for each predator category and productivity category?

Results of the glmm

Results of the permanova and betapart

**Discussion**