UNIVERSITY OF CALIFORNIA

Santa Barbara

Demystifying Species Interactions: Conservation and Theory from the Model Island System of Palmyra Atoll

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Ecology, Evolution, and Marine Biology

by

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ACKNOWLEDGEMENTS

I have so much appreciation for all the wonderful collaborators, mentors, mentees, colleagues, funders, friends, and family who supported my PhD journey. My appreciation cannot be captured in words on these pages.

Thanks to amazing field crews for three field seasons on Palmyra Atoll as well as the many people who supported the laboratory portions of this dissertation. A huge thank you to Devyn Orr and Austen Apigo for your help in developing ideas and methods for all three of my dissertation chapters. Devyn, I can't imagine a better way to brainstorm about science than trail running in Montaña de Oro. Austen, thank you for your amazing teaching abilities and your endless patience as I learned new skills and second-guessed every step of the process. So many giant field crews helped support this work, and a huge thank you to the 2014, 2015, 2016, and 2017 crews that helped collect the data for this project. Many thanks to Tessa Chou and Emily Lutz for working so hard in the lab extracting DNA from lots of spiders. Thank you to Ryoko Oono for allowing me to use lab equipment in her lab for multiple years to do DNA extraction and PCRs.

Many thanks to the US Fish and Wildlife Service and Nature Conservancy on Palmyra Atoll for supporting the field work for this research. I am also so grateful for funding and support from the National Science Foundation, UCSB Faculty Senate, and National Geographic Society. Also thanks to the Biological Nanostructures Laboratory within the California NanoSystems Institute, supported by the University of California (UC) Santa Barbara and the University of California Office of the President. We especially thank Dr. Jennifer Smith, manager of the Biological Nanostructures Laboratory for her assistance in

preparing and troubleshooting our samples. I acknowledge the use of computational facilities at the Center for Scientific Computing (CSC), which was purchased with funds from the National Science Foundation (CNS-1725797) and is supported by the California NanoSystems Institute and the Materials Research Science and Engineering Center (MRSEC; NSF DMR 1720256) at UC Santa Barbara.

I have had the privilege of a bounty of mentors who have helped develop my scientific thinking as well as supported me as a scientist. Thank you to my advisor, Hillary Young, for helping develop my research ideas, my identity as a scientist, and for helping me develop as a scientist-storyteller. Thank you to my committee members, Holly Moeller, Douglas McCauley, and Adrian Stier, for providing invaluable feedback on scientific theory and for your professional development advice as I navigated the PhD and beyond. A huge thank you to Rodolfo Dirzo; you continue to inspire me to think about my power as a scientist in helping the world be more equitable, and you constantly remind me that I'm an ecologist because nature is truly wonderful. I am also so blessed with the mentorship of the NSF AGEP HSI Alliance team, including Carlos Nash, Erich Fleming, Angela Winek, and Mary Hegerty. Thank you all so much for providing an incredible community of support that gave me a lot of confidence in my teaching abilities. Finally, thank you to Dan Preston for letting me adopt you and your lab group in the final year of my PhD and providing great and supportive input on my dissertation chapters.

A huge thank you to collaborators who helped collect and contextualize data for all of my chapters. The list is long! Thanks to Devyn Orr, An Bui, Rodolfo Dirzo, Maggie Klope, Douglas McCauley, Carina Motta, Hillary Young, Austen Apigo, Bart DiFiore, Elizabeth Forbes, Michelle Lee, Daniel Preston, Rachel Behm, Taylor Bogar, Jasmine Childress, Kevin Lafferty, John McLaughlin, Marisa Morse, Kevin Park, Katherine Plummer, David

Weber, Ronny Young, Cora Johnston, and Magalay Espinoza and more! The Young, McCauley, and Preston labs also helped immensely with idea generation, writing, and editing my dissertation, including Georgia Titcomb, Kate Culhane, Caroline Owens, Chelsea Steel, John Schroeder, Ruby Harris-Gavin, Stephanie Copeland, Benjamin Boyce, Zoe Zilz, Kirsten Butner, Ana Guerra, Molly Hardesty-Moore, Lacey Hughey, Erin Dillon, Erin Crone, Erin Sauer, Daniel Trovillion, Landon Falke, Diana Perales Macedo, Gaby Shay, Catherine Lewis, and Luis Abreu-Socorro.

So many wonderful friends and colleagues helped me stay healthy and happy during my PhD. The list includes so many of the people already mentioned and more. To my Santa Barbara crew – Devyn, Colin, Ere, Chris, Ana, An, Heili, Elizabeth, Georgia, Austen, Lacey, Molly, Erin, Caroline, Kate, Michelle, Jake, Jasmine, Kelly, Mallory, and Cory – thanks for the birthday beach barbeques, camping trips, Wildcat nights, Galentine's Days, bike rides, trail runs, lunch on the beach, paddleboarding, and musubi potlucks. In particular, so much love to the Chard House, Yaas Queen, and WE CAN DO IT. Thank you to the NSF AGEP cohort for the wonderful friendship and support - Monica, Roselia, Natalia, and Carlos. Thanks to my Flagstaff friends for keeping me grounded and reminding me to visit the desert as much as possible – Deja, James, Stephanie, Mike, Cerissa, Emily, Ryan, Tim, Jesse, and Rachel. Thank you to friends from college – Emma and Amanda, the adventure continues! Thanks to my DND group (Emma, Flo, Leah, Diana, and Maggie) for being so silly. Thank you Madison running and game night crews – Niko, Erin, Daniel, Abby, and Cori! Thanks to the Boulder group for staying weird – Adam, Joe, Tasha, Sarah, Robbie, Teal, Chris, and DakotaRae.

Wow and how to thank my family for the lifetime of love and encouragement. To my parents, who trained me to be an ecologist at an early age by letting me play in irrigation

water and encouraging me to collect bugs and rocks. Elena and Dini – thanks for being there for all the adventures. Also thank you for bringing two hilarious little niblings into my life- Amalia and Layla have made me laugh and brought so much joy as I cross the finish line of my PhD. I only wish I lived closer to them both. I have been so blessed with a massive family, so thank you to all the tias, tios, cousins, and new family members we have adopted. Especially thank you to Tia Nitschka, Tia Lisa, Tia B, Izzy, Mia, Randy, Tia Sonia, Tia Paula, Tia Alex, Oma Martha, Sydni, Kalee, Kathleen, JK, Uncle Curtis, Uncle Steven, Terri, Howard, Dori, Heather, Quinn, Jamie, Kayleigh, Tio Todd and Tia Mary, Peg, Jay, Wendy, and Julia.

Finally thank you to my wonderful husband, Kyle, for the love and support throughout my PhD. Thank you for the adventures, the encouragement to go outside because I'll be happier, and the thousands of hours of board games. I love you.

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Graduate Opportunity Fellowship, University of California, Santa Barbara, 2015 – 2017

ABSTRACT

Demystifying Species Interactions: Conservation and Theory from the Model Island System of Palmyra Atoll

by

Ana Miller-ter Kuile

The world's lands and oceans are currently being irrevocably altered by anthropogenic change, including the combined impacts of species extinctions, species invasions, land use change, and climate change. In the face of these shifts, understanding the fate of biological communities as a whole becomes imperative to slow or mitigate the extinction of species and the loss of ecosystem functions. Understanding how biological communities have and continue to respond to human activity includes both a) considering the conservation outcomes of interventions to curb biodiversity loss as well as b) using new and emerging methods to understand how individuals and species interact to shape biologically complex interaction webs (e.g. food webs). In this dissertation, I use the island ecosystem of Palmyra Atoll (Central Tropical Pacific) to understand both a) the ecosystem-level effects of the loss of plant-herbivore interactions following an invasive rodent eradication and b) the patterns and biological rules that shape individual- and species-level predator-prey interactions by developing and using emerging DNA metabarcoding methods. Together, this work aims to paint a better picture of the ecosystem-wide effects of species interactions and what losing them might mean for ecosystems beyond Palmyra Atoll.

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CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Roughly 75% of the earth's land surface is actively managed by humans (Venter et al. 2016, Figure 1) and humans have altered habitat characteristics and species compositions of ecosystems globally (Crutzen 2002, 2006). With this change occurring rapidly across the globe, we are experienceks rates of biodiversity change (both introductions and losses) at unprecedented rates (Barnosky et al. 2011, Hughes et al. 2013). This global change does not only mean changes in biodiversity, but also a shift in species interactions, often with cascading losses of interactions (Valiente-Banuet et al. 2015, Figure 2). These species interactions shape ecosystem functions, including nutrient storage and cycling (Schulze and Mooney 2012), and these functions often change even before species or interactions completely disappear ("functional extinction"; e.g,. Galetti et al. 2013, Rozas-Davila et al. 2016). For example, a reduction in bird or rodent seed predators and dispersers shapes plant community composition and biomass in ecosystems from the Australian desert shrubland to the Brazilian Atlantic Forest (Galetti et al. 2013, Gordon and Letnic 2016); the functional loss of pollinating birds in New Zealand leads to a reduction in plant reproductive success due to lost pollination interactions (Anderson et al. 2011). Introduced species also interact in ecosystems where they are transplanted, either competing with species in that ecosystem (sharing interactions) or introducing new interactions. For example, black rats (*Rattus rattus*) introduced across the islands of the globe (Atkinson 1985) serve as both seed predators and dispersers, often competing with or replacing native seed interactors ((Shiels and Drake

2011). At the same time, black rats are introduced predators of many island native fauna, including invertebrates, reptiles, and birds (Jones et al. 2016).

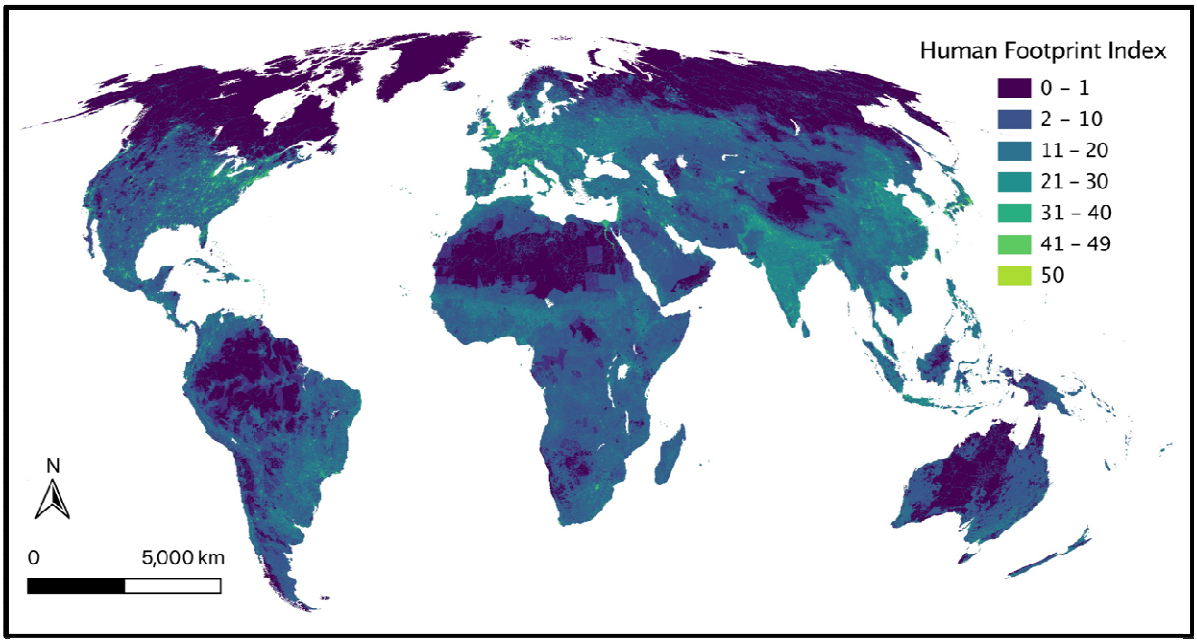


Figure 1: Human Footprint Index

Over 75 percent of the world's un-iced land surface is managed by humans, including urban development, croplands, and roads. The human footprint index ranges from 0 – 50, with zero being no human impacts. The index incorporates a) extent of built environments, b) human population density, c) crop and pasture area, d) night light pollution, and e) navigation corridors, including roads, railways, and navigable waterways (data source: Venter et al. 2016)

Because species losses and additions and the re-shuffling of interactions among them is changing at a global scale and a rapid rate, we often have a lack of knowledge of how interactions or ecosystems are shifting or will continue to shift. This makes it challenging to know both the changes that have occurred for already-altered systems that we are aiming to restore to previous ecological baselines or for systems where we want to predict how future changes could alter ecosystem functions (Cardinale et al. 2012). Sometimes, this is because changes occurred before ecological data collection efforts (Rozas-Davila et al. 2016). In conservation settings when the goal is restoring previous ecological states, monitoring the

effects of introduced or recovered species is often limited by the amount of money available for the restoration effort (Holmes et al. 2015). When the goal is to predict interactions and how they might change, we are limited by interactions we can see, and so the interactions of cryptic and small-bodied organisms are often unknown (Sheppard and Harwood 2005). These challenges are multi-facetted and so require a multitude of approaches – both using datasets that already exist to understand the effects of species interaction in management contexts and for developing new tools for determining interactions where we are data limited.

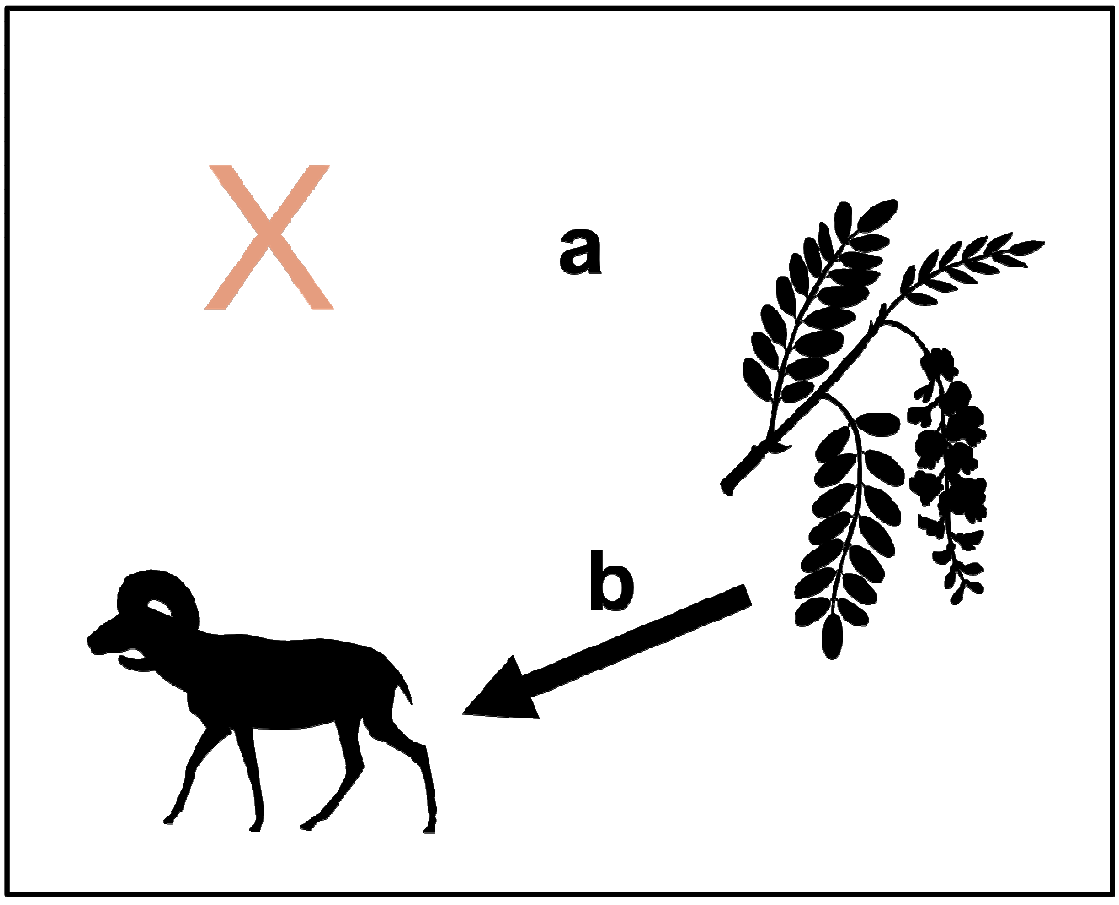


Figure 2: Re-shuffling interactions

The loss and gain of species can lead to the loss or gain of interactions. The extinction or functional extinction of bird seed dispersers (here, palila (*Loxiode bailleui*), a honeycreeper from Hawai'i) is due to the introduction of mammal herbivores such as domestic sheep. The seed predation (and

likely dispersal) interaction (a) is lost, to the mutual determent of palila and plant interactors (e.g. mamani (*Sophora chrysophylla*)). Meanwhile, a new interaction, the herbivory of mamani seedlings (b) by sheep, emerges, shifting plant community composition and ecosystem function. (vector art: phylopic.org)

In this dissertation, I approach the challenge of re-shuffling species interactions from both angles: understanding the effects of past and ongoing restoration and also developing new tools for understanding and predicting species interactions across ecosystems.

1. In my first chapter, I explore how invasive species serve as novel seed predators, and how removing these invasive species has ecosystem-level consequences.
2. In my second chapter, I test a novel way of using DNA from diets to determine predatory interactions between invertebrates, specifically a model spider species, and their prey, to develop methods that can be scaled up to understand predatory interactions in the wild.
3. In my third chapter, I use these new DNA methods to understand how predatory interactions between invertebrate predators, including spiders, insects, and centipedes, and their prey can be predicted by a set of predator traits and used to predict cryptic interactions.

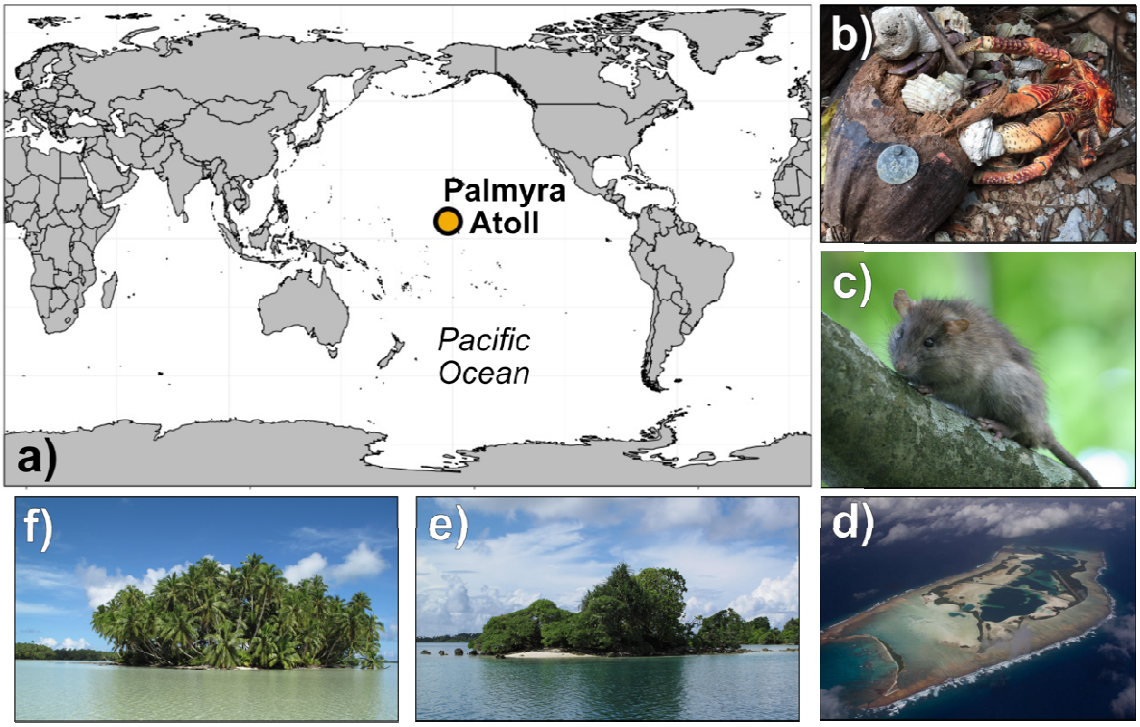
1.2 PALMYRA ATOLL AND THE HISTORY OF ISLAND ECOLOGY IN THEORY AND CONSERVATION

I conducted research for all of these chapters on Palmyra Atoll (Figure 3), which is a small (11.9 km^2) uninhabited coral reef atoll located in the Central Pacific Ocean (5°53′1″N 162°4′42″W). Palmyra Atoll is primarily forested (90% forests) with canopies primarily composed of a handful of species, including *Cocos nucifera* (Family: Arecaceae), *Pisonia grandis* (Family: Nyctaginaceae), *Pandanus tectorius* (Family: Pandanaceae), *Heliotropium*

foertherianum (Family: Boraginaceae), and *Scaevola taccada* (Family: Goodeniaceae) (Young et al. 2010b, 2010a, 2017, Miller-ter Kuile et al. 2020). Like many tropical atoll systems, Palmyra Atoll is relatively species-poor, with roughly 400 species of macroscopic terrestrial plants and animals (McLaughlin et al. unpublished data). The animals of Palmyra are all invertebrates apart from three species of gecko (Briggs et al. 2012) and include the world's largest terrestrial arthropod, the coconut crab *Birgus latro* (Nigro et al. 2017).

Palmyra Atoll has been a part of the human story for thousands of years – oceanic voyagers traveling across the Pacific likely visited Palmyra as a fishing area and for harvesting terrestrial resources – including those naturally present on the island (e.g. coconut crabs) or those brought to the island for cultivation (e.g. potentially *Pandanus* and *Cocos* fruit and vegetation, Gunn et al. 2011). Palmyra Atoll was unlawfully claimed by the United States government in the 1856 Guano Island Act as Western industrialized nations sought to claim resources of phosphorous-rich guano sources before the development of synthesized fertilizers (Burnett 2005). Palmya Atoll then became a coconut plantation for the production of copra during the late 1800's and early 1900's (Rock 1859, Dawson 1959, Harries 1978) during which time much of the forests of Palmyra were converted to monodominant stands of coconut palms. According to a botanist who visited the island at that time: "The coconuts are in splendid condition; they have no enemies on Palmyra…" (Rock 1859). During World War II, Palmyra was established as a military base for the United States. The military era created the Palmyra we see today: built up land with straight coastlines along the interior and exterior that are relics of airstrips and roads. Military occupation also likely brought black rats to the island, which thrived on young coconuts in the canopies of coconut palms across the island. Black rats were not the only invasive species brought to the island during this time; a survey of Palmyra Atoll's biodiversity estimated that 50-75% of the current

arthropod species on the island are likely introduced (Handler et al. 2007). More recently, Palmyra has become a US Fish and Wildlife Refuge with a field station operated by The



Nature Conservancy. With wildlife and ecosystem conservation missions, these organizations have conducted various ecosystem conservation efforts, including the eradication of rats from the atoll in 2011 and an ongoing coconut palm eradication effort (Howald et al. 2004, Wolf et al. 2018).

Figure 3: Palmyra Atoll and its Ecology

(a) Palmyra Atoll is in the central tropical Pacific Ocean. (b) The island is home to roughly 400 macroscopic plants and animals, including the world's largest terrestrial arthropod, the coconut crab *Birgus latro* (photo; An Bui). (c) Black rats (*Rattus rattus*) were introduced to the atoll during World War II and eradicated from the atoll in 2011 (photo: Graham Carroll). (d) Palmyra Atoll is a small (11.9 km^2) coral-derived atoll consisting of a ring of smaller islets around three central lagoons (photo: Alex Wegmann. (e) The atoll is 90% forested, with multiple native tree and shrub species, including *Pisonia grandis* (Family: Nyctaginaceae), *Pandanus tectorius* (Family: Pandanaceae), *Heliotropium foertherianum* (Family: Boraginaceae), and *Scaevola taccada* (Family: Goodeniaceae; not in picture). (f) Approximately 60% of the tree cover on the atoll consists of either monodominant or mixed *Cocos nucifera* (coconut palms) canopy. Coconut palms, which were likely

present on the island before colonization by the United States, were heavily planted for copra production in the late 1800's and early 1900's, likely tipping the ecological scale in their favor.

Palmyra Atoll can be seen as a model for understanding conservation interventions because we have data tracking the ecosystem both before and after a conservation intervention, the eradication of black rats from the island in 2011 (10 years of data from 2007-2017; see Young et al. 2017, Wolf et al. 2018, Miller-ter Kuile et al. 2020). datasets are often rare with costly eradication-based conservation interventions and so invaluable evidence for the ecosystem-level effects of invasive species and their eradication. The conservation challenges and actions facing Palmyra are similar to many islands in the Pacific and so the case study of Palmyra can be valuable evidence for the outcomes of ongoing and future eradication-based conservation efforts (Atkinson 1985, Jones et al. 2016). Palmyra can also be seen as a model for understanding ecological theory, building on a vast literature using islands as model ecosystems (Simberloff and Wilson 1969, Gravel et al. 2011). Palmyra Atoll and other islands lie somewhere between a petri dish and a mainland ecosystem in ecosystem complexity. The relatively species-poor habitat falls in a middle ground between simplicity and complexity in a way that is useful for understanding the patterns of natural ecosystems and generating ecological theories that can be used to predict the structure and dynamics of more complex systems (Young et al. 2013a).

CHAPTER 2: IMPACTS OF RODENT ERADICATION ON SEED PREDATION AND PLANT COMMUNITY BIOMASS ON PALMYRA ATOLL

Publication Note: The content of this chapter is published in "Impacts of rodent eradication on seed predation and plant community biomass on a tropical atoll." A. Miller-ter Kuile *et al.*, *Biotropica*, Copyright © 2020, Wiley)

2.1 ABSTRACT

Invasive rodent eradications are frequently undertaken to curb island biodiversity loss. However, the breadth of rodents' ecological impact, even after eradication, is not always fully recognized. For example, the most widespread invasive rodent, the black rat (*Rattus rattus*), while omnivorous, eats predominantly seeds and fruit. Yet, the effects of seed predation release after eradication on plant communities and ecological functions are not well understood, posing a gap for island restoration. We examined the role of seed predation release following black rat eradication in changes to tree composition and aboveground biomass across an islet network (Palmyra Atoll) in the Central Pacific. We conducted repeated surveys of seed, juvenile, and adult tree biomass and survival in permanent vegetation plots before and after the eradication of rats. We observed a 95% reduction in seed predation for an introduced, previously cultivated tree population (*Cocos nucifera*). Juvenile tree biomass of all species increased 14-fold, with *C. nucifera* increasing the most, suggesting that eradication increased this tree's competitive advantage. Indeed, based on stage-structured demographic models, rat eradication led to a 10% increase in *C. nucifera* population growth rate. The effect of invasive rodent seed predation varies considerably

among the plant species in a community and can shift competitive dynamics, sometimes in favor of invasive plants. These bottom-up effects should be considered in evaluating the costs and benefits of eradication. Documenting the variation in invasive rodent diet items, along with long-term surveys, can help prioritize island eradications where restoration is most likely to be successful.

