Target Journal: Biology Letters (max 2500 words (abstract-conclusion + figure captions))

Working Title: Something about combining isotope and DNA information to find out where shifts in food web structure occur

Author List: Ana Miller-ter Kuile1,2,3, Austen Apigo1, An Bui1, Kirsten Butner1,4, Jasmine Childress1, Stephanie Copeland1, Bart DiFiore1, Elizabeth Forbes1,5, Maggie Klope1, Carina Motta1,6, Devyn Orr1,7, Katherine Plummer8, Daniel Preston9, Hillary Young1

Author Affiliations

1 Ecology, Evolution, and Marine Biology Department, University of California, Santa Barbara, Santa Barbara, California, USA

2 School of Informatics, Computing, and Cyber Systems, Northern Arizona University, Flagstaff, Arizona, USA

3 USDA Forest Service Rocky Mountain Research Station, Flagstaff, Arizona, USA

4 KIRSTEN AFFILIATION

5 ELIZABETH YALE affiliation

6 CARINA AFFILIATION

7 DEVYN USDA Agricultural Research Station, Burns, Oregon, USA

8 Department of Biology, Stanford University, Stanford, California, USA

9 DAN DEPT Colorado State University, Fort Collins, Colorado USA

**Abstract (200 words)**

**Keywords (3-6)**

**Background**

Baby:

Environmental context can shift community composition across trophic groups (e.g. Young et al. 2013, WOLF DNA paper, Other food web papers). For example, as [give an example here from the literature about community composition shifts]. As ecological communities shift, interactions among organisms may also face new constraints – [give a couple example of interaction shifts, not necessarily trophic]. 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 context due to habitat loss, invasive species, and climate change, will be important for predicting and mitigating ongoing and future biodiversity loss.

Zoomed in Baby: Different types of data have helped us track trophic shifts across habitats. For example, based on stable isotope analyses, we know that top predators in higher-productivity environmental contexts have higher isotopic trophic levels (Young et al. 2013). Based on DNA diet analyses, we know that wolf diet shifts across islands in \_\_ due to shifts in prey availability. We also know that spider [species] diet does not shift across environmental contexts even with shifting prey bases [cite]. It is clear, then, that shifts in diet across habitat contexts are not universal, even as underlying community composition shifts.

Werewolf: The multitude of relationships between environmental context and community composition shifts and predator trophic responses suggest that examining on indicator of trophic dynamics (e.g. stomach contents vs. DNA diet vs. isotopic trophic positions) may not illuminate the full picture of changes in trophic dynamics across environmental contexts. It may be that combining data types will help illuminate underlying shifts in community structure (ee.g. ttrophic positions/trophic niches) while also providing the mechanisms by which these may occur (shifts in top predator diet versus shifts in diet lower in food chains).

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 context can alter trophic dynamics. We examine stable isotope and diet DNA data from a top predator (the spider *Heteropoda venatoria*) and the diet DNA data of one of this predator’s key diet groups – other spiders in the order Araneae to determine how diet shifts given environmental context and where in the food chain these changes occur (e.g. at the top of the food chain or lower) Specifically, we ask 1) Does top predator isotopic trophic niche shift across environmental context?, 2) Do DNA diet trophic niches of top predators shift across environmental contexts? 3) Do DNA diet niches of top predator diet groups (other predators) lower in the food chain help explain any discrepancies between top predator isotopic and DNA diet trophic niches? Combining stable isotope-based trophic data with diet DNA data provides a unique opportunity to evaluate the mechanisms by which trophic shifts occur. Specifically examining whether top predators increase their trophic positions by switching prey sources or whether increases in trophic positions of top predators are due to diet shifts among lower-level consumers.

**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 (e.g. *Pisonia grandis*) 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 intermediate predator species on the island and are predated by *H. venatoria* (Miller-ter Kuile et al. 2021, 2022).

*Predator collection and sample processing:*

We collected all predator individuals for isotope and DNA diet samples across various islets that comprise two habitat contexts: habitats dominated by 1) native vegetation (*P. grandis*; indicative of higher ecosystem productivity, Young et al. 2013) and 2) invasive vegetation (*C. nucifera*; indicative of lower ecosystem productivity, Young et al. 2017). For isotope samples, 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 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 niche shifts with environmental context, we calculated two common trophic niche metrics (KIN and SEA – define, cite). We extracted the areas estimated within the 95% confidence interval for both metrics and used a generalized linear model to examine how habitat context shapes isotopic niche space. To complement this niche space analysis, we also examined how each isotopic signature (d13C and d15N) shifted individually with environmental context using a set of linear mixed effects models. We used a Gaussian error distribution for all linear models and random effects of islet and year to account for spatial and temporal non-independence among data points within the mixed effects models.