2.2. INTRODUCTION

We are in the midst of a global biodiversity crisis, with islands facing some of the most alarming losses of endemic species (Pimm et al. 2014). This loss is fueled by a combination of factors; one of the most detrimental being invasive mammals (Tershy et al. 2015). Invasive rodents, in particular, are found on over 80% of the world's major islands and are implicated in the extinction or suppression of 34 plant and 101 animal species (Atkinson 1985, Towns et al. 2006). Rodent eradications on over 400 islands worldwide have led to the recoveries of species across taxonomic groups and the scale of planned and attempted eradications continues to increase (DIISE 2015, Jones et al. 2016).

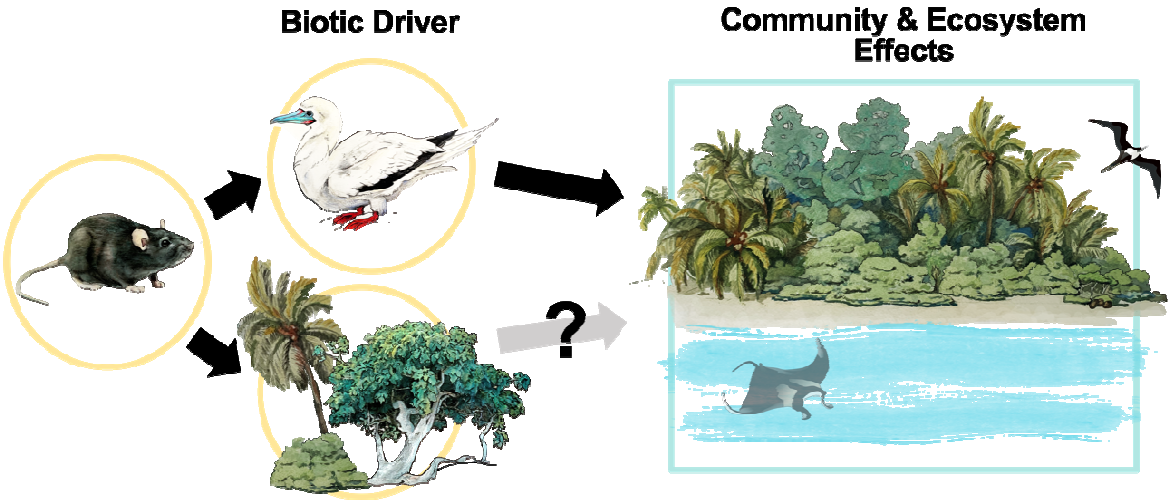


Figure 4: Ecosystem Trajectories After Rat Eradication

Invasive rodents are omnivores that predate important biotic drivers for island ecosystems (here, seabird and tree communities). Rodent eradications are usually undertaken to protect seabirds from rat predation, recovering the ecological processes seabirds regulate (e.g., nutrient subsidies and soil turnover; top pathway). However, less is known about how rodent eradications may directly impact (through seed predation) plant communities and the ecological processes they regulate (bottom pathway). (illustrations by Devyn Orr; vector images by Tracey Saxby and Sally Bell, Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/imagelibrary/).

The main goal of eradications is typically the recovery of nesting seabird populations (Jones et al. 2008, 2016). Nesting seabirds provide nutrients with their guano and perturb soil while creating burrow nests (Jones 2010, Buxton et al. 2016). Seabird recovery, thus, can cascade to restore nutrient and soil cycling regimes which maintain island plant and consumer communities, and such recoveries have occurred across the globe (Croll et al. 2005, Fukami et al. 2006, Mulder et al. 2009, Le Corre et al. 2015).

Although the management focus of eradications is typically seabird communities, diet studies of island rodents suggest that the majority of their diet is plant-based (Shiels & Pitt, 2014). The most widespread rodent in the tropics, the black rat (*Rattus rattus*), predominantly eats seeds and fruits, including both native and introduced grasses, forbs,

shrubs, and trees (Shiels et al. 2014). Black rat seed predation is responsible for the suppression and extinction of plants across the tropical Pacific (Meyer and Butaud 2009) through the effects of seed predation on plant demography, community composition, and biomass (Maron and Pearson 2011, Maron et al. 2018). Black rats also disperse invasive plant species, increasing competitive pressure on native plants (Shiels and Drake 2011, Hays et al. 2018). Plant community responses to seed predation release following rodent control or eradication, however, are relatively under-documented (Figure 4; but see Grant-Hoffman et al. 2010a, 2010b, Pender et al. 2013). This is surprising since changes to plant community composition and abundance can alter ecological functions and processes such as carbon storage, nutrient cycling, and decomposition (Chave et al. 2006, Sayer et al. 2011), as well as shape above- and below-ground consumer communities at a comparable magnitude to seabird-driven ecosystem recovery (i.e. Fukami et al. 2006, Drake et al. 2011, Young et al. 2013a). While the release of native plants from seed predation may lead to the recovery of island ecosystems (Campbell and Atkinson 2002, Grant-Hoffman et al. 2010a, 2010b, Pender et al. 2013, Wolf et al. 2018), release of non-native, invasive plants may lead to the establishment of additional invasive species (Bergstrom et al. 2009), or alternative ecological states (Suding et al. 2004) that endanger additional native species and create the need for supplementary management.

These ecological outcomes, including whether islands recover following eradications or not, likely vary depending on plant community composition and ecological pressure exerted by rodent seed predation (Grant-Hoffman & Barboza, 2010). On the world's tropical atolls, which are both heavily invaded by black rats and altered by human agriculture and agro-forestry, eradication responses may follow general patterns that could favor introduced plants (Harper and Bunbury 2015, Thaman 2016). In particular, the coconut palm (*Cocos*

nucifera), which was planted across atolls in the late 1800s and early 1900s for copra production, may be a driver of post-eradication island recovery (Dawson 1959, Thaman 2016). Black rats are known to heavily predate the seeds of *C. nucifera* across the Pacific, targeting pre-mature seeds in the canopy (Wegmann 2009, Harper and Bunbury 2015). Black rat seed predation is not only likely to regulate *C. nucifera* populations, but also the populations of other seed predators that may target seeds of *C. nucifera* and other plants once they reach the ground (this includes the largest terrestrial arthropod, the coconut crab *Birgus latro*) (Nigro et al. 2017). Additionally, depending on whether black rats preferentially target *C. nucifera* seeds or the seeds of other introduced or native species, plant community composition may shift in favor of the introduced *C. nucifera* or in favor of native species. The expansion of *C. nucifera* from agricultural planting (even on isolated and uninhabited atolls) negatively influences nesting seabirds and the island food webs seabird nutrients support, and so increased expansion of this species may exacerbate these impacts (Wegmann 2009, Young et al. 2010b, 2017, Thaman 2016).

In this study, we examined the role of black rats (*Rattus rattus*) as seed predators on trees at Palmyra Atoll in the Central Tropical Pacific. Palmyra is a model system of the ecological shifts caused by *C. nucifera* invasion (Young et al. 2017) and provides an opportunity to assess the impacts of black rat seed predation in the absence of other canopy-feeding seed predators (Wegmann 2009). Following black rat eradication on Palmyra Atoll in 2011, juvenile individuals of all tree species increased in abundance (Wolf et al. 2018); however, the mechanisms for this increase and its effects on species-level biomass and long-term ecosystem recovery have not been explored. We used multi-year data, which is often unavailable in eradication efforts, from both before and after black rat eradication to demonstrate that 1) the loss of black rat seed predation is a mechanism for increases of *C.*

nucifera and it seems that native seed predators (*Birgus latro*) do not equal black rat in their seed predation ability, 2) increases in juvenile abundance lead to increases in juvenile biomass that vary across species with important impacts on ecosystem function, and 3) long-term effects of seed predation on tree community composition, aboveground biomass, and post-eradication ecosystem recovery, with shifts toward *C. nucifera* dominance, revealed via stage-structured demographic models.

2.3. METHODS

2.3.1. Study system

We conducted this study on a low-lying, currently uninhabited coral atoll (Palmyra) in the Northern Line Islands (5°53′1″N 162°4′42″W). The atoll's 230 ha of land area consists of a ring of islets surrounding three central lagoons. Palmyra Atoll has a wet tropical climate (an average 4,488 mm annual rainfall; mean annual temperature 27°C) and approximately 90% of the atoll is forested, with canopies of five species which occur in monoculture to mixed-stand gradients of *Cocos nucifera* (Arecaceae) (65% of total tree canopy area), *Pisonia grandis* (Nyctaginaceae) (12%), *Scaevola taccada* (Goodeniaceae) (12%), *Heliotropium foertherianum* (Boraginaceae) (12%), and *Pandanus tectorius* (Pandanaceae) (10%) (Young et al. 2010b, Lafferty et al. 2018). Similar to many islands in the Pacific, it is believed that *Cocos nucifera* arrived from Asia with humans in the last 1500 years (Matisoo-Smith and Robins 2004, Gunn et al. 2011). On Palmyra, cultivation of *C. nucifera* for copra production between 1850 – 1970 increased what had likely been scattered coastal populations of this species to populations of 4,000 adult trees in the 1850s (Dawson 1959). This population has grown to more than 53,000 reproductive adults on the atoll in 2005 and *C. nucifera* continues to expand its range into forest patches of native broadleaf tree species

(Wegmann 2009, Young et al. 2017). Black rats were likely introduced to the atoll during World War II military occupation (starting in 1941) and were successfully eradicated with aerially-distributed anticoagulant rodenticide (brodifacoum) over a one-month period, June-July 2011 (Wegmann 2009, Young et al. 2017, Wolf et al. 2018). Black rats likely died almost immediately due to the potency of brodifacoum rodenticide and the success of aerial baiting (Parkes et al. 2011); rat absence was verified with post-eradication baiting in summer 2012 and no rats have been observed since the eradication (Wolf et al. 2018).

2.3.2. Permanent plot setup

In 2007, before black rat eradication, we established seven 300-m^2 vegetation monitoring plots. These plots were established to track population dynamics among tree species over time, with a focus on understanding long-term dynamics of the dominant

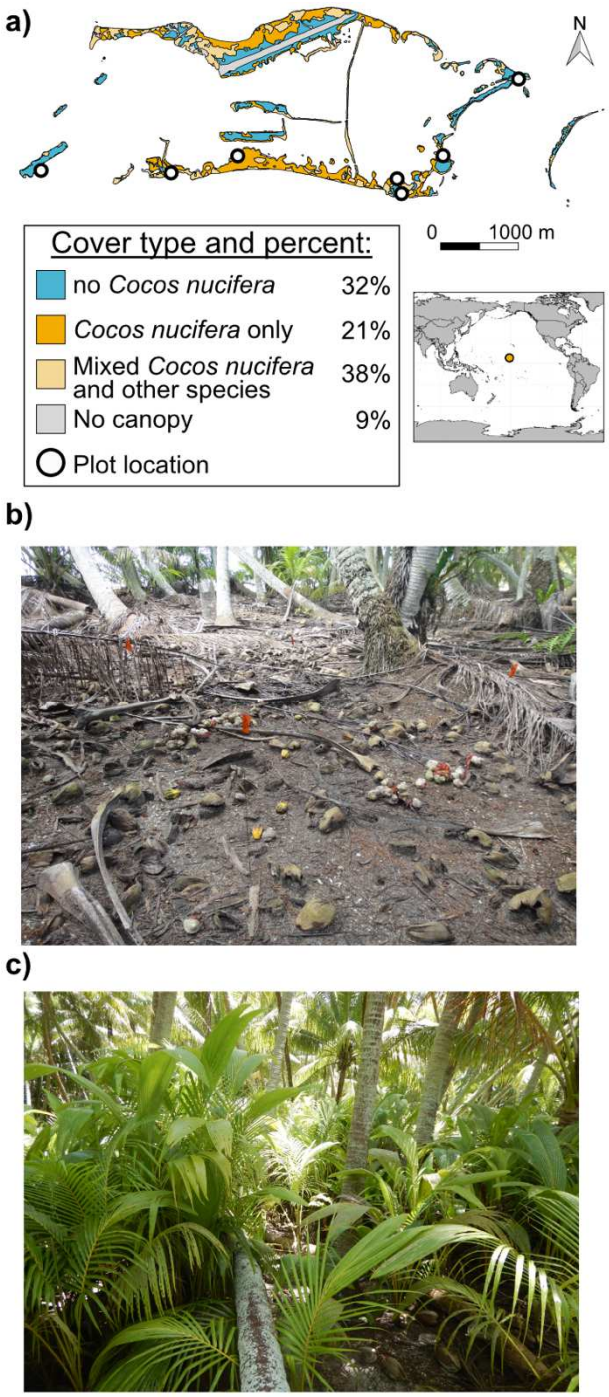


Figure 5: Palmyra Forests Before and After Rat Eradication

(a) Palmyra Atoll, Central Pacific, with vegetation plot distribution across the atoll. The palm tree *Cocos nucifera* occurs in mixed and monoculture stands in 65% of the forested portions of the atoll. Repeated photos taken in the same season (August-September 2010 and 2015) of one vegetation plot one year before (b) and four years after (c) rodent eradication illustrate how rodent presence limits juvenile tree recruitment. (For reference, fronds on the ground in photo (b) are 2 meters in length; tallest understory palms in left of photo (c) are 2.5 meters in height and the average height of seedlings in this photo is 1.5 – 2 meters).

introduced *C. nucifera* (65% of atoll forest canopy cover; Figure 5) within the atoll ecosystem (see Young et al. 2017). Therefore, plot locations were selected either in areas already dominated by *C. nucifera* (i.e., 100% cover based on adult basal area), or areas considered potential expansion fronts, given the presence of adult *C. nucifera* in or adjacent to each plot (0-37% basal area of *C. nucifera*, with at least one subadult *C. nucifera* present in the plot).

2.3.3. Permanent plot tree measurements

At the initial survey (2007) we permanently tagged each adult tree (individuals with diameter $\geq$ 10 cm either at breast height (DBH; 1.3 m) or below the base of their crowns). We measured each tree DBH and determined aboveground biomass using diameter-based allometric equations (Chave et al. 2005; Appendix 1A) We also counted, measured (diameter at DBH or trunk base), and tagged all subadult trees (hereafter 'juveniles'; < 10 cm DBH and taller than 1 m). We revisited each plot in four pre-eradication years (2007-2010) and four years post-eradication (2014- 2017) during the summer season. In each of these years, we confirmed if all previously tagged adult trees were present and alive, re-counted previously marked juvenile trees, and counted and tagged all newly recruited juveniles in each plot.

2.3.4. Permanent plot seed counts and seed predation

For the large-seeded *C. nucifera* (2-3 kg fruit wet weight; (Harries 1978, Dransfield and Cooke 1999), we counted the number of predated and viable drupes (hereafter referred to as 'seeds') on the ground using a series of 24 1-m^2 seed count quadrats in each plot. For this tree species on Palmyra Atoll, predation always leads to seed death because seed predators

burrow into the seed embryo. We included both predation of green, immature seeds (often attributed to rats gnawing into seed embryos while seeds are in the canopy) and predation of ripe, mature seeds on the ground (most likely from husking by the coconut crab *Birgus latro*) (Harper and Bunbury 2015). Rats are the only canopy seed predators in this system. Seed surveys were conducted in three years (2007, 2015, 2016, n = 144 per year). Seeds of most tree species on the atoll (not including *C. nucifera*) have previously been shown to be heavily predated by rats (Wegmann 2009), though we did not measure predation of these seeds in our plots because these species were not present or because seeds of these species were not observed before rat eradication. We also estimated the number of immature and mature *C. nucifera* seeds in each tree in each plot in three years (2007, 2015, 2016; Appendix 1D)

We established that seed predation was the mechanism of change in *C. nucifera* abundance and biomass by comparing 1) predated and 2) viable seed counts in quadrats across eradication periods using generalized linear mixed effects models. For each of the two seed types ('predated' and 'viable'), we specified a full model with seed number as the response variable, eradication status as fixed effect, plot as random effect, and distributions appropriate per response variable (Zuur et al. 2009). We built all models in the *lme4* package in R (R version 3.5.0, *lme4* v 1.1-17, Bates et al. 2015). We selected the best fitting model by minimizing AIC values (*MuMIn* package v 1.42.1, (Burnham and Anderson 2002)) and conducted post-hoc pairwise comparisons between marginal means of pre-post eradication periods for best-fitting models that included this fixed effect (*emmeans* package version 1.4.5). We verified that model assumptions (including heteroskedasticity and lack of overdispersion and zero inflation) were met using the *DHARMa* package (version 0.2.0; Hartig 2018; Appendix 1B).

2.3.5. Short-term changes in aboveground biomass

We examined whether decreased rat seed predation led to a short-term increase in plant aboveground biomass by measuring changes in the biomass of juvenile trees of all species (non-reproductive trees < 10 cm DBH and taller than 1 m). We converted juvenile tree counts for all species to dry biomass using species-specific equations or conversions based on data collected for juveniles elsewhere on Palmyra Atoll and from literature values (Chave et al. 2005, Young et al. 2011, 2013b, Ashish et al. 2015, Climate Action Reserve 2017). Because *C. nucifera* dry biomass is highly age-dependent, we determined total juvenile tree biomass in plots only for years in which all juveniles had been longitudinally tracked for at least two years and could thus be separated into age classes (pre: 2008, 2009, and 2010; post: 2016; Appendix 1A).

To quantify juvenile tree biomass change, we used generalized linear mixed effects models to determine whether eradication altered juvenile biomass. We specified a full model with total juvenile biomass (in dry grams) as the response variable, eradication status as fixed effect, plot nested within year as the random effect, and a Tweedie error distribution, which is used to measure biomass values in datasets with potential zero-inflation and high skewness (Lecomte et al. 2013, Dons et al. 2016). For all models, we chose models and assessed model fits with the same model selection process used for the seed models. For these models, we used the *glmmTMB* package (version 1.0.0) to accommodate the Tweedie distribution and compared marginal means with the *emmeans* package (version 1.4.5). To verify that changes in juvenile biomass were not due to environmental conditions in the years in which biomass could be assigned, we also ran a repeated measures ANOVA for juvenile tree numbers across all sampling years (2007-2010, 2014-2017) to verify that

changes were consistent and compared annual precipitation (in cm) between pre- and post-eradication years using a Mann-Whitney *U* test, (Lafferty et al. 2018, Appendix 1D).

2.3.6. Demographic modeling and estimates of long-term biomass change

Changes in juvenile abundance and biomass reflect immediate responses to seed predation release but are not necessarily indicative of the longer-term outcomes for the tree community. Because of the long lifespans of the trees on the atoll (70+ year lifespan with a time to maturity of 12 or more years; Navarro et al. 2008, Malhotra and Welfare 2017) compared to the relatively short time period of this study, we used demographic models to predict how these changes may lead to long-term shifts in total plant biomass. Because we had data on all life stages for *C. nucifera* and not for other species, and because this species is the most abundant species on the atoll, we modeled this species only and assume that any changes in this species reflect a magnitude of change that is representative of biomass changes for the tree community. Because *C. nucifera* can persist in the juvenile stage using stores from their large seed for a variable amount of time (3-6 months up to one year, (U. of Hawai'i Cooperative Extension Service 1996)) depending on growing conditions, we used stage-structured (in lieu of age-structured) demographic models, which separate populations of a species into discrete stages in which each stage shares the same probabilities of survival, growth, and reproduction (vital rates) and tracks these population stages over time given specified initial population sizes (Caswell 2001, Rist et al. 2010).

Because rats are known seed predators and seedling herbivores in island ecosystems (Harper and Bunbury 2015), eradication may have had a significant effect on both seed and seedling life stages. Rats may alter 1) fecundity (adult seed production) through pre-dispersal predation, 2) seed survival and germination through post-dispersal predation, and 3) juvenile

survival and recruitment to adulthood through herbivory of seedling apical meristems or of the large seeds attached to seedlings. Indirectly, changes in juvenile abundance may lead to altered intra- and interspecific competitive dynamics (Silva Matos et al. 2013). We determined whether rats had altered each vital rate by determining each rate for each plot within a given year. We then compared vital rates among years using repeated measures ANOVAs or Wilcoxon signed rank tests (Appendix 1C). Based on the results of these tests, we created two vital rate (i.e., Leftkovich) matrices for later use in stage-structured population models, one representing vital rates with rats present in the community and one representing vital rates with rats absent. When vital rates did not vary significantly ($\alpha = 0.05$) among years, we used the same vital rate across all plots and years. When vital rates varied across eradication periods (i.e. with and without rats), we averaged vital rates across plots within each eradication period, resulting in a different value for that vital rate with and without rats present.

To determine whether changed vital rates altered future aboveground biomass, we ran demographic model simulations for each vegetation plot with each of the two vital rate (Leftkovich) matrices ($n = 14$ simulations). We used a starting population vector for all simulations of population sizes for each plot in the pre-eradication time period. We ran each model over one juvenile generation (12 years). We converted the yearly population increase in each life stage from these models to biomass by multiplying the total number of new juveniles by the biomass of one age-one juvenile and the total number of new adults by the biomass of an age-twelve individual minus an age-eleven individual (thus only accounting for the biomass added as it became an adult). We summed this total biomass per plot per rodent status and tested whether the total added plant aboveground biomass was significantly different with and without seed predation using a Wilcoxon signed-rank test. In addition, we

determined population growth rates (λ) for each matrix. We also determined model elasticities, which measure the relative importance of changes in each vital rate compared to all other vital rates and therefore indicate which vital rates determine population growth rate (λ).

2.4. RESULTS

2.4.1. Permanent plot seed counts and seed predation

Rat eradication significantly decreased the total number of predated seeds (Figure 6a) and increased the total number of viable seeds in vegetation plots (Figure 6b). We observed 44 predated seeds in 22 of 144 quadrats (15% of quadrats) pre-eradication and 15 predated seeds in 11 of 218 quadrats (5% of quadrats) post-eradication). Of these, we only observed mature predated nuts (brown with husk tearing, indicative of *B. latro* predation) following eradication and we only observed a total of 11 seeds in this category (of a total of 59 observed predated seeds). We observed 12 viable seeds in 8 of 144 quadrats (6% of quadrats) before eradication, and 87 viable seeds in 54 of 218 quadrats (25% of quadrats) post-eradication. The best model for the number of viable seeds had a negative binomial error structure and included eradication status as the fixed effect and plot as a random effect (*marginal* $R^2 = 0.13$, *conditional* $R^2 = 0.28$, pairwise difference between pre-post eradication marginal means significant with *p-value* < 0.001; Appendix 1B). The best model for the number of predated seeds had a Poisson error structure and included eradication status as a fixed effect and plot as a random effect (*marginal* $R^2 = 0.15$, *conditional* $R^2 = 0.58$, pairwise difference between pre-post eradication marginal means significant with *p-value* <0.001; Appendix 1B).

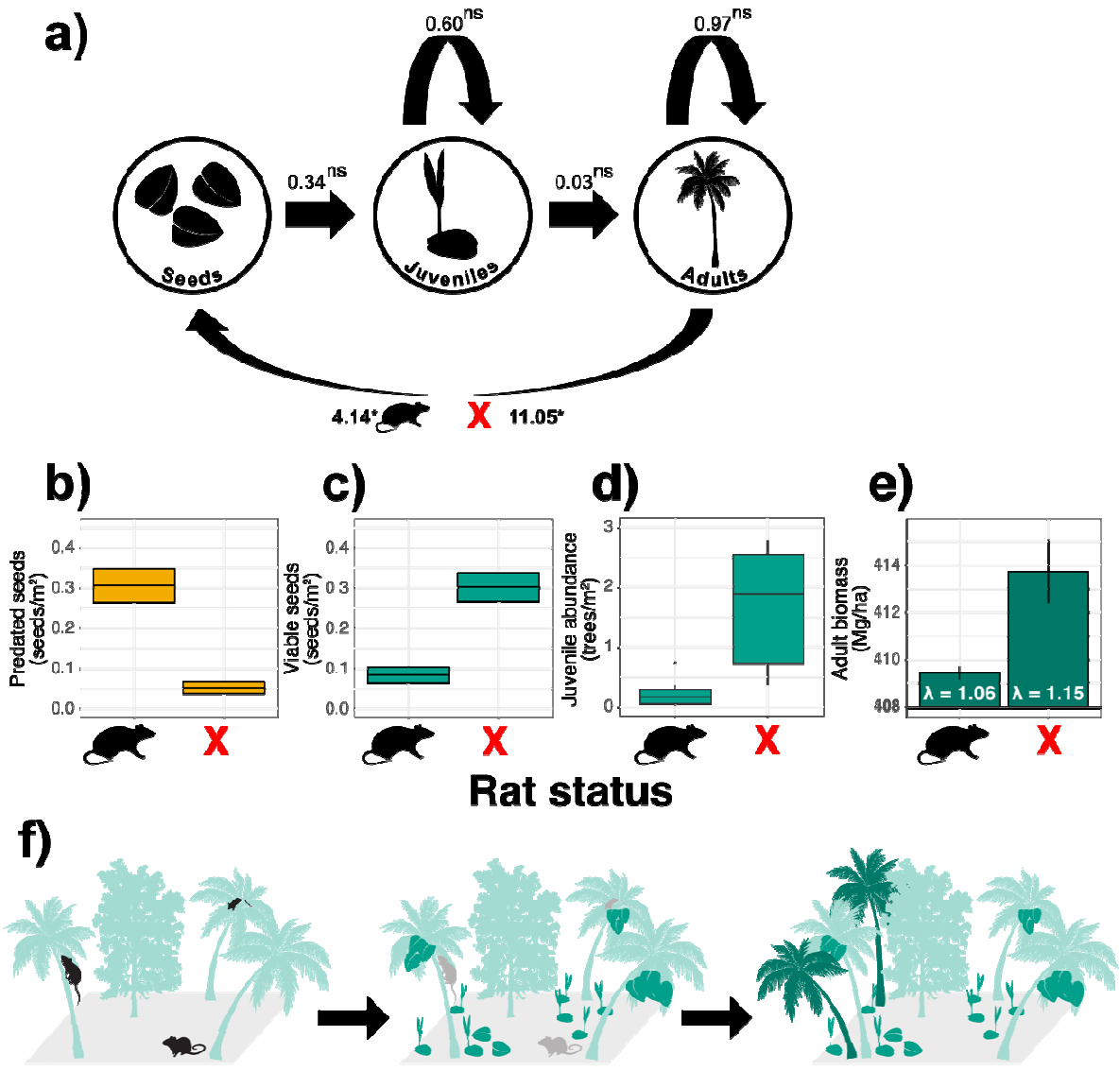


Figure 6: Short- and Long-term Changes to Palmyra Atoll Forests

A stage-structured demographic model for *Cocos nucifera* (a). The value on each arrow indicates a rate of survival or transition within or between stages. Only fecundity (number of mature seeds produced per adult tree per year) significantly changed following eradication. (Statistical significance across years with and without rats denoted with *, statistically indistinguishable change denoted by "ns".) Black rats predated seeds (b), controlling viable seed numbers (c) (values given in terms of the average number of seeds per square meter quadrat). Juvenile abundance (d) increased from an average 0.23 to 1.67 per square meter in each plot. In the first three generations (12 years), projected additional aboveground tree biomass in *Cocos nucifera* (e) significantly increased from an average of 1.42 to 5.73 Mg ha^{-1} as population growth rate (λ) increased. (f) Forest aboveground tree biomass along a temporal trajectory: invaded (left), immediate eradication response (center), and

long-term projections of increased tree biomass in the absence of rodent seed predators (right), where changes at each temporal step are indicated by increasingly darker colors. (vector images: Ana Miller-ter Kuile and Shutterstock)

2.4.2. Short-term changes in aboveground biomass

There was a significant increase in juvenile tree biomass following eradication (Figure 6c), increasing from an average of 0.69 (*SD* 0.50) to 9.48 (*SD* 5.97) Mg ha^{-1} ($n = 27$). This increase was primarily driven by increases in juvenile *C. nucifera*, which accounted for 73% of the total increased juvenile biomass, though two other species (*Pa. tectorius* and *Pi. grandis*) also increased in biomass (Figure 7). Juvenile biomass was best predicted by a model with eradication status as fixed effect and plot as random effect (pairwise difference between marginal means: *p-value* < 0.001; Appendix 1B). This is consistent with results from yearly juvenile abundance repeated measures ANOVA. Precipitation was not significantly different across pre- and post-eradication years (Appendix 1D).

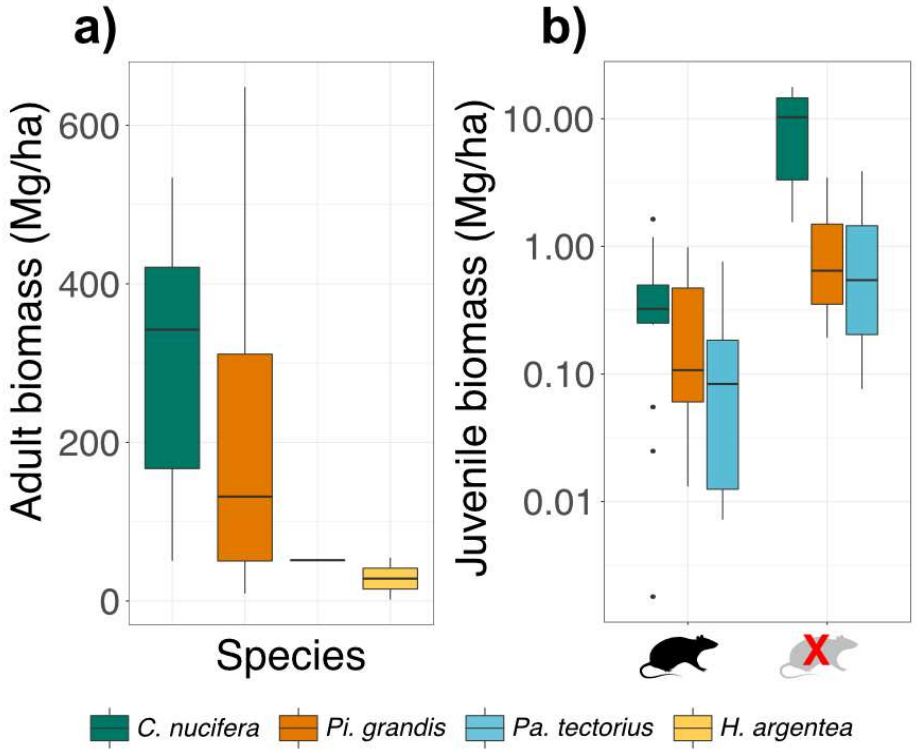


Figure 7: Adult and Juvenile Biomass in Palmyra Permanent Plots

In our plots, *C. nucifera* is the species with highest adult tree biomass (a) and juveniles of this species had the most marked biomass response to black rat eradication (b). (vector image: Shutterstock).

2.4.3. Demographic modeling and estimates of long-term biomass change

Modeled future aboveground biomass significantly increased following eradication (Figure 6d). Fecundity significantly increased following eradication, increasing from 4.14 (*SD* 8.99) to 11.05 (*SD* 12.23) viable seeds per tree per year. No other vital rates significantly changed between pre- and post-eradication (Table 1, Appendix 1C). In the first juvenile generation (12 years), there was an increase in the added aboveground biomass in the plots (Wilcoxon signed-rank test, *p-value* = 0.02), with aboveground biomass added via

recruitment increasing from an average of 1.42 (*SD* 0.82) to 5.73 (*SD* 3.62) Mg ha^{-1} per plot. Examination of elasticities for both models shows adult survival has the greatest effect on population growth rate (Table 1).

Model (growth rate, $\overline{\lambda}$)	Vital rate (per year)	Value	Elasticity
With rats ($\overline{\lambda} = 1.06$)			
	Seed to juvenile transition	0.34	0.06
	Juvenile survival	0.60	0.09
	Juvenile to adult transition	0.03	0.06
	Adult survival	0.97	0.72
	Fecundity	4.04[a]	0.06
Without rats ($\overline{\lambda} = 1.15$)			
	Seed to juvenile transition	0.34	0.11
	Juvenile survival	0.60	0.11
	Juvenile to adult transition	0.03	0.11
	Adult survival	0.97	0.57
	Fecundity	11.05[a]	0.11

[a] Significantly different based on quadrat seed counts

Table 1: Demographic Model Vital Rates

Rates of survival, growth, and reproduction (vital rates), elasticities (relative importance of each rate for population growth), and population growth rates (λ) for stage-structured demographic models of *Cocos nucifera* with and without rat seed predation (vital rate determinations in Appendix 1C).

2.5. DISCUSSION

Black rats, through seed predation, reduced juvenile recruitment and aboveground biomass of the introduced palm tree species *C. nucifera* (a 24-fold increase). Through seed predation or seedling herbivory, black rats also limited juvenile recruitment and biomass of at least two native tree species (*Pa. tectorius* and *Pi. grandis*), but to a lesser degree (11-fold). These data support a previous finding of the recent increases in seedling abundance on Palmyra Atoll (Wolf *et al.* 2018) and further elaborate on the mechanisms driving these increases. Specifically, the present study illustrates that rat seed predation operated as a key control on introduced *C. nucifera* populations. Here we show, based on demographic models informed by repeatedly-surveyed permanent plot data, how increases in fecundity

(undamaged seed production) and juvenile recruitment in *C. nucifera* will lead to an increase in adult recruitment in this species, increasing total aboveground biomass and changing the species composition of biomass pools on the island. In this system, in particular, the negative effects of the non-native *C. nucifera* on island food webs, even in the presence of black rats, is well-established (*C. nucifera* deters nesting seabirds, thus removing guano subsidies; reviewed in Young et al. 2017). This study demonstrates that this negative effect could be compounded by the eradication of an introduced seed predator (black rats), thus highlighting the need for further management intervention. More broadly, while island rodents are known to consume seeds and fruit (Drake et al. 2011), this study provides an important demonstration of how seed predation can influence entire island ecosystems from short-term changes for plant community composition, to long-term shifts in ecological functions related to plant biomass, such as carbon storage and decomposition (i.e., ecosystem-level eradication: Zavaleta et al. 2001).

The ecological and conservation outcomes on Palmyra Atoll highlight the importance of considering seed predation as a critical management element associated with the removal of invasive rodents. On Palmyra, juvenile recruitment and biomass of an introduced tree (*C. nucifera*) responded at greater magnitudes (2-5 times) than other tree species to seed predation release, even though all these tree species were heavily targeted by rodent seed predators and have seen some population recovery following eradication (Wegmann 2009, Wolf et al. 2018). The differential responses of plant species to rat eradication may be due to rat food preference, inability to effectively kill seeds of a species (thus providing a benefit as a seed disperser), the reproductive ability of the tree, the tree's ability to escape predation by other seed predators when rats are not in the system, or a combination of these or other factors (Clark 1981, Hayes and Barry 2008, Young et al. 2013b, Nigro et al. 2017). In this

system, it is likely that the differential advantage of *C. nucifera* comes from the natural history of the tree, since evidence from rat husking stations on Palmyra Atoll suggest that rats predate seeds of most of the canopy trees in this system and cause seed mortality in the majority of predation events for all seed species, thus providing little or no benefit as a seed disperser for this or other tree species (Wegmann 2009). Although we saw some evidence that the coconut crab (*Birgus latro*), the only native seed predator effective at predating *C. nucifera* seeds once they reach the ground, is predating some seeds in the absence of rats, the relative rarity of this predation compared to rat predation suggests that coconut crabs will not compensate for rats in the short term. However, the increased frequency with which coconut crabs are now observed in *C. nucifera* forests following black rat eradication suggests this is a process that warrants longer-term monitoring (Nigro et al. 2017). *B. latro* and other terrestrial crabs in this system (hermit crabs: *Coenobita brevimanus* and *Coenobita perlatus* and land crabs: *Cardisoma carnifex* and *Cardisoma rotundum*) preferentially predate seeds of rare tree species, so the disproportionate immediate benefit to *C. nucifera* may only be exacerbated by seed selection of native seed predators for seeds of rarer canopy trees (Young et al. 2013b, Nigro et al. 2017). The conservation implications of the increased competitive advantage of an introduced tree (*C. nucifera* in this case) means that the detrimental effects of yet another invasive species become magnified by rat eradication (i.e. Bergstrom et al. 2009). On Palmyra, the negative effects of this tree species are already known: *C. nucifera* deters seabird nesting on Palmyra, thus stopping the provision of seabird guano to native plant communities dependent on these subsidies. In this system, in particular, the effects of rat eradication on *C. nucifera* make imperative an extensive secondary intervention to remove and control this invasive species.

While shifts in plant communities led to immediate changes for the island ecosystem (i.e. Wolf et al. 2018, Nigro et al. 2017), our demographic models highlight that the effects of seed predation release on island ecosystems can potentially reverberate through decades (or even longer periods) of succession dynamics. Similar to other systems modeling palm demography, population growth rate (and thus total biomass) is disproportionately determined by changes in the number of seedlings that germinate, survive, and reach adulthood (Pinard and Putz 1992). Increased total tree biomass in *C. nucifera* on Palmyra will likely alter a diverse range of ecological functions on the atoll, including carbon storage, nutrient cycling, and primary productivity (Chave et al. 2006, Sayer 2006, Sayer et al. 2007, 2011, 2012, Bello et al. 2015). Currently, the increase in the proportion of biomass in juvenile life stages (an increase from 0.08 to 1.43%) has likely altered rates of decomposition and soil respiration through an increase in the number of juveniles germinating and then dying in the understory (Quested et al. 2007, Chomel et al. 2016). In the long term, a shift in the species composition, and therefore of productivity and biomass (increasingly more *C. nucifera*-dominated), has the potential to substantially alter rates of decomposition and nutrient cycling (Quested et al. 2007). The demographic models ignore potential responses in other seed predators (here, land crabs) or stochastic events that may alter successional dynamics (i.e. storms, climatic conditions) but highlight that island responses to seed predation release may occur on long time scales and in other island systems successional trajectories may be reasonably predicted using modeling approaches.

These ecosystem-level effects of invasive rodents and their eradication have important implications for the management and restoration of islands and island biodiversity. While the ecological regime-shifting potential of invasive rodents on seabird islands has been observed across many islands in New Zealand (Fukami et al. 2006), this work from Palmyra

Atoll contributes to a smaller body of literature examining ecological regime shifts through the mechanisms of seed predation and the ecosystem effects of rodent seed predator eradication (Grant-Hoffman et al. 2010b, 2010a). In the case of Palmyra, unlike on the islands of New Zealand, the effects of seed predation are decoupled from those of burrowing seabirds since Palmyra does not have nesting populations of these seabirds and demonstrates that seed predation alone can be an important ecological driver. In all these cases, invasive plants often become common on islands following eradications, making subsequent management necessary or advised to curb island ecosystem degradation (Grant-Hoffman et al. 2010a). On Palmyra as with other tropical atolls where abandoned agro-forestry has shifted plant communities in favor of cultivated crops, the effects of rat eradication may increase the rate of spread of naturalized crop plants, putting much of the remaining habitats for important island species (i.e. island endemics and nesting seabirds) in greater jeopardy (Thaman 2016, Young et al. 2017). These studies emphasize the need to view eradications as part of holistic management plans to avoid ecological regime shifts (i.e. Zavaleta et al. 2001).

The observed plant responses on Palmyra suggest important insights for future island rodent eradications. Foremost, it is imperative to revisit the trophic ecology of invasive rodents and refine eradication efforts considering all organisms likely to respond to eradications. Specifically, as seeds and fruit constitute the majority of the diet of black rats (Shiels & Pitt 2014), eradication efforts of this species need to document plant responses. This may change the prioritization of islands for rodent eradication based on the restoration of important elements of the flora that support island fauna (Capizzi et al. 2010, Harris et al. 2012, Holmes et al. 2019). Incorporating plant responses into eradication monitoring may require a combination of field and modeling approaches since many plants, including those

in this study, respond on longer time scales than the time span of most eradication monitoring programs. Appropriate temporal scales need to be built into eradication programs because long-lived trees are foundational species that shape ecosystem structure and function (Ellison et al. 2005). With Palmyra Atoll as a case study, the immediate recoveries of island communities following release from the effects of rats may be outweighed by the long-term expansion of invasive tree species, favoring ecosystem processes that are actively detrimental to native biodiversity (Young et al. 2017). Many islands host multiple invasive species and often these species interact, so eradications should be done with care to ensure recovery of stable and regenerating ecosystems rather than deteriorated alternative ecological states (Suding et al. 2004, Tershy et al. 2015). Indeed, understanding the interactions between invasive species on islands and how these influence ecosystems therein is an important area of current and future study that starts with understanding the breadth of ecological effects of invasive species.

Data Availability: The data that support the findings of this study are openly available in Dryad at doi: https.//doi.org/10.5061/dryad.xsj3tx9cp (Miller-ter Kuile *et al.*, 2020).

Acknowledgements: We thank the National Science Foundation (#1457371, #0639185, #1714426, #1820379, #1620366), the National Geographic Society (# 9698-15), the U.S. Fish and Wildlife Service, and The Nature Conservancy for supporting this research. We thank A. Meyer, S. Kroplidowski, E. Forbes, J. Childress, K. Plummer, D. Weber, M. Lee, A. McInturff, A. Briggs, P. DeSalles, J. McCallen, K. Rodman, G. Titcomb, J. Schroeder, and K. Culhane for help on various aspects of this research and manuscript preparation.

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AM-tK, DO, HY, and RD conceived the ideas and designed methodology; HY, RD, and DM received funding for this project; AM-tK, DO, AB, RD, MK, DM, CM, and HY collected data; AM-tK, DO, and MK analyzed the data; AM-tK led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CHAPTER 3: EFFECTS OF SURFACE STERILIZATION ON DIET DNA METABARCODING DATA OF INVERTEBRATE PREDATORS

3.1 ABSTRACT

DNA metabarcoding is an emerging tool used to quantify diet in environments and consumer groups where traditional approaches are unviable, including small-bodied invertebrate taxa. However, metabarcoding of small taxa often requires DNA extraction from full body parts, and it is unclear if surface contamination from body parts alters presumed diet presence or diversity. We examine four different measures of diet (presence, rarefied read abundance, richness, and species composition) for a terrestrial invertebrate consumer (the spider *Heteropoda venatoria*) both collected in its natural environment and fed an offered diet item in contained feeding trials using DNA metabarcoding of full body parts (opisthosomas). We compared diet from individuals surface sterilized in 10% commercial bleach solution followed by deionized water with a set of unsterilized individuals. We found that surface sterilization did not significantly alter any measure of diet for either consumers from a natural environment or in feeding trials. The best-fitting model predicting diet detection in feeding trial consumers included surface sterilization, but this term was not statistically significant (ΔAICc = 1.59 compared to the null model, β = -2.3, p-value = 0.07). Our results suggest that surface contamination does not seem to be a significant concern in this DNA diet metabarcoding study for consumers in either a natural terrestrial environment or in feeding trials. As the field of diet DNA metabarcoding continues to progress into new environmental contexts with various molecular approaches,

we suggest ongoing context-specific consideration of the possibility of surface contamination.

3.2 INTRODUCTION

Biological communities and ecosystem function are shaped by interactions between organisms (Brown et al. 2001, Hooper et al. 2005, Schleuning et al. 2015). Among the many interaction types, consumptive interactions (including herbivory, predation, and parasitism) can shape the stability of biologically diverse communities (Ings et al. 2009, Delmas et al. 2019). Until recently, these consumptive interactions were most often measured by visual observations of feeding or by gut dissection or inspection of fecal contents (Hyslop 1980, Duffy and Jackson 1986, Baker et al. 2014, Nielsen et al. 2018), which made it challenging or impossible to conduct diet analyses for many consumer groups. Specifically, these diet analyses are not possible for consumers that a) are too small for dissection and food identification, and b) have feeding habits or food items which make diet visually unidentifiable (Sheppard and Harwood 2005). This group of consumers, which includes terrestrial insects, spiders, and other arthropods, form the base of most terrestrial food webs and are integral to maintaining biodiversity and ecosystem functioning in ecosystems worldwide (Wilson 1987). For these consumer groups, the use of high-throughput sequencing methods for determining gut contents is one of the most promising emerging approaches. High-throughput sequencing (hereafter referred to as "diet DNA metabarcoding") can identify a suite of diet species at once and provides a comprehensive and efficient method for determining intra-population, intraspecific, and interspecific diets (Pompanon et al. 2012, Quéméré et al. 2013, Soininen et al. 2015, Lucas et al. 2018). These methods have already illuminated new interactions and ecological trends in a variety of

environments (e.g. host-parasitoid: (Wirta et al. 2014); plant-herbivore: (Kartzinel et al. 2015); host-parasite: (Schnell et al. 2012), predator-prey: (Toju and Baba 2018).

As diet DNA metabarcoding methods continue to advance, however, they need to be validated so that the ecological inference made from them is robust. Focusing on the challenges of small organisms where small body size has limited other diet analysis methods, DNA diet analyses are often performed on full organisms or body parts without gut dissection (e.g. Jacobsen et al. 2018, Toju and Baba 2018). The necessity to use full organisms or body parts increases the possibility of surface contamination altering the detection and species composition of presumed diet items. Surface sterilization, the use of chemical treatments or physical action to remove surface contaminants, is systematically used in other fields to reduce the risk of contamination in DNA metabarcoding datasets (e.g. fungal endophyte research; Zimmerman and Vitousek 2012, Burgdorf et al. 2014). However, surface sterilization has not been systematically used in diet metabarcoding studies. While some fields have developed informed protocols based on decades of research into best practices and study-specific considerations (Hallmann et al. 1997, Brown et al. 2018), the field of diet DNA metabarcoding has not developed a similarly systematic approach (e.g. ethanol washes in (Doña et al. 2019), bleach washes in Anslan et al. 2016, no sterilization in Wirta et al. 2014, Jacobsen et al. 2018). The lack of systematic surface sterilization in diet DNA metabarcoding when using full individuals or body parts may be due to the desire to avoid destruction of DNA in relatively permeable animal cells (Greenstone et al. 2012). However, without considering surface sterilization as a treatment for surface contamination, we have limited ability to confidently assign DNA sequences to ingested diet items (Linville and Wells 2002, Greenstone et al. 2011, 2012).

In this study, we look at the effects of surface sterilization on our understanding of consumer diets where the DNA of full body parts (no internal dissection) is used for diet DNA metabarcoding. Targeting the CO1 gene region, we produced high throughput sequencing results from the full body parts (opisthosomas) of an invertebrate consumer species (the spider, *Heteropoda venatoria*). We surface sterilized half of the consumers prior to DNA extraction using a series of washes in a 1:10 dilution of bleach (10% commercial bleach) and deionized water; we left the other half of consumers unsterilized. We first determined how surface sterilization influences potential diet from consumers collected in their natural environment, comparing surface sterilized individuals to those which were not surface sterilized to ask whether surface sterilization influences 1) detection, 2) rarefied abundance, 3) richness, and 4) composition of potential diet items. We then performed a laboratory feeding trial in which we fed consumer individuals a specific diet item, comparing surface sterilized individuals to those which were not surface sterilized to ask whether surface sterilization influenced 1) detection or 2) rarefied abundance of offered diet items. Exploring these questions in both natural and contained settings help address whether surface contamination could alter ecological interpretations of community-scale species interactions and whether surface sterilization needs to be incorporated into standard protocols in this field.

3.3 METHODS

3.3.1 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 characterization of consumer and

potential diet items in DNA metabarcoding data (Handler et al. 2007). We targeted a generalist, active hunting spider species (*Heteropoda venatoria*) as the consumer for this project because a) it occurs in high abundance on the atoll and is therefore easy to collect, b) it is a generalist species that feeds on a wide suite of other organisms (including insects, spiders, other invertebrates, and two vertebrates, geckos in the genus *Lepidodactylus*), c) due to its small size and arachnid feeding habits, there are no viable non-genetic methods of diet analysis, and d) it is the only species in its family on the atoll, meaning consumer DNA can be differentiated from potential diet DNA. We collected consumer individuals during two summers. In 2015, we collected individuals in natural habitats across the atoll. In 2017, we collected consumer individuals which we kept in container environments in the lab (explained below). All individuals were collected individually in sterilized collection containers to avoid contamination (Greenstone et al. 2011).

	Surface sterilized		*Unsterilized*	
Environment	Extracted	Amplified	Extracted	Amplified
Natural environment	22	**18**	25	**19**
Feeding trial	10	**8**	14	**11**

Table 2: Sample Sizes for Surface Sterilization Study

Sample sizes for successfully extracted and PCR amplified samples of surface sterilized and unsterilized *H. venatoria* individuals in the natural environment and feeding trial studies. Bold numbers indicate final sample sizes for statistical analyses.

3.3.2 Natural environment consumer collection

In 2015, we collected consumers (n = 47) in natural environments in order to test whether DNA metabarcoding would detect potential diet DNA from consumers which fed on available diet items and came into contact with natural environmental surfaces. We

collected and froze all individuals in separate containers at -80°C immediately following collection until surface sterilization and DNA extraction in 2019 (see below).

3.3.3 Feeding trial consumer set-up and feeding

In 2017, we conducted laboratory trials (n = 26) in order to test whether DNA metabarcoding would detect DNA from a diet item a consumer was offered in a contained environment. We created feeding environments out of one-liter yogurt containers with holes for air transfer. We placed an individual *H. venatoria* in each container and after a 12-hour period alone in the containter, all *H. venatoria* individuals were offered one individual of a large grasshopper species (*Oxya japonica*), which is a common introduced species on the island and a likely diet item (Handler et al. 2007). We left all containers for 24 hours, after which we immediately froze (at -20°C) *H. venatoria* individuals which had killed an *O. japonica* individual (n = 25 of 26 trials); consumption of killed individuals was not easily detectable and thus not considered in analyses. All containers were cleaned between each trial with a 10% bleach solution and kept closed to avoid contact of other organisms with the inside of the containers.

3.3.4 Both natural environment and feeding trial consumers: surface sterilization

Because we planned to extract DNA from entire body parts (opisthosomas) of consumer individuals (following methods from Krehenwinkel et al. 2017, Macías-Hernández et al. 2018), we wanted to determine whether surface sterilization of *H. venatoria* consumer individuals altered common diet DNA measures (detection, abundance, richness, and composition). We used a surface sterilization treatment to remove possible contaminants from some consumer individuals from both the natural environment and feeding trials while

leaving some individuals unsterilized. We used surface sterilization techniques common in other fields of molecular ecology (i.e. plant endophytes Schulz et al. 1993, Burgdorf et al. 2014) by submerging and stirring each full consumer in 10% commercial bleach by volume (0.5% sodium hypochlorite) for 2 minutes and then washing each consumer by submerging and stirring in deionized water for 2 minutes. Similar or longer periods of bleach washing at equal or greater concentrations have led to undetectable DNA degradation in similar soft-exoskeleton consumers (e.g. maggots and beetle nymphs; Linville and Wells 2002, Greenstone et al. 2012). Natural environment consumers (2015) had been frozen at -80ºC since collection; these consumers were surface sterilized following the bleach wash protocol in a sterilized laminar flow hood in 2019 just before DNA extraction (n = 22 surface sterilized, n = 25 not surface sterilized; Table 2). We surface sterilized feeding trial consumers (2017) following the bleach wash protocol in the lab on the atoll in 2017 following freezing at -20°C and then stored each sample in individual vials of 95% ethanol in a -20ºC freezer until DNA extraction because no -80ºC freezer was available at the field station that year (n = 10 surface sterilized; n = 14 not surface sterilized). Prior to DNA extraction, all samples from both 2015 and 2017 were allowed to dry for 1-3 hours in a sterilized laminar flow hood and the opisthosoma was removed from every consumer individual for DNA extraction using a sterilized scalpel in a sterilized laminar flow hood. For all sterilization steps, forceps, scalpels, and laboratory surfaces were sterilized with either ethanol and flame (scalpels and forceps) or 10% bleach (surfaces) between handling each individual.

3.3.5 DNA extraction and removal of consumer DNA with Ampure XP beads

We extracted DNA from each *H. venatoria* consumer opisthosoma following a modified CTAB extraction protocol (Fulton et al. 1995). At least twenty-four hours following extraction, we quantified DNA using a Qubit (Invitrogen) fluorometer with the high sensitivity double-stranded DNA quantification kit using 1µL of DNA template per reaction. We used methods developed by Krehenwinkel et al., (2017) to isolate a proportion of lower molecular weight DNA with Ampure XP beads prior to PCR. Ampure XP beads preferentially bind to heavier molecules of more intact consumer DNA, leaving the smaller fragments of presumed semi-digested diet DNA in the supernatant (Appendix 2E). Thus, by keeping the supernatant, we aimed to work with a sample that had a larger proportion of lower molecular weight diet DNA after removing consumer DNA that bound to beads (Krehenwinkel et al. 2017). To do this, we diluted each DNA sample to 20ng/µl (creating a total sample volume of 40µL), mixed each sample using Ampure XP beads (0.75x bead-to-DNA ratio), and kept the supernatant from this step. With the supernatant, we repeated the CTAB protocol steps for precipitating DNA pellets with isopropanol and 5M potassium acetate and cleaned DNA pellets with ethanol washes (Appendix 2F). After at least another twenty-four hours, we quantified DNA again using a Qubit fluorometer (following the same methods as above) and diluted all samples to 10ng/µL prior to PCR steps. All DNA pellets were stored in and diluted with TE buffer.