To examine how diet DNA shifts with habitat context for both top and intermediate predators, we determined shifts in DNA diet niche using a metric for beta diversity: distance based redundancy analyses, comparing individual predator diet composition between the two environmental contexts 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 environmental contexts, 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 capscale() function in vegan (cite version) to run distance-based redundancy analyses, using a set of distance matrices generated by the beta.pair() function in the package betapart (cite version). These distance matrices included one for overall beta diversity, one for nestedness, and one for the turnover components of beta diversity.

**Results**

152 top predators for isotopes (88 from *P. grandis*, 64 from *C. nucifera*). 34 DNA samples from top predators (21 from *P. Grandis*, 13 from *C. nucifera*), comprising 68 unique interactions.

25 itnermedate predators samples for Araneae diet DNA (23 from *P. grandis* and 8 from *C. nucifera*) comprising 66 interactions total.

Neither measure of isotopic niche showed clear changes in niche area across environmental contexts (habitat context p-value = 0.51); however, d15N clearly increased and d13C decreased in PG habitats (d15N: p-value < 0.001, β = 1.93 CI = 1.56 – 2.31 NEED R2; d13C: p-value = 0.04, β = -1.00 CI = -1.96 to -0.05 NEED R2).

For top predators, beta diversity was not clearly different across *P. grandis* and *C. nucifera* habitats (p-value = 0.242); however, intermediate predator (other Araneae) beta diversity shifted with environmental context (p-value = 0.002), with shifts attributed to turnover (p-value = 0.001) but not nestedness (p-value = 0.981) among environmental contexts.

From the community composition of diet – it looks like intermediate predators have a larger contribution of more predatory diet items in their diet in PG habitat, including more Araneae, Coleoptera, Hymenoptera, Diptera; whereas in CN habitat they eat more Blattodea, Thysanoptera, and Psocoptera. For top predators, the majority (75-90%) of diet stays the same across environmental context. Majority of diet is Araneae, Blattodae, Dermaptera, Diptera, and Orthoptera across both.

**Placeholder figures:**

Chart

Description automatically generated

Figure 1: A) Isotopic Niche, B) DNA niche, C) Niche area (two methods), D) Diet composition of top predators. While top predators increase their d15N (trophic position) and decrease their d13C (become more marine food web dependent) in PG habitat (A) – this does not mean an a shift in their DNA diet niche (B) or their overall isotopic niche area (C). The majority (75-90%) of their diet across environmental context comprises Araneae, Blattodea, Dermaptera, Diptera, and Orthoptera.

Chart, bar chart

Description automatically generated

Figure 2: Intermediate predators (other Araneae) have a substantial shift in DNA diet niche space (beta diversity), with PG Araneae eating more Araneae, Coleoptera, Diptera, and Hymenoptera – all Orders that include predatory species on Palmyra Atoll.

**Discussion**

How cool is it to combine datasets!?

**Conclusions**

We learned a thing about food webs.

**Data Availability Statement**

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 article acceptance.

**Acknowledgements**

**Funding**

**References**

**To-Do and assigned folks:**

* Write abstract
  + Ana
* Write intro
  + Ana
* Fill out information for isotope methods
  + Steph, Katie
* Fill out information for DNA methods
  + Carina, Maggie
* Fill out results section
  + Austen – check and recommend changes
* Update figures to be more pretty (color palette consistency, size consistency, order diet composition graphs to be in order of decreasing abundance in samples)
  + Help me, An!
* Get R2 values for models
  + Ana
* Write discussion – make topics of major paragraphs for folks to fill in?
  + Ana – others – if lit reviewing, fill in info in specific paragraphs
* What else needs to be supplemental in this paper? – comb through for gaps
  + Jasmine, Devyn
* Lit review of diet shifts/lack of shift with changing habitats
  + Dan, Bart, Hillary
* Lit review of studies combining DNA and isotope data – what is the framing?
  + Kirsten, Elizabeth, Maggie
* Formatting – scientific names, sections, references – someone come up with a list
  + An,
* Edit a general draft intro/discussion
  + Hillary