3.3.6 PCR amplification, library preparation, and sequencing

We amplified the CO1 gene with general metazoan primers (Yu et al. 2012, Leray et al. 2013, Krehenwinkel et al. 2017). The CO1 gene is well-represented in the GenBank sequencing database (Porter and Hajibabaei 2018). We performed all PCR preparation steps

in a UV-sterilized biosafety cabinet. We used a standard desalted primer set tested by Krehenwinkel et al., (2017) for use in diet analyses of invertebrate predatory consumers, including spiders (Table 3). These primers included overhang adapters compatible with the Illumina indexing PCR (Illumina 2009) that immediately followed CO1 amplification.

We amplified the CO1 gene in each sample by PCR in a 25µL reaction volume that included 9µL nuclease free water, 12.5µL GoTaq Green Master Mix (Promega Corp.), 1.25 µL of each of the primers (at 10mM), and 1 µL of DNA template (at 10ng/µL). When DNA concentrations were lower than 10ng/µL, we added more DNA to the sample to equal 10ng of total template and reduced the amount of water added. Each sample was run in duplicate until after Illumina indexing PCR, and we ran a duplicated negative sample each PCR run. We ran each reaction with an initial denaturation step at 95°C for 3 minutes, and then 35 cycles of: 1) denaturation at 95°C for 30 seconds, 2) annealing at 46°C for 30 seconds, and 3) elongation at 72°C for one minute. We ended each PCR run with a final elongation step at 72°C for 5 minutes and then held samples at 4°C until placed in a 4°C refrigerator. To remove reaction dimer before attaching Illumina P5/P7 indices, we removed lower molecular weight amplicons (~200 bp) with Ampure XP beads at a 0.8x bead-to-DNA ratio. Samples were re-suspended from beads using a 10mM TRIS resuspension buffer.

Primer	Sequence (5' – 3')	Source
mlCOIintF	**TCGTCGGCAGCGTCAGATGTGTATAAGAGA CAG**GGWACWGGWTGAACWGTWTAYCCYCC	Yu et al. 2012
Fol-degen-rev	**GTCTCGTGGGCTCGGAGATGTGTATAAGAG ACAG**TANACYTCNGGRTGNCCRAARAAYCA	Leray et al. 2013

Table 3: Primers with Illumina Overhang Adapters

Primers with Illumina overhang adapters (in bold) used to amplify the CO1 region in this study.

We then attached Illumina index primers with an additional PCR step (Nextera XT Index Kit v2). Each total reaction volume was again 25µL, with 5µL of nuclease free water, 12.5µL GoTaq Green Master Mix, 1.25µL of each primer (at 10mM), and 5µL of PCR product. These were run in a standard PCR protocol for these primers: an initial denaturing step at 95°C for 3 minutes, followed by 10 cycles of: 1) denaturation at 95°C for 30 seconds, 2) annealing at 55°C for 30 seconds, and 3) elongation at 72°C for 30 seconds. We ended each run with a final elongation step at 72°C for 5 minutes and then held samples at 4°C until placed in a 4°C refrigerator.

We verified PCR amplification by visualizing 3-4 µL of each PCR product in a 1.5% agarose gel using GelRed (Biotium) at 100V for 30-40 minutes. Gels were visualized with a Bio-Rad Gel Doc XR+ imager using Image Lab 5.0. We kept samples for which both duplicates successfully amplified during the PCR steps and only kept samples on PCR runs in which both negative control duplicates resulted in no product detection by gel electrophoresis. For successful samples, we combined duplicates and mixed with an Ampure XP bead-to-DNA ratio of 0.7x. We determined the average length of the gene region using an Agilent TapeStation with a D1000 ScreenTape System following the standard protocol from the quick start guide. We then quantified these final PCR products using a Qubit fluorometer and a high sensitivity kit with 1µL of sample per reaction tube and diluted each sample in 10mM TRIS to a final concentration of 5nM.

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; Toju et al. 2012, Clark et al. 2016, Apigo and Oono 2018). 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. Our full protocol from DNA extraction through submission for Illumina sequencing can be found in Appendix 2F.

3.3.7 Sequence merging, filtering, and clustering with UNOISE3

We merged, filtered (max ee = 1.0), and denoised (clustered) our sequences around amplicon sequence variants (ASVs) using the UNOISE3 algorithm (unoise3 command in the open-source USEARCH 32-bit version 11.0.667; Edgar 2016). This ASV denoising approach incorporates sequence abundance, quality, and error rates to cluster reads in high throughput sequencing data into a smaller subset of biological units (Appendix 2E). Prior to denoising with UNOISE3, we used cutadapt (version 1.18, Martin, 2011) to remove primers from each sequence. We also repeated analyses with the DADA2 algorithm run through R (dada2 package version 1.1.14.0; Callahan et al., 2016) and with a data cleaning step run through BBSplit (Bushnell 2019) to remove consumer DNA prior to ASV assignment (because ASV assignment is abundance-sensitive). We chose to consider analyses from the UNOISE3 algorithm only because UNOISE3 assigned more sequence reads to positive controls than DADA2 (on average, 3x as many reads per positive control) and the cleaning step paired with either DADA2 or UNOISE3 did not increase potential diet DNA detection (summary and comparisons in Appendices A and B).

From the output, we created a list of unique ASVs and a matrix of ASV abundances across samples. We matched ASVs 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 (LCA=naïve, MinScore = 50.0, MaxExpected = 0.01, TopPercent = 10.0, MinSupportPercent = 0.05) and selecting the subtree with all possible diet items for this species (Kingdom: Animalia, Clade: Bilateria). For taxonomies which were not assigned below the order level (n =24), we submitted each ASV individually to the BLAST Basic Local Alignment Search Tool and assigned them a family based on the best sequence match in the database, given that the top ten database matches were from the same family. For BOLD taxonomic assignment, we used the BOLD IDEngine of the CO1 gene with Species Level Barcode Records (accessed February 5-16, 2020; 3,825,490 Sequences, 216,704 Species, and 95,537 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 et al. 2017a).

3.3.8 Detection of potential diet items

For consumers from both the natural environment and feeding trials, we wanted to know whether surface sterilization altered the detection of potential diet items for each consumer. For natural environment consumers, we examined all potential diet items (which could represent either diet or surface contaminants). For feeding trial consumers, we focused our

detection analysis on the offered diet item we provided the consumers in the feeding trial environment (*O. japonica*, which all consumers were observed to have killed, but not necessarily ingested). Samples were rarefied (McKnight et al. 2019) because sequencing depth, or the total number of DNA sequences assigned per sample, can vary considerably in high throughput sequencing runs (Appendix 2E). We rarefied 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 and Chao 2017). We rarefied using the rrarefy() function in the vegan (version 2.5.6) package in R. We rarefied to 16,004 reads per sample for the natural environment and 55,205 reads per sample for the feeding trial consumers. We rarefied these separately because samples had been preserved in different ways and for different times, which can have large effects on DNA extraction outcomes (Murphy et al. 2002).

Following rarefying, we selected all ASVs that matched all potential diet items for the natural environment consumers (Kingdom: Animalia; Clade: Bilateria, excluding consumer DNA) and just the offered diet item for the feeding trial consumers (including species: *Oxya japonica*, genus: *Oxya*, family: Acrididae, excluding those which only matched to order: Orthoptera with no lower taxonomies). Because the consumer species *H. venatoria* is the only species in the family Sparassidae on Palmyra Atoll, removing consumer DNA meant excluding all ASVs that received a family-level taxonomic assignment of "Sparassidae". All ASVs received a family-level taxonomic assignment, so we chose to combine ASVs at the family level. Furthermore, because family-level taxonomic assignments are common in other diet metabarcoding studies (Kartzinel et al. 2015) and in the field of predator-prey interactions more broadly (Brose et al. 2019), this level allows diet data to be most comparable to studies across environments. We combined family-level taxonomic units by

combining ASVs that matched at the family level into one combined taxonomic unit with cumulative read abundance (i.e. all ASVs matched to *diet family A* were combined into one *diet family A* taxonomic unit with cumulative read abundance).

3.3.9 Abundance of potential diet DNA

To test whether surface sterilization altered the abundance of DNA representing all potential (natural environment) or offered (feeding trial) diet items, we assessed per sample offered or potential diet DNA abundance for both sets of consumers (natural environment and feeding trial) separately. For this analysis, we used only consumer individuals for which we detected potential or offered diet DNA (33 of 37 for natural environment; n = 14 out of 19 for feeding trials), to test whether contaminants altered potential diet abundance only when potential diet DNA is present.

3.3.10 Potential diet richness and composition in natural environment consumers

In addition to allowing detection of potential diet items, DNA metabarcoding also enables the analysis of potential diet communities, allowing explorations of individual-, population-, and species-level diet richness and composition. If surface contaminants alter these metrics, ecological interpretation of these community-level data could be misleading, either by increasing the richness of consumptive interactions attributed to a consumer, or by hiding interactions that occur more rarely or further back in time (e.g. (MacKenzie and Kendall 2002, Macías-Hernández et al. 2018). We determined the potential diet richness and composition in natural environment consumers. For per sample potential diet richness and composition, we performed analyses used both taxonomic units combined at the family level (described above) and with the original number and composition of ASVs matched to potential diet items.

3.3.11 Statistical analyses

For potential diet detection and rarefied abundance in both sets of consumers (natural environment and feeding trial) we used generalized linear models to assess the effect of surface sterilization treatment. For potential prey detection, all potential (natural environment) or offered (feeding trial) diet item detection (presence-absence per sample) was the response variable in the full model with surface sterilization treatment as a fixed effect and a binomial distribution. For diet abundance, we treated the number of all potential (natural environment) or offered (feeding trials; *O. japonica*) diet DNA reads per sample as the response variable, surface sterilization treatment as a fixed effect, total read abundance of the sample (constant across all) as an offset term, and a Poisson or negative binomial distribution (to correct for overdispersion when needed). We also examined the abundance of other potential diet items for the feeding trial consumers as well as DNA which was sequenced but was not diet (e.g. fungi and potential endoparasites) with results in the Supplemental Information (Appendix 2E). We assessed differences in per sample potential diet richness among sterilization treatments for the natural environment consumers using generalized linear models with the number of potential diet items per sample as the response variable (both family-level taxonomic units or ASVs), surface sterilization treatment as the fixed effect and a Poisson or negative binomial distribution (to correct for overdispersion when needed). We assessed differences in potential diet item composition with family-level taxonomic units between surface sterilized and unsterilized consumers using a presence-absence PERMANOVA model fit with a binomial mixed effects model with surface sterilization treatment as a fixed effect, a random intercept term for potential diet item, and a random slope term for surface sterilization treatment. Incorporating a random intercept term for potential diet item combined with a random slope term for surface sterilization treatment

allows the effect of surface sterilization treatment to vary by potential diet item, such that some potential diet items may increase in presence with surface sterilization (i.e. hidden by contaminants), while others may decrease in presence (i.e. potential diet item is a contaminant; Zuur et al., 2009). We also assessed ASV composition as a representation of potential prey composition using a canonical correspondence analysis (CCA) with surface sterilization as a predictor variable. We repeated the natural environment consumer potential diet item PERMANOVA with abundance data (Poisson distribution), conducted both presence-absence and abundance based PERMANOVA analyses on all potential diet items (including offered item) for feeding trial consumers, and repeated each analysis using the adonis() function from the vegan package (version 2.5.6) in R (dist = "jaccard" with binary = TRUE for presence/absence and dist = "bray" for abundance; Appendix 2D & E).

For all generalized linear models and mixed models, we performed model selection by comparing the full model (including the fixed effect of surface sterilization treatment) to a null model without this effect. All models were called in the glmmTMB package (version 1.0.0, Brooks et al., 2017) in R (version 3.6.1) We chose the best fitting model based on size corrected AIC values (MuMIn package version 1.43.15). For responses for which the best model included the surface sterilization treatment term, we examined the model summary to determine the standardized coefficients (β, or the degree of change in the response with every unit change in the predictor variables, with positive or negative values depending on the response direction) and p-value of the significance between marginal means of the levels of the surface sterilization fixed effect. We assessed model fit using diagnostics in the DHARMa package (version 0.2.7), including tests for heteroskedasticity, and for count models (Poisson or negative binomial), zero inflation and overdispersion (Bolker et al. 2009,

Zuur et al. 2009). We performed the CCA analysis using the vegan package in R, comparing a model with surface sterilization as a fixed effect to a null model using an ANOVA. All raw data, data cleaning, and data analyses are available online (Miller-ter Kuile 2020a, 2020b), and model outputs for primary and supplemental models can be found in Appendices C and D.

3.4 RESULTS

3.4.1 PCR success, sequence merging, filtering, and clustering with UNOISE3 and DADA2

We successfully extracted DNA from 100% of samples (n = 72). Amplification success across all samples was 78%, with 56 of 72 initially extracted samples successfully amplified and sequenced (natural environment: n = 18 surface sterilized, n = 19 unsterilized, feeding trials: n = 8 surface sterilized, n = 11 unsterilized; Table 2). The Illumina MiSeq run yielded 33,332,804 unpaired reads and had a Q30 quality score of 78.03%. After quality filtering and denoising with UNOISE3, 8,029,959 paired-end reads corresponded to 176 ASVs. Seventy-three percent (128 of 176) of ASVs matched to a taxonomic assignment. Twenty-three percent of the total ASVs corresponded to potential diet items (41 of 176) and eight percent (14 of 176) corresponded to consumer DNA (the remaining 73 ASVs corresponded to non-diet items, including fungi, bacteria, and human DNA). ASVs that matched to the consumer comprised the majority of each sample (98 ± 0.6% of rarefied abundance compared to 1.5 ± 0.6% for potential diet and 0.3 ± 0.1% for non-diet). Eighty-five percent of the potential diet ASVs received a species-level taxonomic assignment (35 of 41) from either the BLAST or BOLD taxonomic assignments, and every potential diet species received a family-level and order-level taxonomic assignment. In MEGAN, the family-level assignments family-level

assignments corresponded to 100% coverage results (LCA parameters: MinScore = 100, MaxExpected = 0.01, TopPercent = 10.0, MinSupportPercent = 0.05, LCA = naïve) suggesting evidence of no mitochondrial pseudogenes (NUMTs) at the family level (Saitoh et al. 2016). There were no conflicting taxonomic assignments at the family level or higher between the BOLD and BLAST assignments.

3.4.2 Detection of potential diet items

We detected potential diet in 89% (33 of 37) of natural environment consumers and offered diet (*O. japonica*) in 74% (14 of 19) of feeding trial consumers. For natural environment consumers, family-level taxonomic units corresponded to 20 families of potential diet items. The best model for potential diet detection in natural environment consumers was the null model that did not include surface sterilization treatment as a fixed effect (Figure 1, Appendix 2D). For feeding trial consumers, one ASV matched to the offered diet (Species: *O. japonica*, Genus: *Oxya*, Family: Acrididae), and the best model for diet detection included the fixed effect of surface sterilization treatment, though the model without the surface sterilization term was within two AICc values (ΔAICc = 1.59) and the surface sterilization term was not statistically significant in the full model at an $\alpha = 0.05$ (surface sterilization parameters: $\beta = -2.3$; p-value = 0.07). We detected *O. japonica* in 50% of consumers that had been surface sterilized compared to 91% of those consumers that were not surface sterilized.

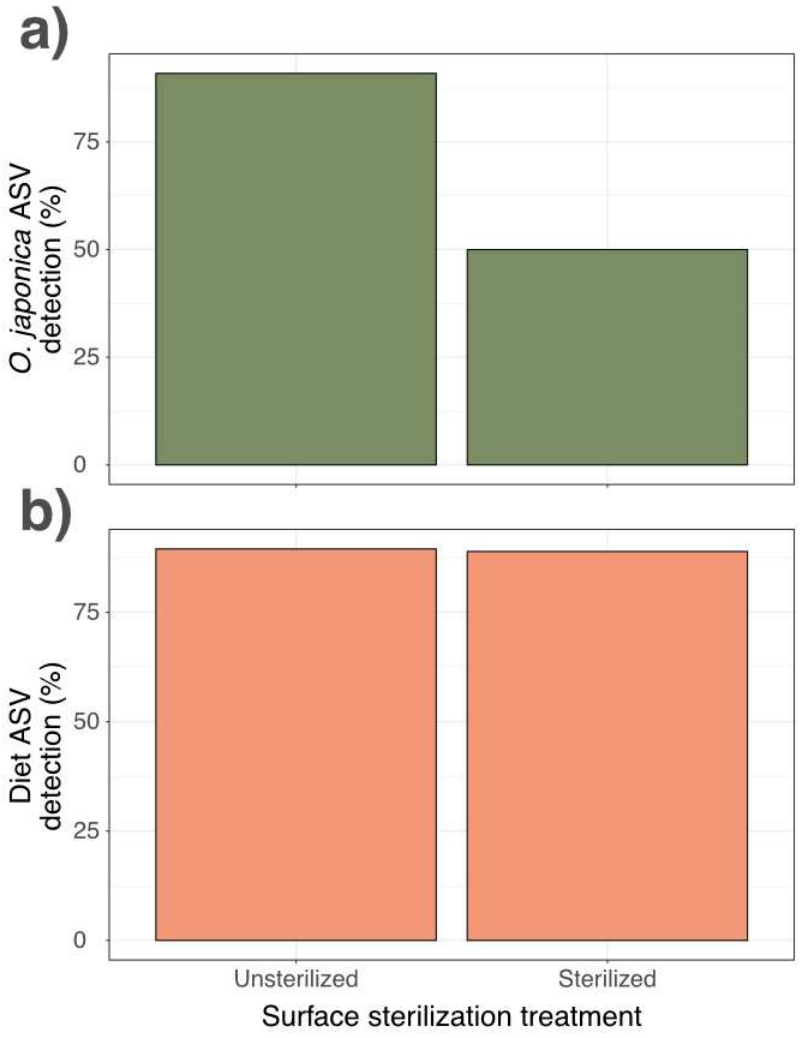

Figure 8: Detection of Diet DNA

a) Detection of all potential diet DNA in natural-environment consumers that were and were not surface sterilized. Detection of diet DNA did not change with sterilization treatment.b) Detection of offered diet (*Oxya japonica*) DNA in feeding trial consumers that were and were not surface sterilized. While the best-fitting model based on AICc values indicated an effect of surface sterilization treatment (a decrease from 91% without surface sterilization to 50% with surface sterilization), the effect of this term in the model was non-significant at a cutoff of $\alpha = 0.05$ (p-value = 0.07).

3.4.3 Proportion of potential diet DNA

For natural environment consumers, potential diet rarefied DNA sequence reads represented 2.0% (± 1.0%) of total per-sample DNA sequence abundance (Figure 9). In feeding trial consumers, offered diet rarefied DNA sequence reads (*O. japonica*) represented 0.8% (± 0.7% SE) of total per-sample DNA sequence abundance. For both the natural environment and feeding trial consumers, the null models that did not include surface sterilization treatment as a fixed effect were the best models of diet DNA read abundance.

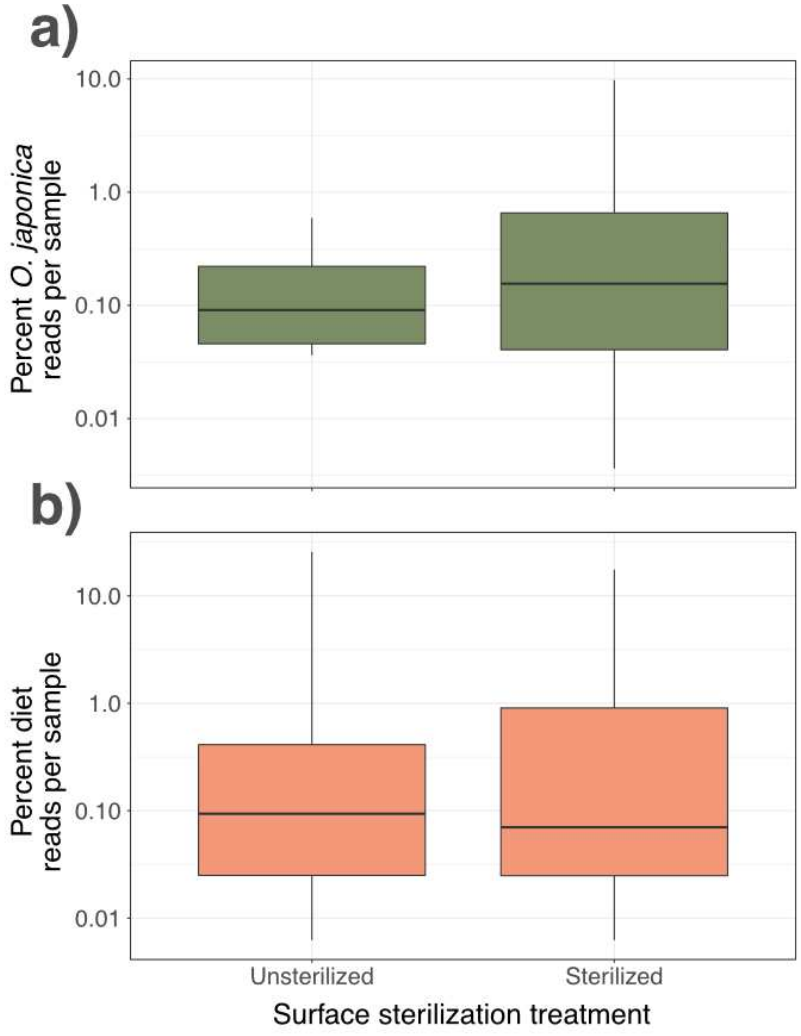

Figure 9: Proportion of Diet DNA

Neither the a) proportion of total potential diet DNA in natural environment consumers or b) proportion of offered diet item DNA in feeding trial consumers significantly changed with surface sterilization treatment.

3.4.4 Potential diet richness and composition in natural environment consumers

For family-level taxonomic units, potential diet richness per natural environment consumer was an average 2.08 (± 0.26) families per individual sample, with a maximum of 5 diet families in one consumer diet (Figure 10). Richness of potential diet ASVs for these consumers was similar, with an average of 2.32 (± 0.31) potential diet ASVs per sample with a maximum of 7 ASVs in one consumer (Figure 10). The best models for per sample potential diet richness for both the family-level taxonomic units and ASV-level were the null models which did not include surface sterilization treatment as a fixed effect. The best models for potential diet composition for family-level taxonomic units (mixed model PERMANOVA) and ASV-level taxonomic units (CCA) also did not include surface sterilization treatment as fixed effects (Figure 11, Supplementary Figure 2.E.1).

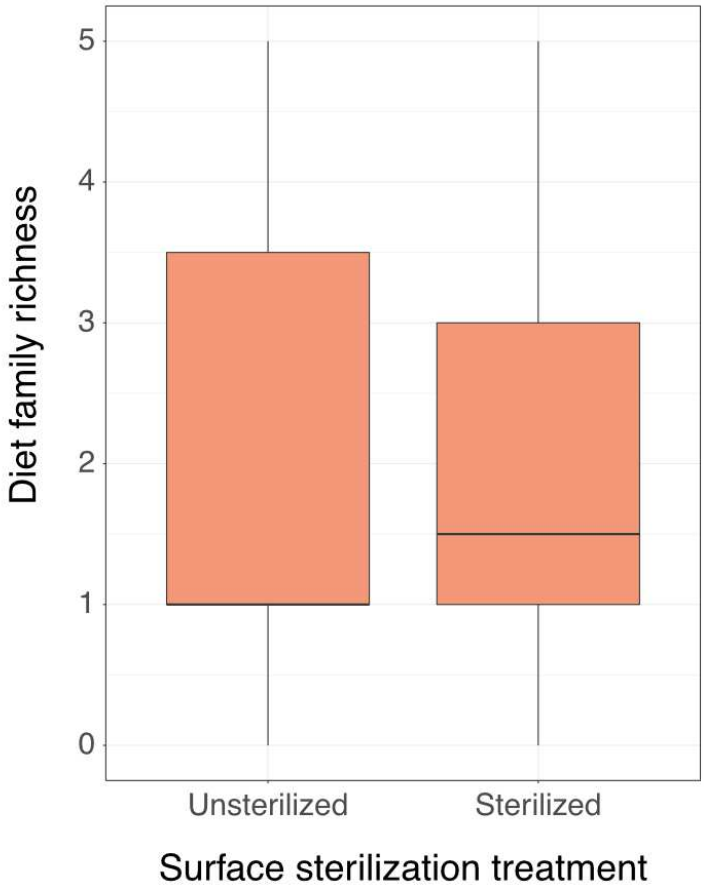

Figure 10: Diet DNA Richness

In natural environment consumers, surface sterilization did not alter per sample diet richness of either family-level or ASV-level taxonomic units.

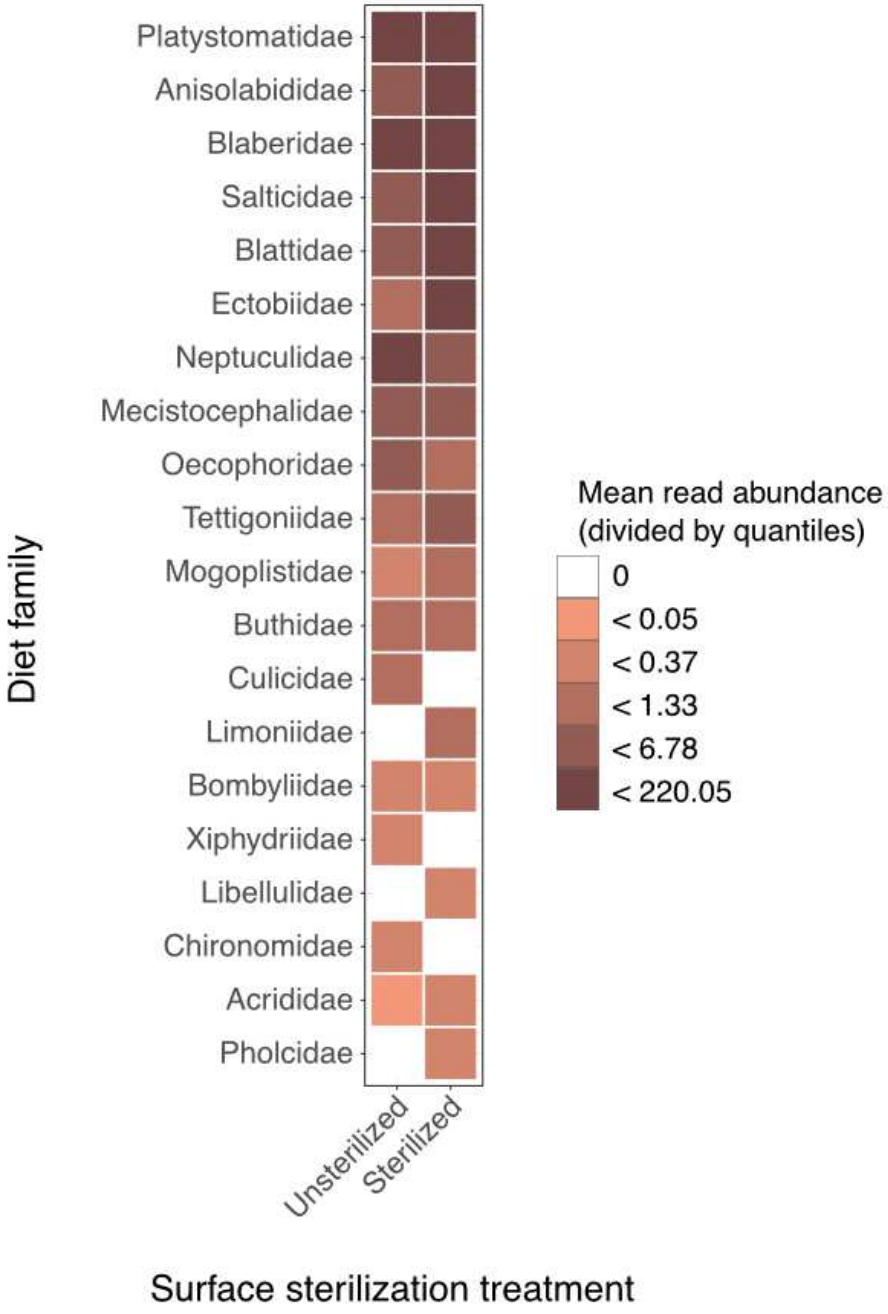

Figure 11: Prey DNA Composition

For natural environment consumers, surface sterilization did not alter the composition (either with a presence-absence of abundance model) of potential diet items either of family-level taxonomic units or ASV-level taxonomic units. In this figure of family-level taxonomic units by surface sterilization treatment, presence is indicated by a colored box and abundance is indicated by color depth (divided by quartiles due to wide variation in DNA sequence abundance). We observed similar results for ASV-level composition (Appendix 2E).

3.5 DISCUSSION

Surface sterilization does not change diet measures in diet DNA metabarcoding data for the predatory consumer *H. venatoria* in either natural settings or a feeding trial environment, suggesting that surface sterilization is not a necessary step for this consumer. Our results suggest that various measures of diet, including potential diet detection, rarefied abundance, richness, and composition, are not significantly altered by surface sterilizing consumers prior to DNA metabarcoding. For potential diet richness and composition, in particular, these results did not change when considering potential diet in combined family-level taxonomic units (making them comparable with food web studies in this field, e.g. (Brose et al. 2019) and when considering richness of molecular taxonomic units (ASVs). We detected diet across 84% of the total consumers in our study (n = 47 of 56), including 20 diet families. Diet DNA metabarcoding has high potential to contribute diet information for small consumers for which diet has been challenging to determine because of small consumer size or feeding habits. Furthermore, it appears that current protocols that do not include surface sterilization steps are sufficient to determine potential diet for these consumers.

The field of diet DNA metabarcoding has not universally adopted surface sterilization practices into common protocols, in particular for studies including DNA extraction of full organisms or body parts without dissection (Wirta et al. 2014, Jacobsen et al. 2018). We demonstrate that surface sterilization does not seem necessary in most or all of these types of studies to avoid contamination effects. The evident lack of the effects of surface contaminants in our study contrast with obvious surface contaminants that alter ecological interpretations in other fields using high-throughput sequencing to determine community

diversity, particularly fungal endophyte studies (Burgdorf et al. 2014). One reason for this difference may be that fungal spores are widespread on and in the surfaces of most environments and organisms (Després et al. 2012, Philippot et al. 2013, Colston and Jackson 2016) and likely to contaminate studies targeting specific subgroups of these communities. Indeed, even in our dataset, some sequences matched to fungal taxonomies. The fact that these non-target sequences did not alter our DNA metabarcoding data by hiding target diet DNA, even with the relative rarity of diet DNA compared to consumer DNA (0.006 – 26% of each sample, similar to other studies; Krehenwinkel et al., 2017), is likely due to the differences in biomass of these sources of DNA in our samples and the specificity of our DNA size-selection protocol and PCR primers (Elbrecht et al. 2017a, Krehenwinkel et al. 2017). Therefore, our results are promising both in validating the robustness of findings from past diet DNA studies that have not implemented surface sterilization treatments, but also highlight that diet DNA metabarcoding using broad, universal primer sets (e.g. those in this study) is an effective tool even when DNA sequence data contain potential environmental contaminants (Appendix 2E).

While we saw no widespread support of the necessity for surface sterilization in our study, because our model results from the feeding trial environment suggested a model including the effect of surface sterilization on diet detection performed slightly better than one without this effect (ΔAICc = 1.59), there is potential that more contained environments may be more prone to contamination than open terrestrial environments. We see this result as an ideal starting point for the next steps in validating diet DNA metabarcoding data in similar contexts. Specifically, because this study had a relatively low sample size (n = 8 and 11 in each treatment group) and because we did not confirm ingestion, repetition of a similar feeding trial including crossed treatments of surface sterilization with different forms of

potential diet item contact (e.g. prey ingested, no prey offered, prey contact on outside but no ingestion, similar to Greenstone et al., 2012) would provide additional evidence of the effects of surface sterilization or surface contamination in this more contained environmental context. Further exploration of these results might reveal that the decision to surface sterilize prior to diet DNA metabarcoding may matter more in some environments and experiments than others (e.g. where diet items are in high density or consumers have long handling times(Samu and Biro 1993, Scharf et al. 1998, Abrams and Ginzburg 2000, Jeschke et al. 2002). Furthermore, as earlier studies in molecular diet methods targeting particular consumer-diet pairs explored (e.g. Greenstone et al. 2012), the field of diet DNA metabarcoding is ripe for a comparison of surface sterilization techniques. This current study was not designed to look for the negative effects of bleach sterilization, for example; thus future work should explicitly explore the potential negative effects of surface sterilization treatments on DNA degradation versus removal due to physical or chemical treatments. Nor was the current study an examination of whether primers that target various gene region lengths (e.g. shorter gene-length arthropod primer sets in Zeale et al. 2011, Krehenwinkel et al. 2017, Thomsen and Sigsgaard 2019) nor methods used to block consumer DNA (Krehenwinkel et al. 2019) may reveal nuances not detected in this current study.

Diet DNA metabarcoding is providing the first glimpse at comprehensive diet for a suite of consumers important to the field of food web ecology and to the maintenance of biodiversity on the planet (Wilson 1987, Hallmann et al. 2017, Nielsen et al. 2018). Being able to determine consumptive interactions for many species and environments for the first time will continue to build a better picture of the complex structure of nature and how natural systems will change with anthropogenic disturbance (Dunne et al. 2002, Ives et al. 2005, Tylianakis et al. 2008, Rudolf, V. H W, Lafferty 2011, Brophy et al. 2017, Harvey et

al. 2017, Pilosof et al. 2017). Like any method for determining consumptive interactions in nature, DNA metabarcoding continues to be refined, especially as new tools and data are continually emerging (Kvist 2013, Jain et al. 2015, Gómez-Rodríguez et al. 2017, Zinger et al. 2019). This study builds on past efforts to refine the field of diet DNA metabarcoding by using surface sterilization to pinpoint potential sources of error in diet DNA data. Here we found that, on the whole, surface sterilization seems to be unnecessary in two contexts (terrestrial environments and feeding trial containers) when extracting DNA from body parts of invertebrate taxa. However, continued context-specific refinements of surface sterilization protocols, along with other steps in diet DNA metabarcoding studies, will continue to increase the validity and widespread utility of diet DNA metabarcoding across consumer groups and environments.

Data Accessibility Raw sequence data are available on GenBank (reviewer link: https://dataview.ncbi.nlm.nih.gov/object/PRJNA639981?reviewer=2k2u1qmrtehqbsmc05vdqivkor) and will be made publicly available following acceptance of this manuscript. Data and analyses are currently available in a GitHub repository (https://github.com/anamtk/DNA_Diet_Methods.git).

Acknowledgements Field work for this project was funded by the National Science Foundation (DEB #1457371), National Geographic Society, and a Faculty Research Grant from the UC Santa Barbara Academic Senate. We would like to thank field technicians Carina Motta and Michelle Lee for help collecting samples for this project and laboratory technicians Emily Lutz and Tessa Chou for helping prepare genetic samples. We would like to thank the U.S. Fish and Wildlife Service and Palmyra Atoll Research Consortium for supporting field work for this project. We would like to thank Dr. Ryoko Oono for use of her laboratory space and equipment. We acknowledge the use of the Biological Nanostructures

Laboratory within the California NanoSystems Institute, supported by the University of California (UC) Santa Barbara and the University of California Office of the President. We especially thank Dr. Jennifer Smith, manager of the Biological Nanostructures Laboratory for her assistance in preparing and troubleshooting our samples. We acknowledge the use of computational facilities at the Center for Scientific Computing (CSC), which was purchased with funds from the National Science Foundation (CNS-1725797) and is supported by the California NanoSystems Institute and the Materials Research Science and Engineering Center (MRSEC; NSF DMR 1720256) at UC Santa Barbara. We thank D. Orr, E. Forbes, H. Lowman, A. Bui, D. Preston, D. Trovillion, E. Crone, E. Sauer, L. Falke, B. DiFiore, C. Jerde, M. Lee, and R. Ramiro for help in aspects of this manuscript, including statistics, bioinformatics, framing, and editing.

Author Contributions

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AM-tK, AA, and HY conceived the idea for this study. AM-tK collected field samples and conducted mesocosm study. AM-tK and AA designed laboratory analyses for this study. AM-tK performed all lab processing and data analyses for the study. AA and HY provided feedback on data analysis methods. AM-tK led the writing of the manuscript. All authors contributed to editing of the manuscript.

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CHAPTER 4: PREDATOR-PREY INTERACTIONS OF TERRESTRIAL INVERTEBRATES ARE DETERMINED BY PREDATOR BODY SIZE AND SPECIES IDENTITY

4.1 ABSTRACT

Predator-prey interactions are shaped by a combination of predator traits, including body size and hunting strategy. However, applying a traits-based approach is challenging for most invertebrate taxa because predator-prey interactions are difficult or impossible to observe directly with traditional approaches. In this study, we combined diet DNA metabarcoding data of 173 individual invertebrate predators from nine species with community body size data to explore how predator traits and identity shape interactions. This dataset includes a total of 305 predator-prey interactions. We found that 1) prey size scales with predator size, with species-specific variation to a general size scaling relationship. We also found that 2) while predator hunting traits, including web and venom use, are thought to shape predator-prey interaction outcomes, predator species identity more strongly influences 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 and biomass-dominant animal taxonomic group.

4.2 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). 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). 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).

Although a few general rules sometimes predict patterns in empirical food webs, we have a dearth of observed interaction data from many predators, in particular 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). 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, Digel et al. 2014, Rudolf et al. 2014). 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. 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 and a majority of animal species diversity (Mora et al. 2011, Costello et al. 2013, Bar-On et al. 2018, Stork 2018).

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. We combined these data, which included 305 unique predator-prey interactions, with an extensive dataset of body sizes for both predator individuals and the prey groups identified in their 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 larger prey 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?

4.3 METHODS

4.3.1 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 12). 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, Appendix 3B).

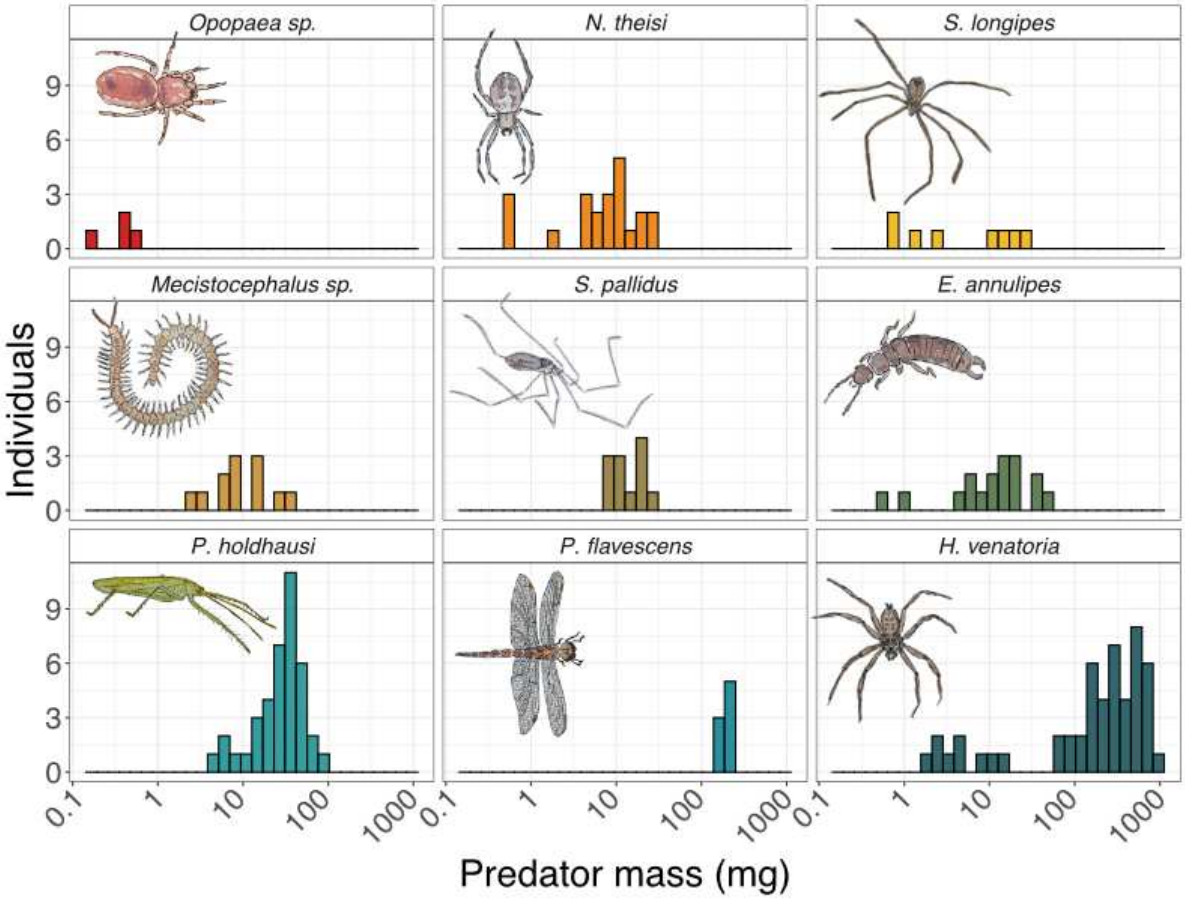

Figure 12: Predator Size Distributions of Predator Individuals

Predator size distributions of predator individuals across the nine predator species. The x-axis scale depicts absolute values but has been $\log_{10}$ transformed. Predator individuals span from $2x10^{-1}$ mg (*Opopaea sp*.) to $9.9x10^{2}$ mg (*H. venatoria*) in wet weight. The facets in this figure have been ordered by increasing predator species mean size.

4.3.2. 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, Appendix 3A & B). 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, Appendix 3B). 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.

4.3.3 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 ('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 et al. 2020). We verified ASV specificity based on positive control samples (Appendix 3B)

4.3.4 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 masses were taken as the average mass for individuals across species within each family (Appendix 3B).

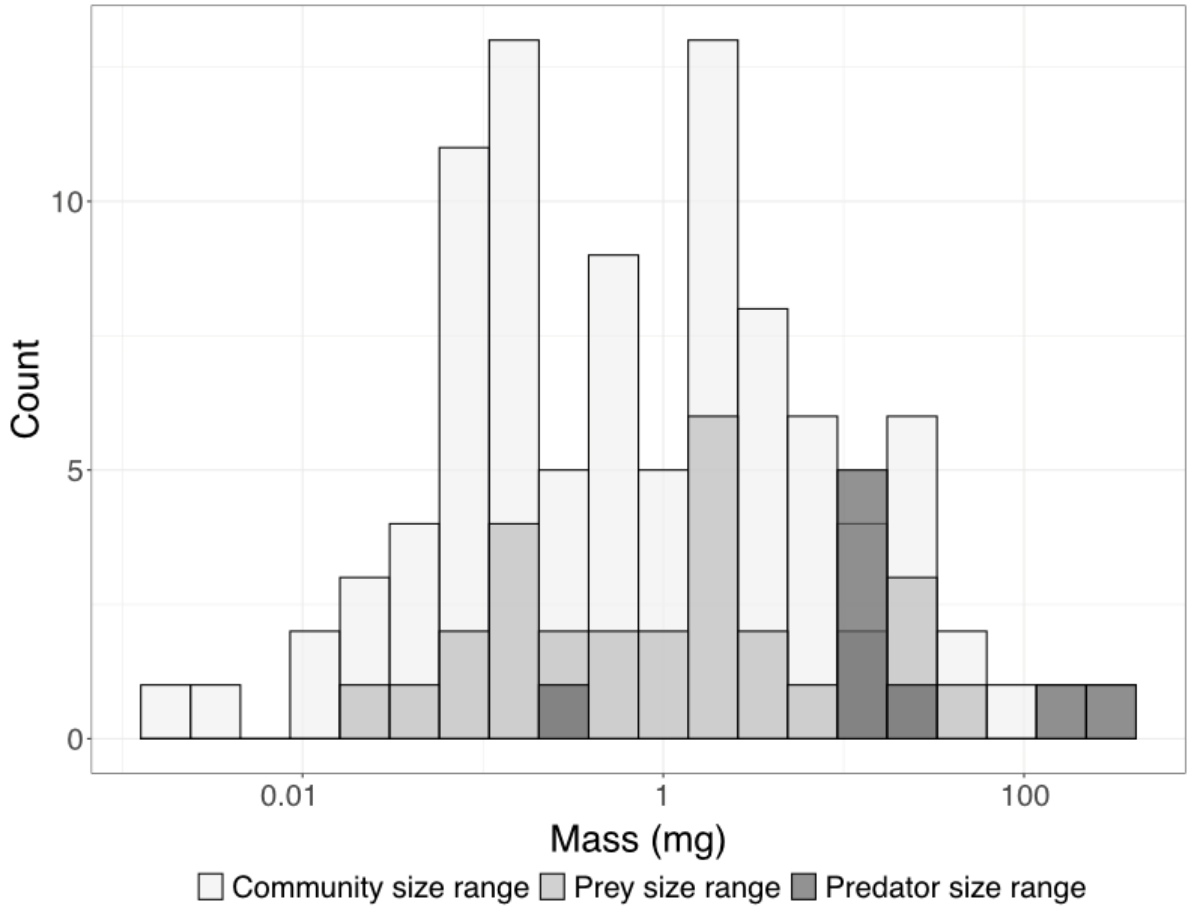

Figure 13: Palmyra Community Size Spectra

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 species detected in DNA data (medium grey) represent species across much of the range of the community size spectrum.

4.3.5 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 $\log_{10}$ prey mass (in mg) and predictor variables of $\log_{10}$ predator mass (in mg), species identity, and their interaction,

with a random effect of predator individual. Then, to explore whether predator hunting traits or predator phylogeny influences predator-prey size ratios, we divided predators 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, and 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 phylogeny 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 except the predator species model, predator individual and predator species identity were considered random effects. For the predator species model, only predator individual was considered as a random effect.

4.3.6 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 for best-fitting 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).

4.4. RESULTS

4.4.1 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 and our code and analyses are currently hosted in a GitHub repository. Neither of these sources is anonymous, and because of double-blind peer review, we do not provide links to them here. 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 14, Appendix 3B). Although the predators used in this study represent species at the larger end of the community size spectrum (Figure 12), prey item size distribution resembled the community-wide size distribution (Figure 13). 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 14).

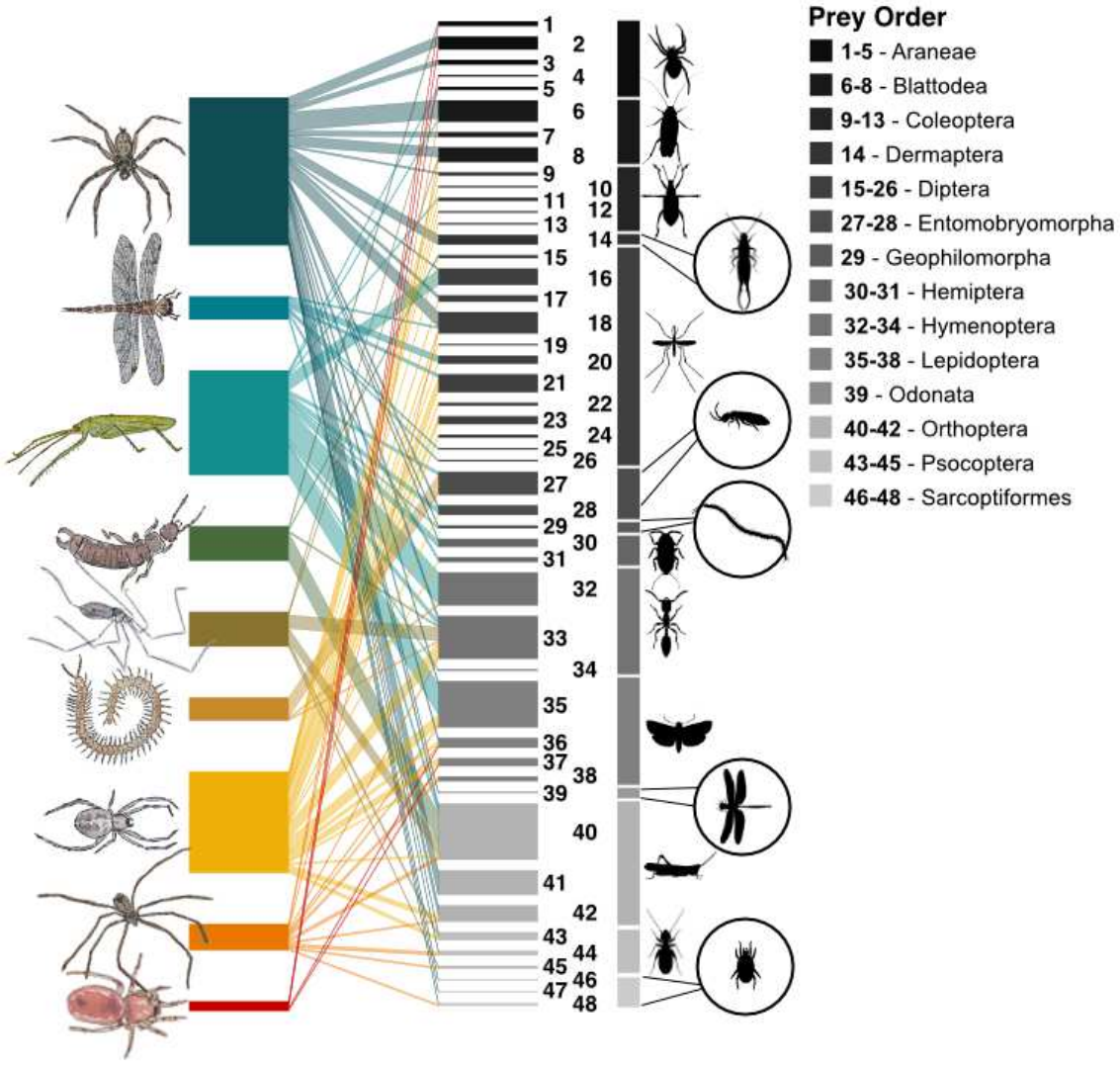

Figure 14: Predator-prey Interaction Bi-plot

Prey community (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 item in that predator species sample, and the width of the prey bar corresponds to the number of times that prey item occurs in any predator's diet. Prey items correspond to 48 families of 14 orders of arthropods, including arachnids, collembola, and insects.

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

The best performing model predicting prey size included the terms of predator mass and predator species identity, but not their interaction ($\log_{10}$(prey mass) = 0.32*$\log_{10}$(predator mass), with variation in by-species intercepts (Figure 15, Appendix 3B) ($\beta = 0.32$, p-value = 0.001). The predator trait or phylogeny model that most explained variation in predator:prey size ratio was the phylogeny model that included predator species as a predictor, with statistically significant post-hoc differences between Arachnida and Chilopoda predators and no others (Figure 16, Appendix 3B).

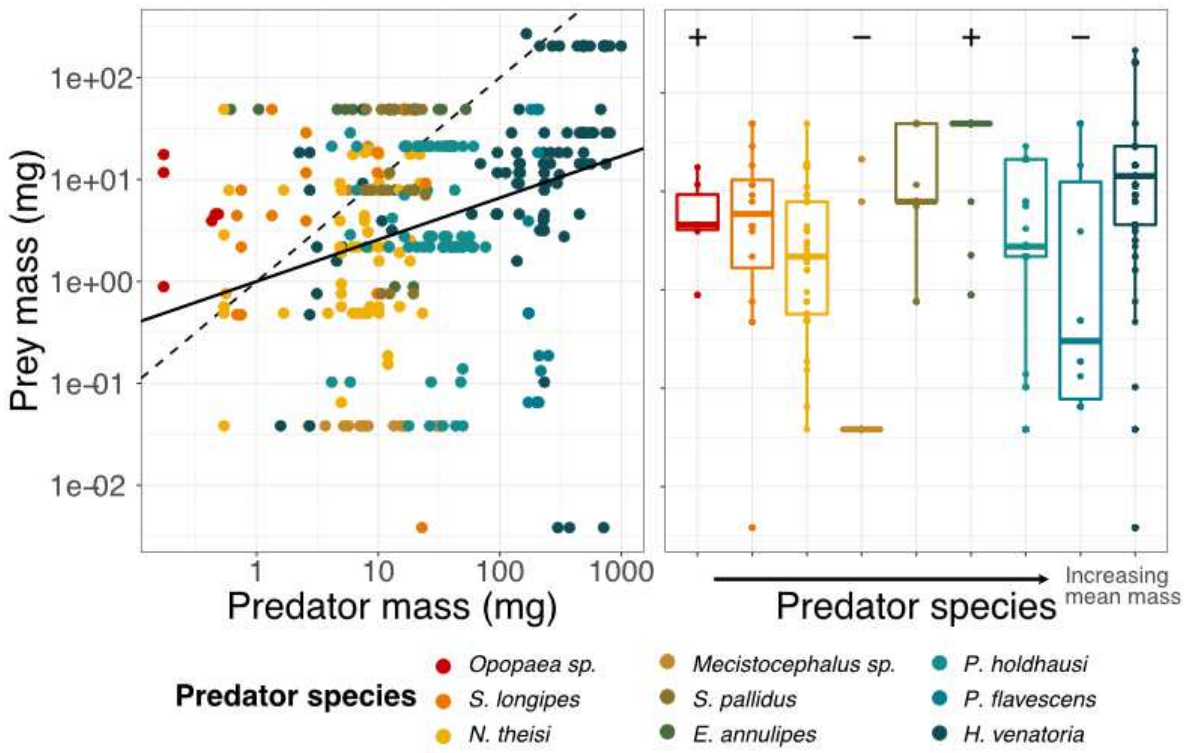

Figure 15: Predator-prey Size and Species Relationships

A $\log_{10}$-$\log_{10}$ transformed relationship shows that larger predators eat larger prey (panel (a), $\log_{10}$ prey size = 0.32*$\log_{10}$ predator size), 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 $\log_{10}$ transformed. In panel (b), "+" and "-" symbols indicate species that either have significantly higher ("+") or lower ("-") prey sizes relative to predator body size and the general predator-prey body size patterns.

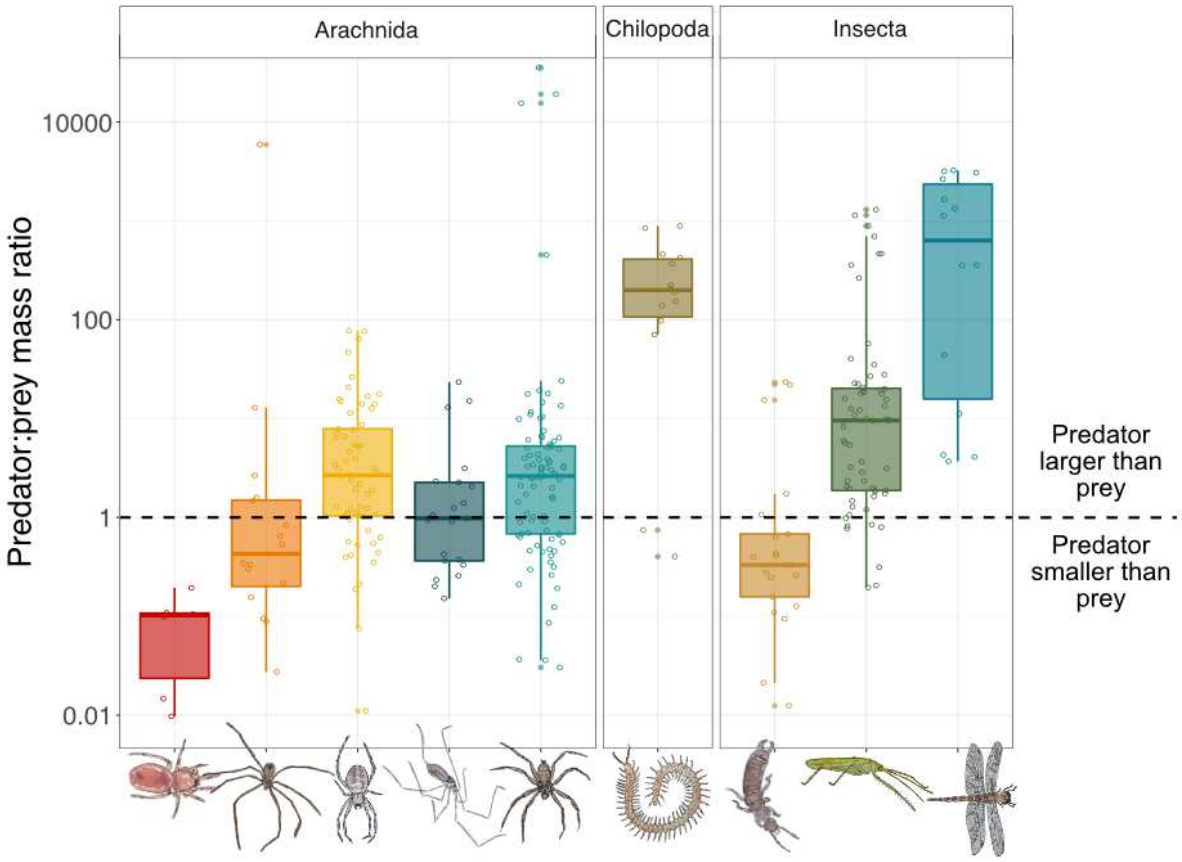

Figure 16: Predator:prey Size Ratios

Predator species identity was a stronger predictor of predator:prey size ratios than specific hunting traits (e.g. web or venom use) or broader predator taxonomies (e.g. Class). In this figure, the dashed line indicates interactions where predators are the same size as prey. Any interactions below that line indicates that predators are smaller than prey, while any interactions above that line are when predators are larger than prey. The y-axis is presented with absolute values but displayed on a $\log_{10}$-transformed scale to demonstrate the spread in the data. While arachnids (all spiders in the order Aranea, lightest grey boxes) tend to eat relatively larger prey items relative to their own body size than non-spider predators (medium and dark grey boxes), patterns across species vary, emphasizing a need for phylogenetic information in predator-prey interactions along with traits such as body size and hunting strategies. Twenty-four percent (72/305) of the interactions in our dataset corresponded to predators eating prey items larger than themselves (interactions below the dashed line), contrary to assumptions about size-based predation interactions.

4.5 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 community 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 larger prey, however, individuals of some predator species eat proportionally smaller or larger prey than would be expected by one general cross-species relationship. Then, we 2) demonstrate that predator phylogeny, specifically predator species, is a strong driver of predator:prey size ratios; no hunting strategies related to hunting activity or tools (e.g. webs and venom) relaxed size constraints consistently across species that possessed those traits. Rather, 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 traits, 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*.) have smaller prey items on average 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 predator and prey size, these constraints vary depending on predator identity, or, more broadly, potentially to predator traits related to prey handling and attack efficiency. Although these traits (body size and handling efficiency) may vary across individuals within a species over life stages, our dataset demonstrates that some traits that limit prey size selection may be conserved across life stages, lending credence to phylogenetic approaches to inferring feeding interactions (Gray et al. 2015). While we did not see evidence that these phylogenetically constrained traits were specifically related to tools such as webs or venom, determining what allows predators to relax size constraints is a fruitful area of future study.

Traits related to 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. It may be that particular invertebrate predators rely on scavenging as opposed to active predation, a phenomenon which may explain why the presumed predator earwig in our dataset (*E. annulipes*), which uses neither venom or webs, fed on relatively large prey (mean ± standard error of predator-prey mass ratio = 4.35 ± 1.99:1) (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). While it may be unclear which traits or species attributes mediate prey size selection in invertebrate predators, almost a quarter (24% or 72/305) of the

interactions in our dataset occurred with larger prey than predators, violating assumptions that predators generally eat prey smaller than themselves (Nakazawa et al. 2013). These patterns may highlight distinct rules governing predator-prey interactions of small-bodied consumers with implications for biomass cycling and food web dynamics (Schmitz 2008).

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. 2017b). Combining these field-based empirical observations with future experimental feeding trials could help to constrain prey sizes or determine preferences for live versus dead prey (Wilson and Wolkovich 2011). 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 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 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), studies like the present, conducted in multiple localities, are warranted.

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.

Acknowledgements: This project was funded by the National Science Foundation (DEB #1457371), National Geographic Society, and a Faculty Research Grant from the UC Santa Barbara Academic Senate. We would like to thank J. Smith, M. Espinoza, C. Johnston, C. Steel, E. Lutz, and T. Chou for help with field and lab work. We would like to thank the U.S. Fish and Wildlife Service and The Nature Conservancy on Palmyra Atoll for supporting field work for this project. R. Oono provided laboratory space and equipment and we

acknowledge the use of the Biological Nanostructures Laboratory within the California NanoSystems Institute, supported by the University of California (UC) Santa Barbara and the University of California Office of the President. We acknowledge the use of computational facilities at the Center for Scientific Computing (CSC), which was purchased with funds from the National Science Foundation (CNS-1725797) and is supported by the California NanoSystems Institute and the Materials Research Science and Engineering Center (MRSEC; NSF DMR 1720256) at UC Santa Barbara. We thank C. Owens, R. Harris-Gavin, S. Copeland, G. Titcomb, H. Lowman, C. Jerde, M. Lee, and R. Ramiro for help in aspects of editing this manuscript.

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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.

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APPENDICES

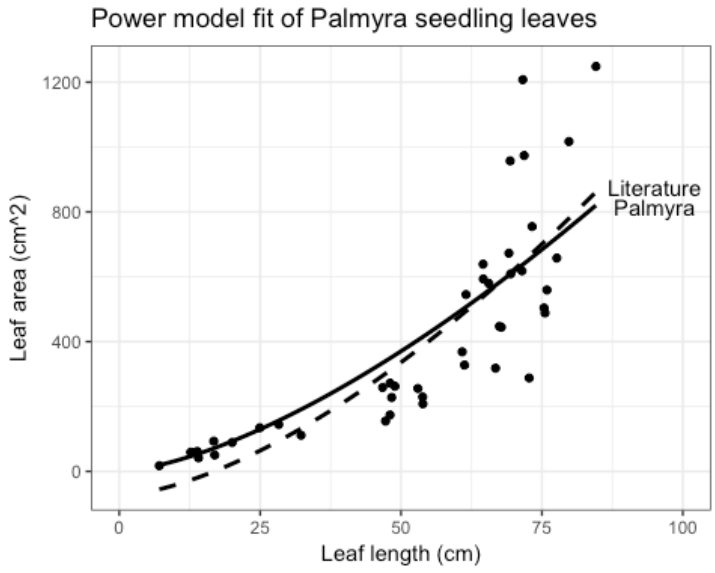
APPENDIX 1: Chapter 2

Appendix 1A: Species-specific biomass equations/values

Species	Size	Biomass (in grams)	Source
Pisonia grandis	Adult and Juvenile	$0.32^{(-1.239 + (1.980(\log(D))))} + (0.207(\log(D))^2) - 0.0281(\log(D))^3)$	(Chave et al. 2005, Climate Action Reserve 2017)
Pandanus tectorius	Adult	$0.33^{-1.239 + (1.980*\log(D)} + (0.207(\log(D))^2) - 0.0281(\log(D))^3)$	(Chave et al. 2005, Climate Action Reserve 2017)
Pandanus tectorius	Small Juvenile (basal diameter 0-4 cm)	108.2367	(Ashish et al. 2015)
Pandanus tectorius	Medium Juvenile (basal diameter 4-8 cm)	1013.327	(Ashish et al. 2015)
Pandanus tectorius	Large Juvenile (basal diameter 80-12 cm)	1275.377	(Ashish et al. 2015)
Cocos nucifera	Adult	$0.46^{(-1.239 + (1.980(\log(D))))} + (0.207(\log(CN\$DBH))^2) - 0.0281(\log(CN\$DBH))^3)$	(Chave et al. 2005, de Sousa et al. 2005, Young et al. 2011, 2013b)
Cocos nucifera	Juvenile	$54 \times Time^{2.39}$	(Chave et al. 2005)
Heliotropium foertherianum	Adult	$0.47^{(-1.239 + (1.980(\log(D))))} + (0.207(\log(D))^2) - 0.0281(\log(D))^3)$	(Chave et al. 2005, 2006)

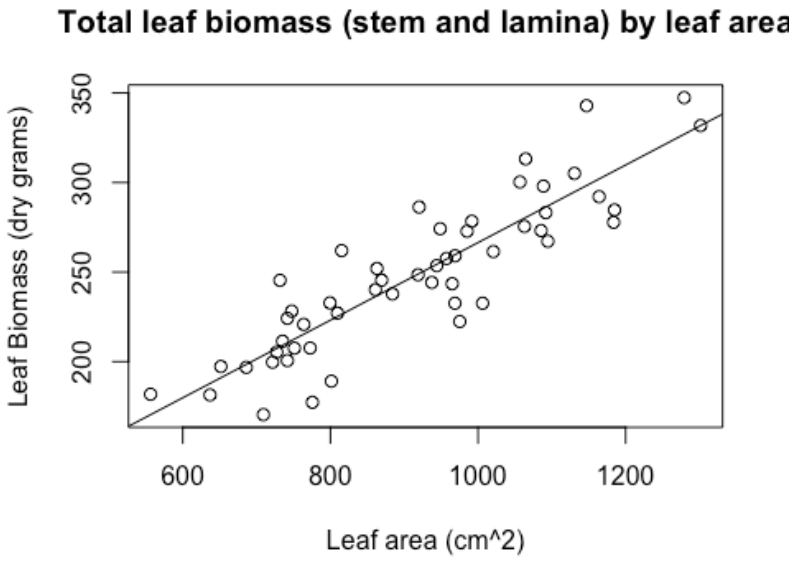
Appendix 1, Table 1: Tree Species Biomass Values

Species-specific biomass scaling relationships drawn from the literature and values from our plots and study site on Palmyra Atoll.



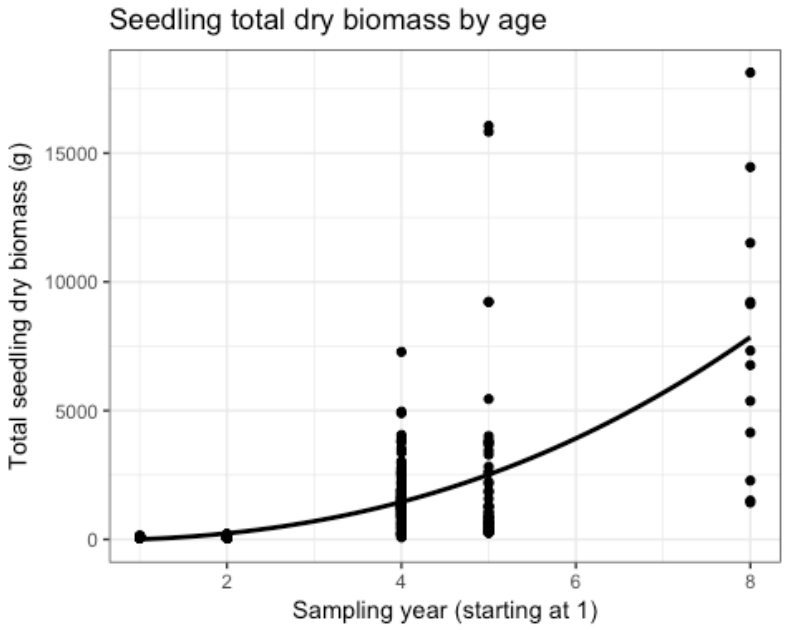
Appendix 1, Figure 1: *Cocos nucifera* Leaf Length to Area Scaling Relationship

Scaling relationship for juvenile *Cocos nucifera* between leaf length and leaf area, which we used to develop biomass relationships.



Appendix 1, Figure 2: *C. nucifera* Leaf Area to Biomass Scaling

Scaling relationship between leaf area and leaf biomass for juvenile *C. nucifera*.



Appendix 1, Figure 3: *C. nucifera* Seedling Age to Biomass Relationship

Relationship between seedling age and total dry biomass for *C. nucifera* seedlings.

Plot	Year	Eradication	Age 0 – 1	Age 2 – 3	Age 4 – 5
Eastern	2009	Pre	2	25	0
Kaula	2009	Pre	3	2	0
Paradise	2009	Pre	2	18	0
Eastern	2010	Pre	32	8	0
Holei 1	2010	Pre	3	12	0
Papala	2010	Pre	24	64	0
Paradise	2010	Pre	6	11	0
Sand	2010	Pre	1	0	0
Eastern	2016	Post	68	55	12
Holei 1	2016	Post	243	378	45
Kaula	2016	Post	310	450	71
Papala	2016	Post	321	434	42
Paradise	2016	Post	238	193	30
Sand	2016	Post	71	30	8

Appendix 1, Table 2: *C. nucifera* Seedlings by Plot by Year

The number of seedlings in each age-class in each plot in each year used to determine *C. nucifera* juvenile biomass in each plot and time period.

Appendix 1B. Statistical models for seed counts and juvenile biomass

Model	***df***	***AICc***
M1 <- glmer(Mature.Intact ~ Rats + (1|Site), data=quad.nuts, family=poisson)	3	515.04
M2<- glmer.nb(Mature.Intact ~ Rats + (1|Site), data=quad.nuts)	**4**	**473.66**
M3 <- glm.nb(Mature.Intact ~ Rats, data=quad.nuts)	3	5201.54
M4 <- glmer.nb(Mature.Intact ~ (1|Site), data=quad.nuts)	3	1716.27

Appendix 1, Table 3: Viable Seed Model Selection

Model (family = 'tweedie')	***df***	***AICc***
M1 <- lglmmTMB(sum_biomass ~ Eradication + (1|Year/Plot), data=B_total)	5	626.13
M2 <- glmmTMB(sum_biomass ~ Eradication + (1|Plot), data=B_total)	**4**	**623.15**
M3 <- glmmTMB(sum_biomass ~ Eradication + (1|Year), data=B_total)	4	623.94
M4 <- glmmTMB(sum_biomass ~ 1 + (1|Plot),data = B_total)	3	663.15

Appendix 1, Table 4: Juvenile Biomass Model Selection

Model	***df***	***AICc***
M1 <- glmer(total_juveniles ~ Eradication + (1|Year/Plot), data=juveniles_full, family=poisson)	**4**	**533.90**
M2 <- glmer(total_juveniles ~ Eradication + (1|Year), data=juveniles_full, family=poisson)	3	5200.94
M3 <- glmer(total_juveniles ~ Eradication + (1|Plot), data=juveniles_full, family=poisson)	3	1715.67
M4 <- glmer(total_juveniles ~ 1 + (1|Year/Plot), data=juveniles_full, family=poisson)	3	553.17

Appendix 1, Table 5: Juvenile Count Model Selection

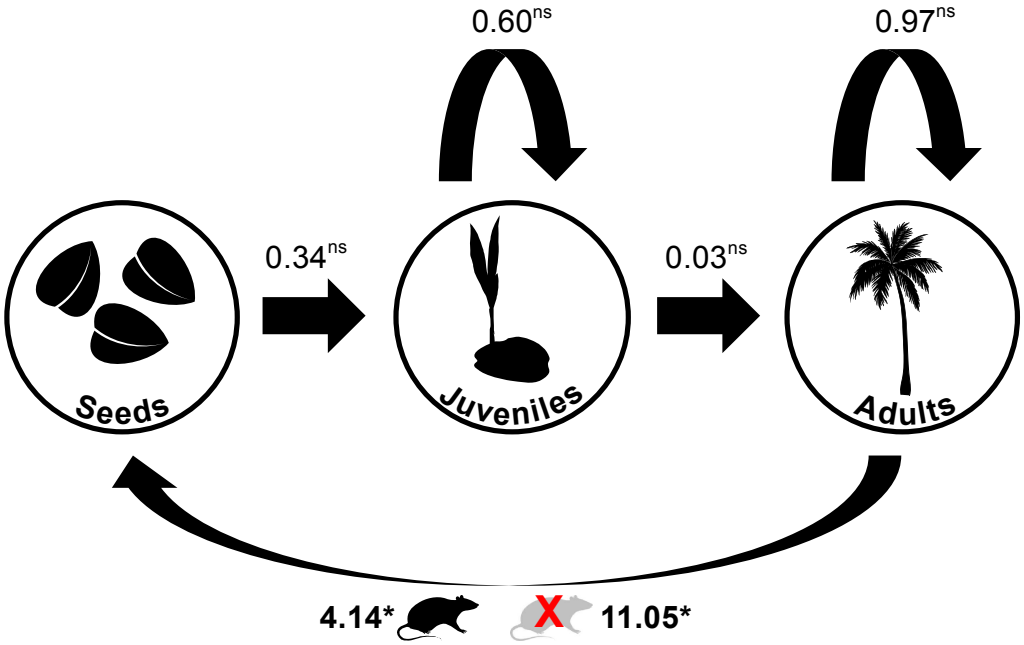
Appendix 1C. Stage-structured demographic model, vital rate statistics, Leftkovich matrices, and model results

1. Description of stage-structured matrix transition models

To determine change over time, we used the matrix model:

$N(t + 1) = An(t)$

where n(t) is a vector of stage abundances at year t, n(t + 1) is the population vector in the following year, and A is the transition matrix for the population (Caswell 2001). The dominant eigenvalue A represents λ, the asymptotic finite rate of increase at the stable stage distribution. Our model is best fit as a stage-structured model (using Leftkovich matrices) since age-specific vital rates are not as useful as stage-specific vital rates, which allow individuals to remain in stages over multiple time steps.



Appendix 1, Figure 4: Stage-structured Demographic Model for *Cocos nucifera*

The value on each arrow indicates a rate of survival or transition within or between stages. All rates were determined using yearly censuses from plots sampled over eight years. We tested whether all rates were influenced by rats, but only fecundity (number of seeds produced per adult tree per year), significantly changed following eradication. (Significance across years with and without rats denoted with *, statistically indistinguishable change denoted by "ns".)

2. Determining vital rates

Fecundity: We based fecundity data on relationships between known values (trees per plot, mature seeds per quadrat in plots, and post-eradication yearly seed counts). Post eradication, in addition to quadrat seed counts, we also counted the total number of seeds in plots, and thus could calculate how many seeds each tree produced each year. Pre-eradication, these data were not collected, but we determined that these values were likely to be significantly different pre-post eradication based on the per quadrat viable seed count (Methods/Results:

Seed Predation Section). To convert quadrat seed counts to total plot seed counts for the pre-eradication period, we found a conversion relationship in the post-eradication period between the average number of seeds per square meter quadrat and the total number of seeds counted in the plot (we determined this to be: 0.006 seeds per m^2 quadrat: 1 total seed in the plot). Assuming that this conversion stays constant regardless of seed count, we used this relationship to predict the total plot seed numbers for pre-eradication by multiplying the quadrat counts by this scaling factor. This gave us the total number of seeds on the ground unsprouted between sampling periods. Before counting these numbers as total yearly fecundity, we also had to account for seeds that fell after the previous year count, but which sprouted before they could be counted as "seeds" (*C. nucifera* drop fruit regularly throughout the year). To determine this number of seeds which had fallen since the last survey, but which had already sprouted, we took the total number of new juveniles counted in each plot each year and subtracting the total number of seeds (either estimated for pre- or counted for post-eradication periods) that were observed in the previous period to give us the total number of seeds that had fallen after the previous year's survey but which had sprouted before our return, thus being missed by the seed count. (so, Fecundity of Plot = total number of seeds for sampling year + (number of new juveniles in sampling year – total number of seeds for previous sampling year)). We divided this plot-level fecundity by the total number of trees in each plot for each sampling year to get per-tree fecundity. We then averaged the fecundity across plots within sampling periods (pre or post) to get the fecundity value for the two model matrices.

Seed survival: Seeds germinate within the year they fall, and so we took seed survival to be 0, since seeds either transition to juveniles or die within the first year.

Seed to Juvenile: To determine the transition probability from the seed to juvenile stage, we used plot data tracking total and new juveniles each year and the number of seeds per plot per year (see *Fecundity* above). We took the total number of juveniles observed as "new" in a sampling period and divided this by the total observed or estimated the number of seeds from the previous year. For the pre-eradication data set, the number of seeds was an average based on estimates used for calculating fecundity, so some values were greater than 1. We excluded these values, as survivorship cannot be greater than 1, and took the average of the remainder for the pre-eradication seed to juvenile transition value. For post-eradication, these values were based on counts, so none were greater than zero. We determined whether there was a significant change in seed to juvenile transition probability before and after eradication using a repeated measures ANOVA. We performed the ANOVA in R (version 3.5.0), with the lme() function in the *nlme* package. We determined model fit using the nagelkirke() function in the *rcompanion* package.

We found no significant change in seed to juvenile transition probability (n=16, p-value 0.87, pseudo R^2 Nagelkerke (Cragg and Uhler): 0.20). We averaged seed to juvenile transition across all years and all plots and used this value in both transition matrices.

Juvenile survival: To determine annual juvenile survivorship, we used data on individual juveniles tracked over time in the plots. We divided the total number of previously-observed juveniles in one year (i.e. not new juveniles) by the total number of juveniles observed in the previous year to determine how many juveniles survive from year to year in plots. We determined whether there was a significant change in juvenile survival probability before and after eradication using a repeated measures ANOVA. We performed the ANOVA in R (version 3.5.0), with the lme() function in the *nlme* package. We determined model fit using the nagelkirke() function in the *rcompanion* package.

We found no significant change in juvenile survival probability (n=22, p-value 0.18, pseudo R^2 Nagelkerke (Cragg and Uhler): -0.78). We averaged juvenile survival across all years and all plots and used this value in both transition matrices.

Juvenile to adult: In either period (pre- or post-eradication), we did not track any individuals long enough to observe the juvenile to adult transition. We have observed that 16 juveniles from another study (Young et al. 2013) become reproductive at age 12 (as of summer 2018 census). To estimate the juvenile to adult transition, we determined the likelihood of juveniles to reach age 5 to be an overestimated but general predictor of the likelihood to reach the adult stage. We measured a total of 232 individuals age 5 or greater in 2016 and used the minimum basal diameter of these individuals (4.5 cm) to estimate how many individuals were in this age class in each plot pre-eradication.

To determine the probability that individuals make it to year 5 in each plot, we first multiplied the average yearly number of juveniles in each plot by 5 to get the cumulative number of juveniles in the juvenile class over a 5-year period. We then divided the total number of age 5+ individuals we observed in each plot by this cumulative number of juveniles produced over a 5-year period to get a juvenile to 5-year survival probability, which we assumed to be reasonable, although likely overestimate of the juvenile to adult transition probability.

We multiplied the average yearly number of juveniles in each plot by 5 to get the total number of individuals to be in the "juvenile" class over a 5-year period. We then divided the total number of age 5+ individuals pre- and post-eradication separately for each model by the total number of juveniles for that period to get the probability of transition to age 5+, which we took to be an (over)estimate of juvenile to adult transition. We had enough data to predict transition probabilities for two years (pre: 2007, post: 2016), so we used Wilcoxon signed

rank tests to determine if the juvenile to 5-year (i.e. adult) transition probability changed pre-post eradication. Based on results from the wilcox.test (paired=T) function in the *stats* package in R, there was no significant difference in juvenile to adult transition pre-post eradication (n = 6 pairs, p-value = 0.84). We used the average across plots and years for the juvenile to adult transition probability in both transition matrices.

Adult survival: We determined adult annual survival rates by tracking the fates of individual trees across years in each plot, and then averaged this annual survivorship across all time periods (2007-2017). This value ranged from .91 to 1.0 and averaged .97 across all trees. We did not compare adult survival statistical significance on a yearly basis, as this number was not expected to be influenced by rats, and because it is a rate which occurs at decadal scales or longer, not on yearly time scales.

3. Transition matrices

We built one summary matrix per population type (pre and post eradication), by taking the values determined for all transition probabilities. Fecundity was the only rate to significantly change pre-post eradication and is indicated with a * in the matrices below.

	Seed	Juvenile	Adult
Seed	0	0	4.14*
Juvenile	0.34	0.60	0
Adult	0	0.03	0.97

Appendix 1, Table 6: Pre-eradication Transition Matrix

	Seed	Juvenile	Adult
Seed	0	0	11.05*
Juvenile	0.34	0.60	0
Adult	0	0.03	0.97

Appendix 1, Table 7: Post-eradication Transition Matrix

Plot	**Seed Population**	**Juvenile Population**	**Adult Population**
Holei 1	0	14.33	30
Papala	13.48	68.33	24
Paradise	0	15.25	4
Eastern	67.41	46.5	3
Sand	0	1.67	17
Kaula	0	1.67	23
Holei 2	0	16	0

Appendix 1, Table 8: Population Vector Input per Plot

$\lambda = 1.06$	**Seed**	**Juvenile**	**Adult**
Seed	0	0	0.06
Juvenile	0.06	0.09	0
Adult	0	0.06	***0.72***

Appendix 1, Table 9: Elasticities of Pre-Eradication Stage-Structure Model

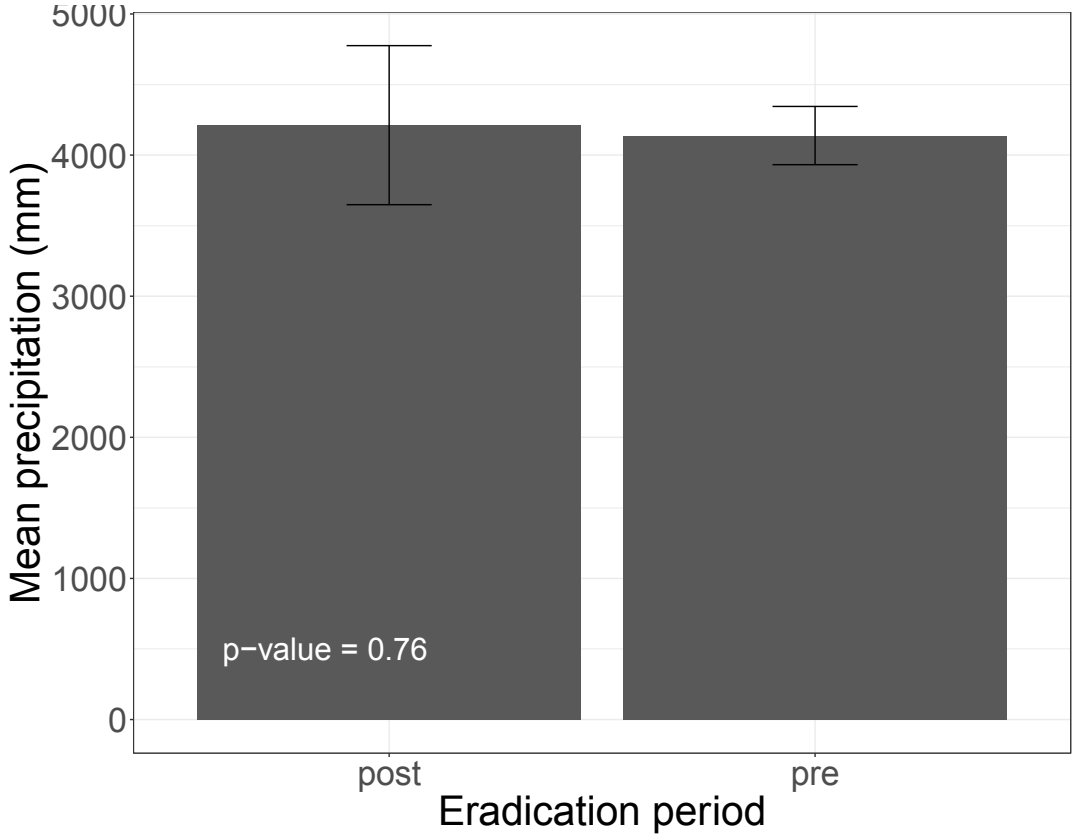
$\lambda = 1.15$	**Seed**	**Juvenile**	**Adult**
Seed	0	0	0.11
Juvenile	0.11	0.11	0
Adult	0	0.11	***0.57***

Appendix 1, Table 10: Elasticities of Post-eradication Stage-Structure Model

Appendix 1D: Repeated measures ANOVA seedling model, annual precipitation model, and number of immature and mature seeds in the canopy

Contrast	Eradication Status	Estimate	SE	Df	t-ratio	p-value
2007 – 2008	Pre-pre	-5.00	94.05	31	-0.05	1.00
2007 – 2009	Pre-pre	12.54	112.23	31	0.11	1.00
2007 – 2010	Pre-pre	-17.13	98.38	31	-0.17	1.00
2007 – 2014	**Pre-post**	**-463.03**	**98.38**	**31**	**-4.71**	**0.0009**
2007 – 2015	**Pre-post**	**-398.86**	**94.05**	**31**	**-4.24**	**0.0032**
2007 – 2016	**Pre-post**	**-489.71**	**94.05**	**31**	**-5.21**	**0.0002**
2008 – 2009	Pre-pre	17.54	112.23	31	0.16	1.00
2008 – 2010	Pre-pre	-12.13	98.38	31	-0.12	1.00
2008 – 2014	**Pre-post**	**-458.03**	**98.38**	**31**	**-4.66**	**0.001**
2008 – 2015	**Pre-post**	**-393.86**	**94.05**	**31**	**-4.19**	**0.004**
2008 – 2016	**Pre-post**	**-484.71**	**94.05**	**31**	**-5.15**	**0.0003**
2009 – 2010	Pre-pre	-29.67	116.54	31	-0.26	1.00
2009 – 2014	**Pre-post**	**-475.57**	**116.54**	**31**	**-4.08**	**0.005**
2009 – 2015	**Pre-post**	**-411.39**	**112.23**	**31**	**-3.67**	**0.014**
2009 – 2016	**Pre-post**	**-502.25**	**112.23**	**31**	**-4.48**	**0.002**
2010 – 2014	**Pre-post**	**-445.90**	**102.67**	**31**	**-4.34**	**0.002**
2010 – 2015	**Pre-post**	**-381.73**	**98.38**	**31**	**-3.88**	**0.008**
2010 – 2016	**Pre-post**	**-472.58**	**98.38**	**31**	**-4.80**	**0.0007**
2014 – 2015	Post-post	64.17	98.38	31	0.652	0.99
2014 – 2016	Post-post	-26.68	98.38	31	-0.27	1.00
2015 – 2016	Post-post	-90.86	94.05	31	-0.97	0.96

Appendix 1, Table 11: Post-hoc Pairwise Means for Seedling Count Model



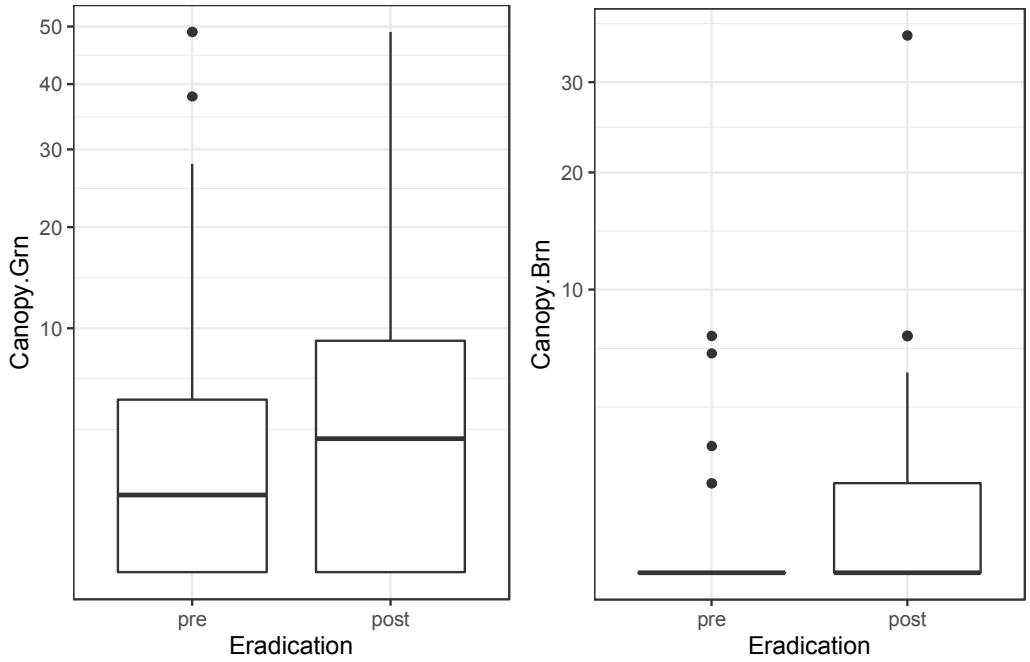
Appendix 1, Figure 5: Annual Rainfall for Palmyra

Annual rainfall data from Palmyra Atoll before and after rat eradication indicating that climatic conditions likely did not influence seed production during the sampling period.

Canopy Seeds

We did not consider canopy seed counts in our main seed counts because the canopy was high above our heads, making counts inaccurate and because it is challenging or impossible to ascertain whether seeds in the canopy have been predated or not. However, in three years (one pre-eradication and two post-eradication) we did count the seeds in the canopy and found marginal increases in green seeds in the canopy (Beta = 0.29, p-value = 0.051) and

significant increases in brown seeds in the canopy (Beta = 1.96, p-value < 0.001) following rat eradication.



Appendix 1, Figure 6: *C. nucifera* Canopy Seed Counts

Counts of per-tree canopy seed counts for *C. nucifera* before and after the eradication of rats. There were significantly more mature seeds (brown) following eradication, suggesting that rats were primarily feeding on seeds while they were in the canopy before they matured.

APPENDIX 2: Chapter 3

Appendix 2A: Comparisons between UNOISE3 and DADA2 with and without BBSplit

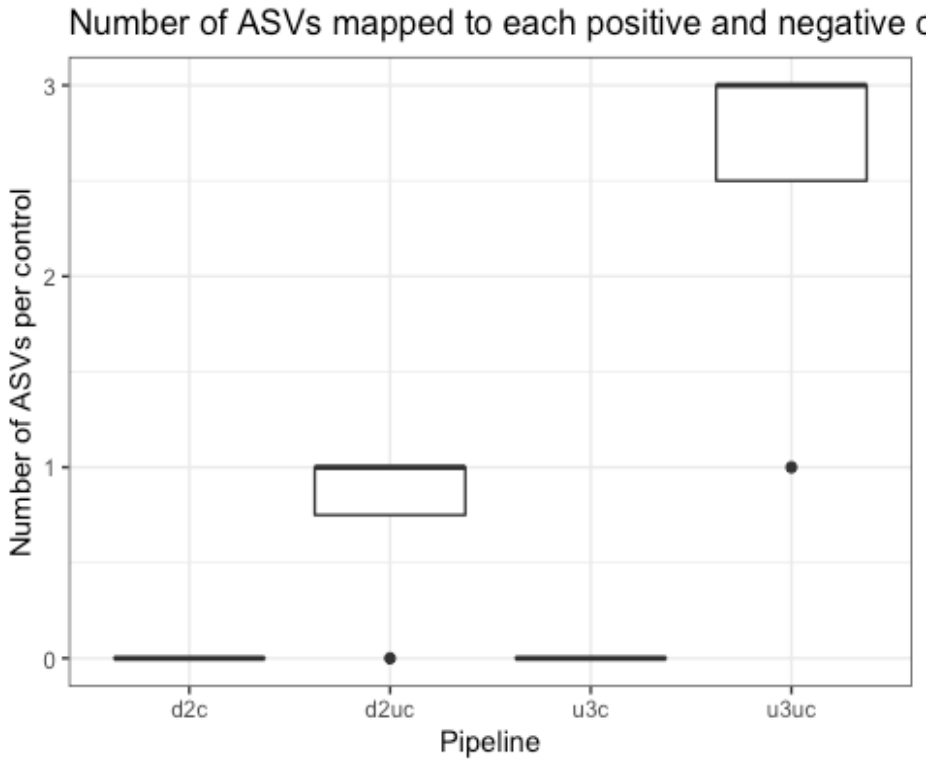
This project creates a workflow for analyzing the diets of invertebrate predators using high throughput sequencing of gut contents. This method provides a promising way to get highly-resolved diet data from consumers across ecosystems.

Bioinformatics challenge: To extract all possible prey items from predator guts when predator and prey are taxonomically similar, it is best practice to use a set of PCR primers that target all possible prey. However, a side effect of this is that these primers will also end up amplifying a large amount of predator DNA. As a result, these datasets are dominated by predator DNA, and so detecting relatively rare prey sequences in these datasets is key. As the molecular ecology field moves toward using amplicon sequence variants (ASVs) as biologically-real units of biodiversity in high throughput datasets, these types of datasets dominated by predator DNA are even more challenging since these ASV clustering pipelines use sequence abundance as a way to cluster sequences into similar, biologically-real groups of sequences. Therefore, any clustering pipeline used for DNA diet data dominated by the predator must

- detect prey sequences that are taxonomically similar to the DNA of predators and
- detect prey sequences that are relatively rare compared to the DNA of predators

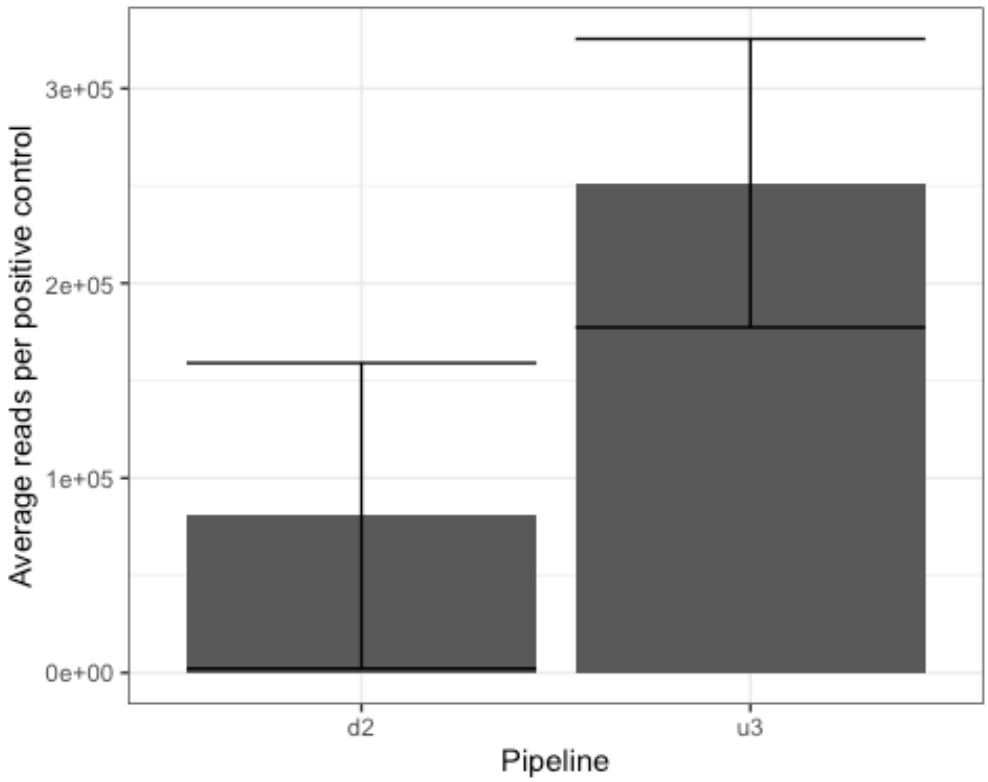
This supplement assesses how different denoising/clustering pipelines perform with these types of datasets and provide a template for other studies interested in interactions

between invertebrate predators and prey, but with the idea that the same sort of process could work well for other study systems (i.e. vertebrates that eat vertebrates) as well.



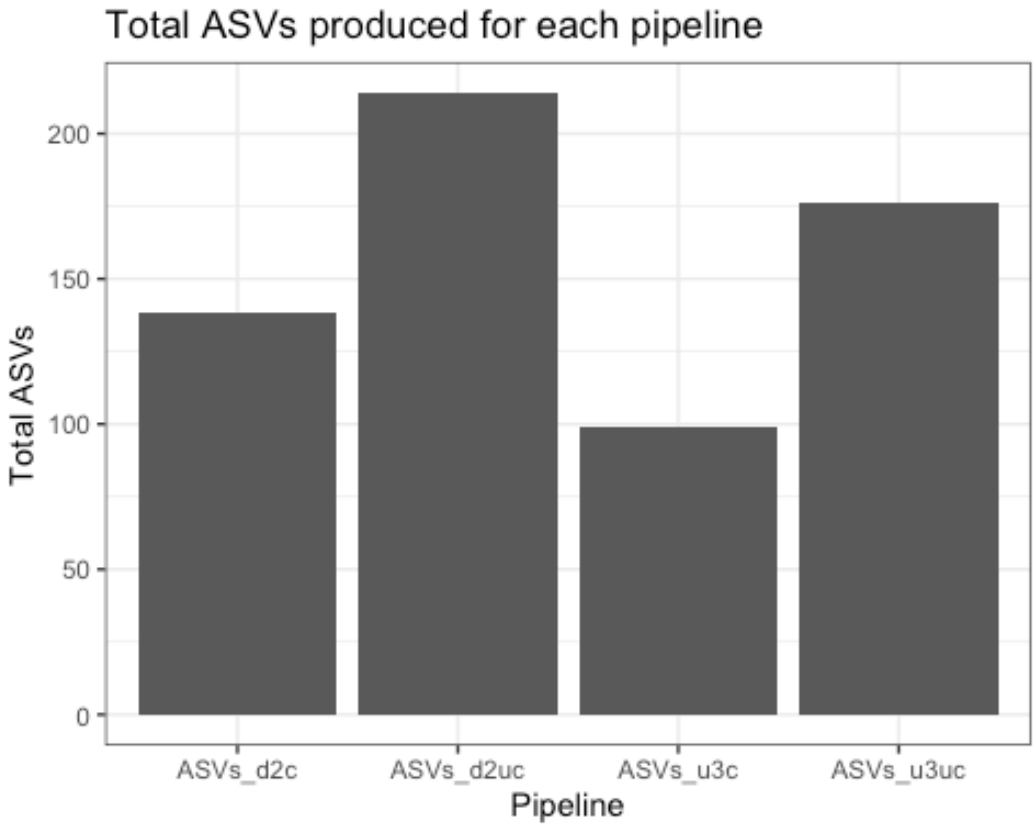
Appendix 2, Figure 1: ASVs in Controls per Pipeline

BBSplit cleaning removes all ASVs for the controls. Furthermore, DADA2 does a better job of assigning fewer ASVs to positive controls than UNOISE3. Furthermore, the negative control had zero reads assigned to any reads in DADA2, while UNOISE3 assigned one ASV a value of 1 read for the negative control.



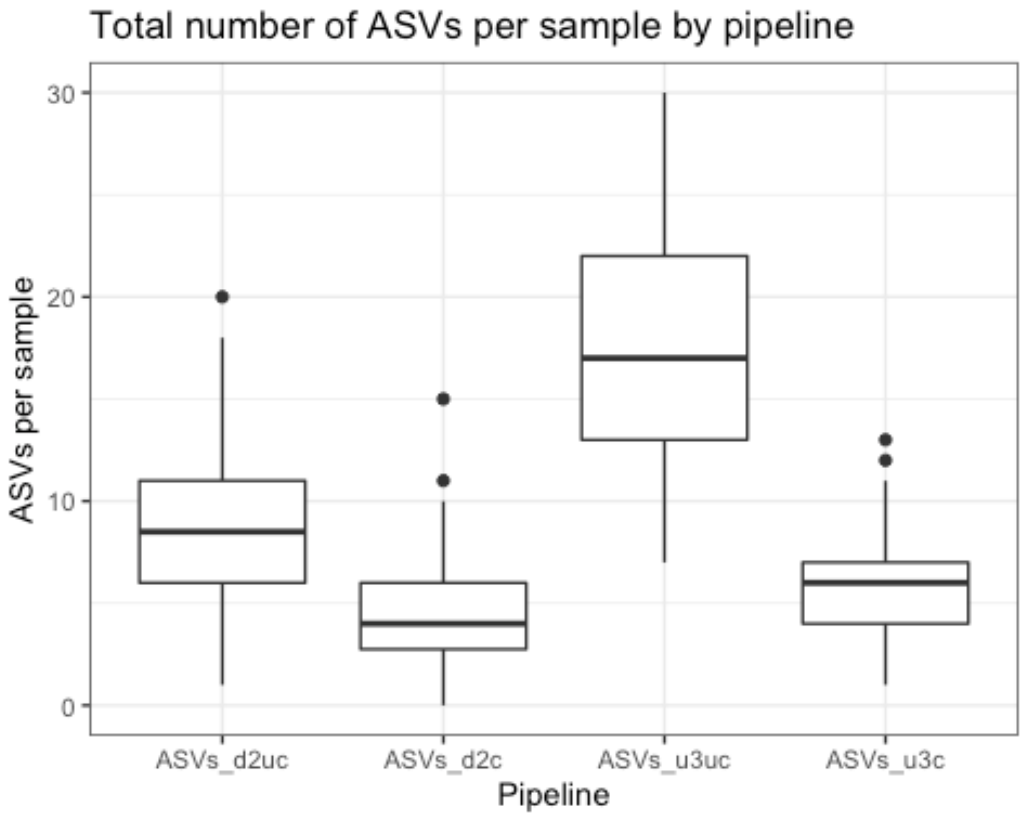
Appendix 2, Figure 2: Positive Controls by Pipeline

UNOISE3 maps more sequence reads to positive controls than DADA2.



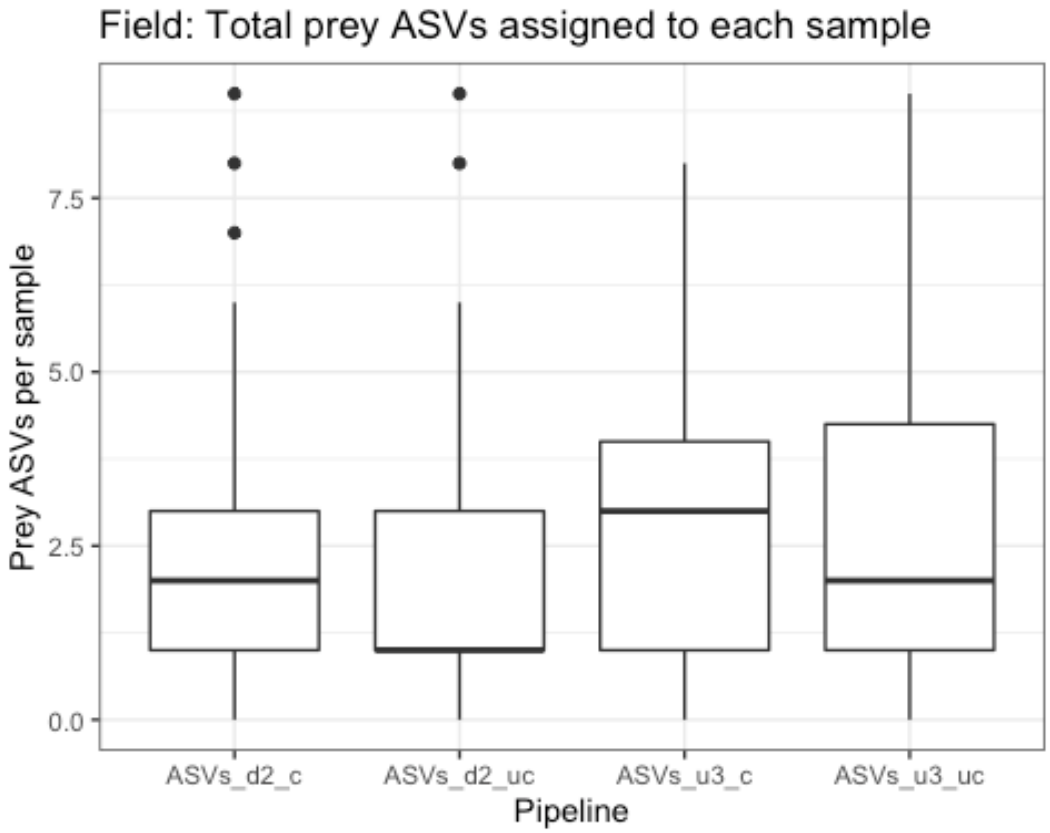
Appendix 2, Figure 3: ASV Count by Pipeline

The uncleaned datasets produce more ASVs, and DADA2 produces more total ASVs than UNOISE3.



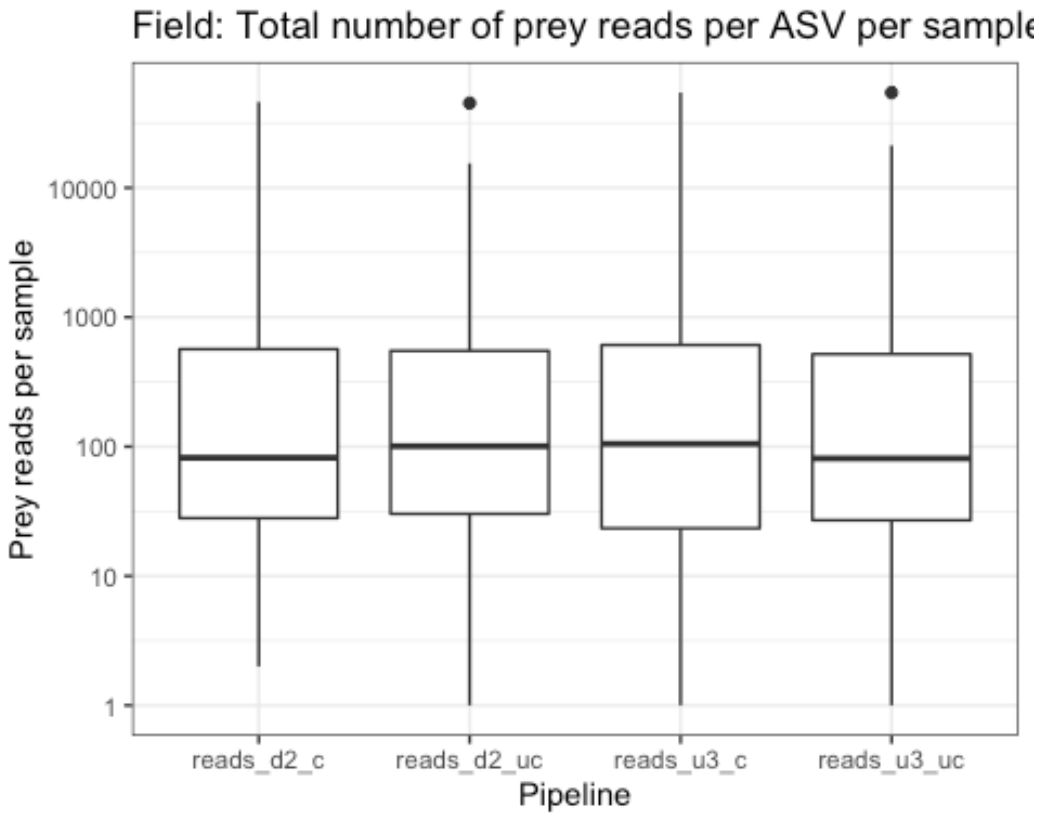
Appendix 2, Figure 4: ASVs per Sample by Pipeline

Both UNOISE3 unclean and clean (e.g. with and without BBSplit) produce more ASVs than their DADA2 counterparts (all pairwise differences significant at alpha = 0.05).



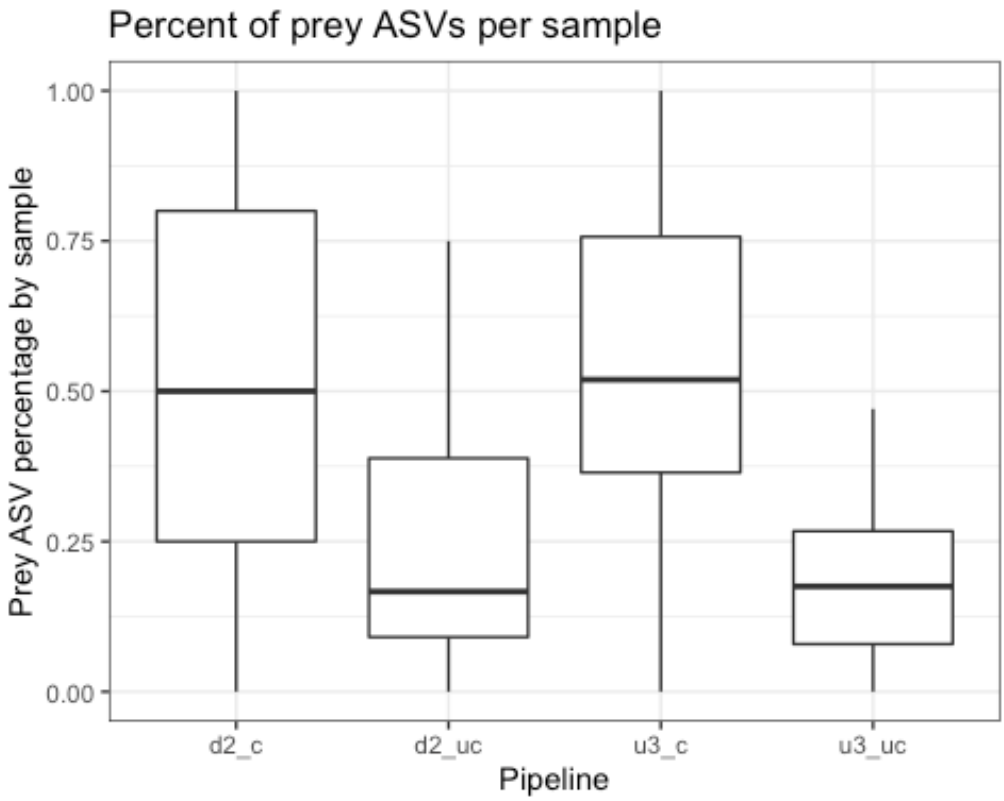
Appendix 2, Figure 5: Prey ASVs per Pipeline

Cleaning does not significantly increase the number of ASVs assigned to prey items for either DADA2 or UNOISE3. However, UNOISE3 assigns more prey ASVs to each sample, suggesting that UNOISE3 is better at detecting a greater prey richness than DADA2 (pair-wise difference = -0.44, SE = 0.07, p-value < 0.0001).



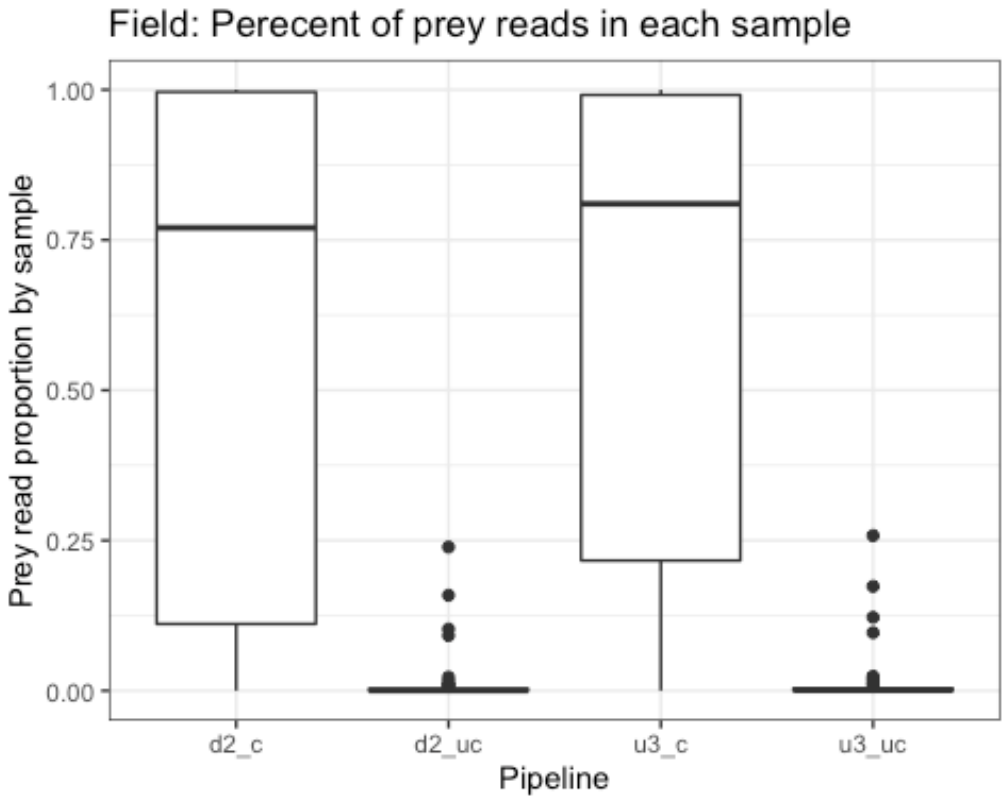
Appendix 2, Figure 6: Prey DNA Reads per Sample by Pipeline

BBSplit cleaning did not significantly increase prey read abundance. UNOISE3 produced more prey reads per ASV per sample than DADA2 (pairwise difference = -0.24, SE = 0.03, p-value < .0001).



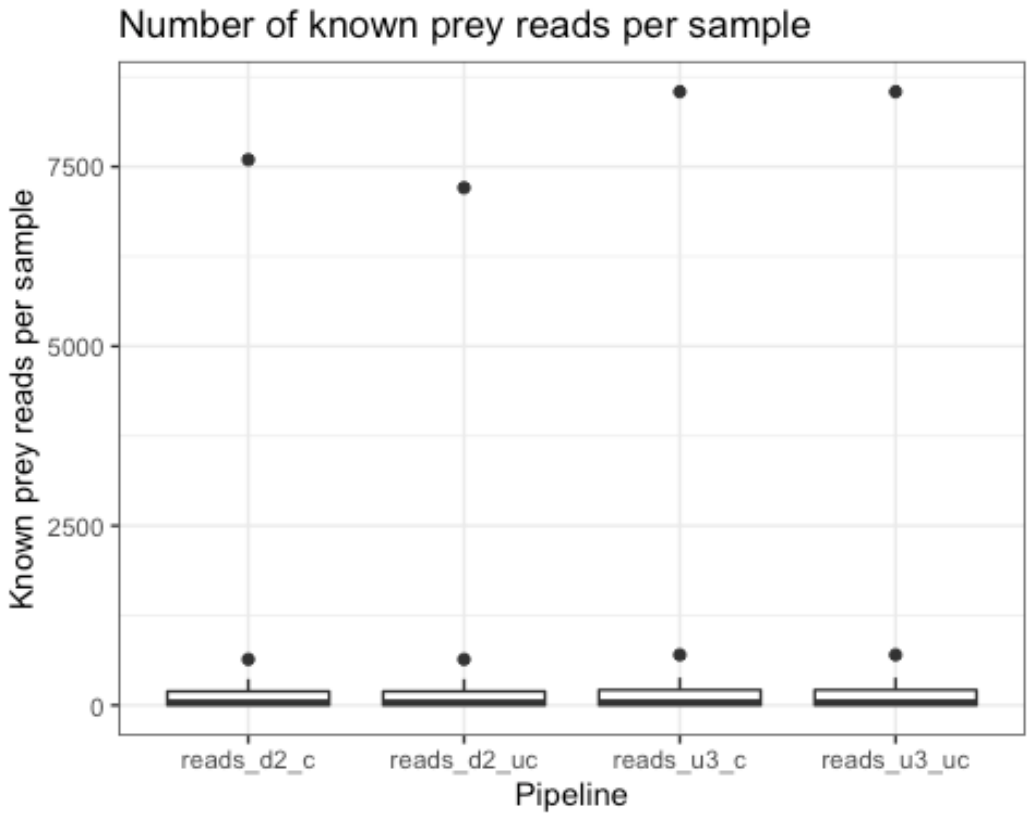
Appendix 2, Figure 7: Prey ASV Read Percent by Pipeline

BBSplit cleaning increases the total proportion of prey ASVs in each sample. DADA2 has a higher proportion of prey ASVs than UNOISE3 for both clean and uncleaned datasets (pairwise difference between DADA2 uncleaned and UNOISE3 uncleaned = 0.26, SE = 0.06, p-value = 0.0004).



Appendix 2, Figure 8: Prey Read Proportion by Pipeline

BBSplit cleaning increased the total proportion of reads that are assigned to prey ASVs. There is no difference in the proportion of prey reads in samples for either DADA2 or UNOISE3.



Appendix 2, Figure 9: Known Prey Reads per Sample by Pipeline

BBSplit cleaned datasets have significantly more reads of the known prey items per sample for DADA2, however cleaning did not increase detection of known prey for UNOISE3. UNOISE3 has better detection of known prey items (pairwise difference between DADA2 and UNOISE3 uncleaned = -0.15, SE = 0.01, p-value < 0.0001).

Measure/Pipeline	DADA2 UC	UNOISE3 UC	DADA2 C	UNOISE3 C
Positive control ASVs		X		
Negative conotrol ASVs	X		NS	NS
Total ASVs	X			
ASVs per sample		X		NS
Prey ASVs		X		
Prey reads		X		
Prey ASV %			X	
Prey read %			TIE	TIE
Known Diet		X		NS
NA	NA	NA	NA	NA
NA	NA	NA	NA	NA

Appendix 2, Table 1: Pipeline Summary Table

From this summary table, the uncleaned pipelines perform equally well or better than the cleaned pipelines. BBSplit cleaning does not add much to our bioinformatic performance nor ecological inference from this dataset. As a result, for analyses of sterilized vs. unsterilized individuals (both field and lab), the best options in terms of time are both unclean DADA2 and UNOISE3. The "winner" between these two pipelines is unclear - UNOISE3 outperformed ecologically (providing more diet data to work with); conversely, DADA2 outperformed bioinformatically (controls mapped more accurately).

Appendix 2B: BBSplit Methods

BBSplit maps sequences to reference databases of sequences provided by the user. The output of BBSplit is a file of sequences that have mapped to each reference database and a file for all sequences that do not map to any reference databases.

We decided to build our BBSplit reference databases based on sequences we already knew to be a part of our dataset (i.e. the output of a previous denoising pipeline). Because dada2 provides more ASVs than unoise3 (Nearing et al., 2018), we chose to use the dada2 ASV list to create our reference databases. We split the output of the MEGAN taxonomic alignment into sequences that mapped to the predator (*Heteropoda venatoria*) and those that mapped to prey. We know that our predator *H. venatoria* is the only member of its genus and family on Palmyra Atoll, and so sequences that matched these higher classifications in MEGAN (Genus: *Heteropoda* or Family: Sparassidae; Handler et al.) were also split into the predator reference file. We then split our dada2 ASV list based on whether sequences mapped to these predator or prey ASV lists from MEGAN. Our final result was a set of two reference files, one including all predator ASVs, and one containing all prey ASVs.

After we had built these reference databases of predator and prey ASVs, we ran the BBSplit program using both the predator and prey reference files to map our trimmed sequences. We kept defaults for most settings of the BBSplit command, including that reads that ambiguously mapped to both databases should go in the best fit database (the default "ambiguous" and "ambiguous2" parameters equal to "best"). We kept this default because we expected that most sequences would be predator sequences. However, because raw sequences or sequences with high error rates had not been denoised yet, would not fit perfectly to the ASV list for predators, but would fit more closely to this ASV list than to the

ASVs in the prey reference file. The output of BBSplit was one set of sequences that mapped to the prey reference ASVs, one that mapped to predator ASVs, and one that did not map to either of these (unmapped).

Although we were most interested in running dada2 and unoise3 again on the prey ASVs split with BBSplit, we also wanted to ensure that the splitting process did an accurate job of removing predator ASVS (i.e. predator sequence file should all map to predator after dada2 and unoise3), and that we weren't missing any prey in the prey sequence file (by looking at the ASV list of the unmapped sequence file after dada2 and unoise3). Therefore, we ran dada2 and unoise3 against each mapped set of sequences: the prey-mapped sequences, the predator-mapped sequences, and the unmapped sequences. As a result, we had a total of six more ASV lists and ASV tables matched to each sample (3 from Dada2 and 3 from unoise3 in USEARCH). We then used BLAST and a database of all nucleotide sequences on GenBank (downloaded on November 20, 2019) and the BOLD IDEngine (accessed February 5-16, 2020) to match taxonomies to each of these ASV files. Again, we selected the subtree in MEGAN with likely prey items (Kingdom:Animalia, Clade: Bilateria) and exported the same files. For BOLD, we again used the Species Level Barcode Records database.

Appendix 2C: Model outputs for GLMMs

Model	df	AICc	Estimate	p-value
With surface sterilization	2	22.54	-2.30	0.07
No surface sterilization	1	24.14	NA	NA

Appendix 2, Table 2: Feeding Trial Prey DNA Detection Model Selection

Model	df	AICc	Estimate	p-value
With surface sterilization	2	29.70	-0.06	0.95
No surface sterilization	1	27.46	NA	NA

Appendix 2, Table 3: Natural Environment Prey DNA Detection Model Selection

Model	df	AICc	Estimate	p-value
With surface sterilization	3	180.22	-0.49	0.34
No surface sterilization	2	177.93	NA	NA

Appendix 2, Table 4: Feeding Trial Prey DNA Abundance Model Selection

Model	df	AICc	Estimate	p-value
With surface sterilization	3	355.85	-0.03	0.91
No surface sterilization	2	353.86	NA	NA

Appendix 2, Table 5: Natural Environment Prey DNA Abundance Model Selection

Appendix 2D: Model outputs for supplementary data analyses

Model	df	AICc	Estimate	p-value
With surface sterilization	3	241.53	-0.41	0.25
No surface sterilization	2	240.06	NA	NA

Appendix 2, Table 6: Feeding Trial Non-diet DNA Abundance Model Selection

Model	df	AICc	Estimate	p-value
With surface sterilization	3	296.03	-0.36	0.31
No surface sterilization	2	294.69	NA	NA

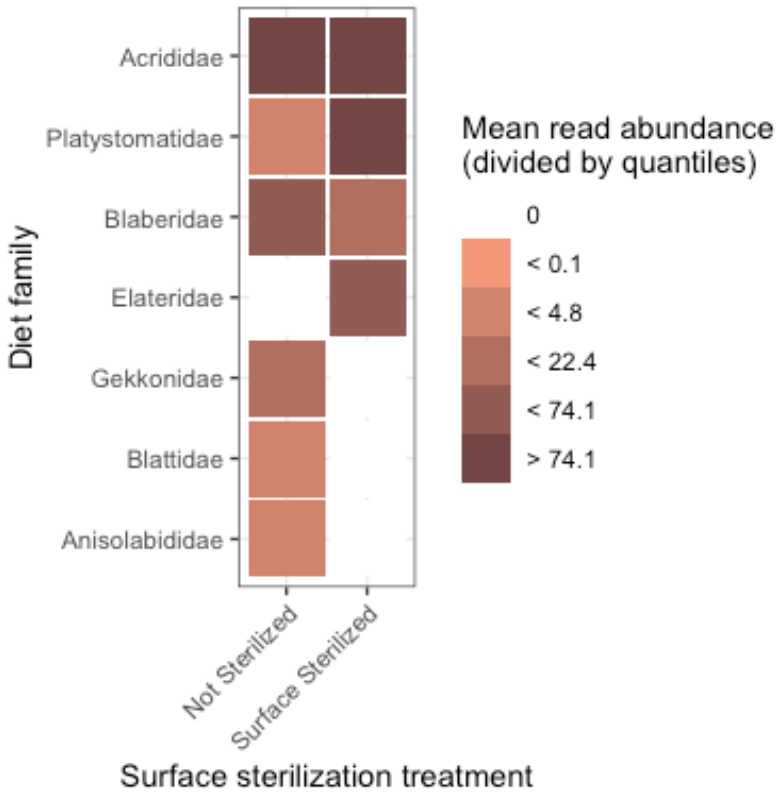
Appendix 2, Table 7: Natural Environment Non-diet DNA Abundance Model Selection

Model	df	AICc	Estimate	p-value
With surface sterilization	6	1107.98	-0.01	0.96
No surface sterilization	3	1101.96	NA	NA

Appendix 2, Table 8: Natural Environment Abundance-based Prey DNA Composition Model Selection

Model	df	AICc	Estimate	p-value
With surface sterilization	6	1107.98	-0.01	0.96
No surface sterilization	3	1101.96	NA	NA

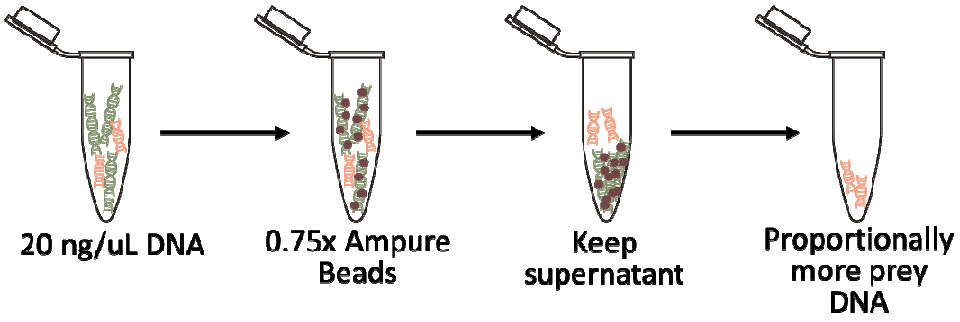
Appendix 2, Table 9: Feeding Trial Prey DNA Abundance-based Composition Model Selection



Appendix 2, Figure 10: Diet Composition of Feeding Trial Spiders

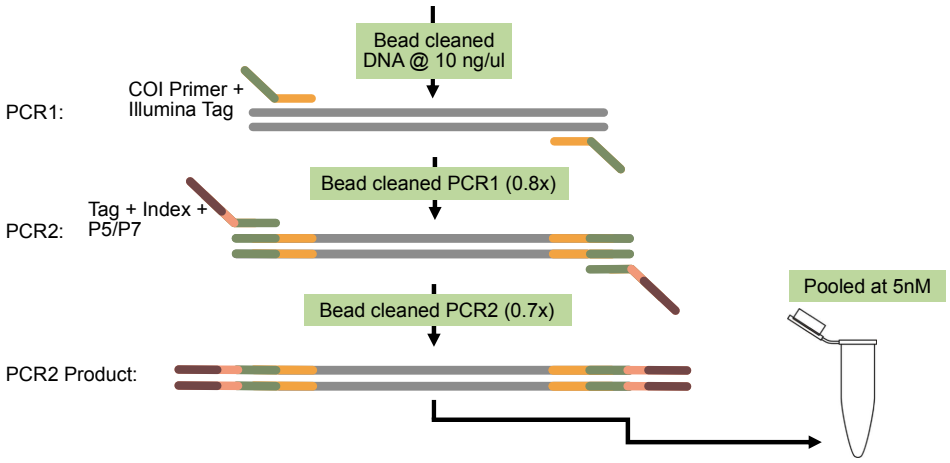
Abundance-based composition of diet families in the feeding trial environment – both presence and abundance-based composition were not influenced by surface sterilization treatment.

Appendix 2E: Supplementary Figures



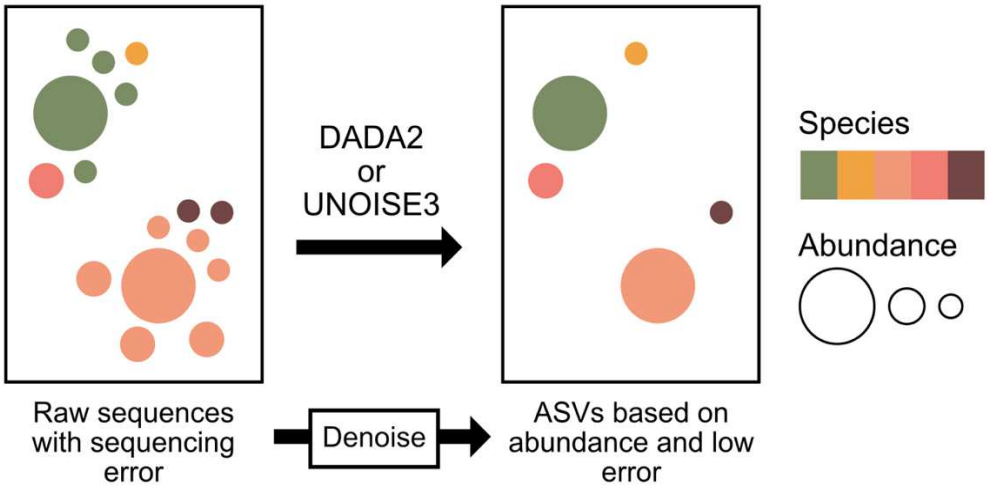
Appendix 2, Figure 11: DNA Cleaning Protocol

Ampure XP bead cleaning of DNA to remove consumer DNA, motivated by results from Krehenwinkel et al. (2016).



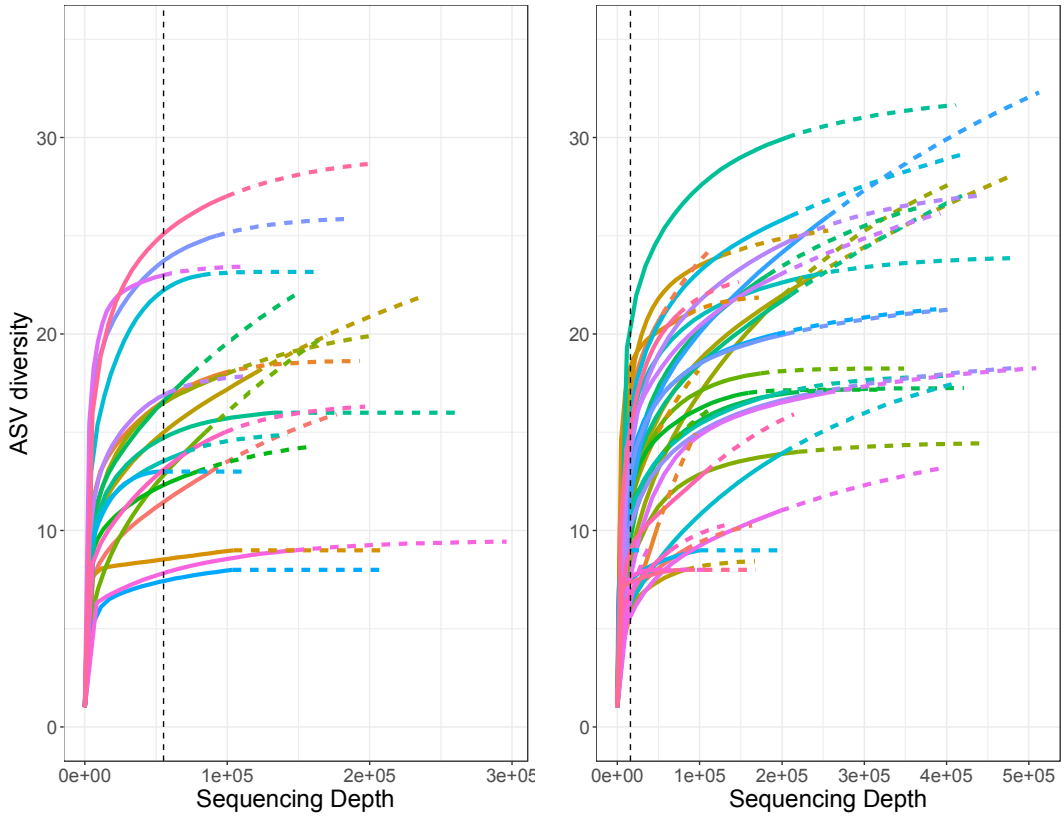
Appendix 2, Figure 12: DNA Protocol Diagram

Library prep, starting with attaching the CO1 primer pair with Illumina tag to diluted, bead-cleaned DNA. Then, this PCR product is bead cleaned at a 0.8x ratio and run through a subsequent PCR step to attach Illumina tag, index, and P5/P7 identifiers. This PCR product is then cleaned again at a 0.7x bead ratio, diluted to 5nM, and pooled for sequencing on an Illumina MiSeq.



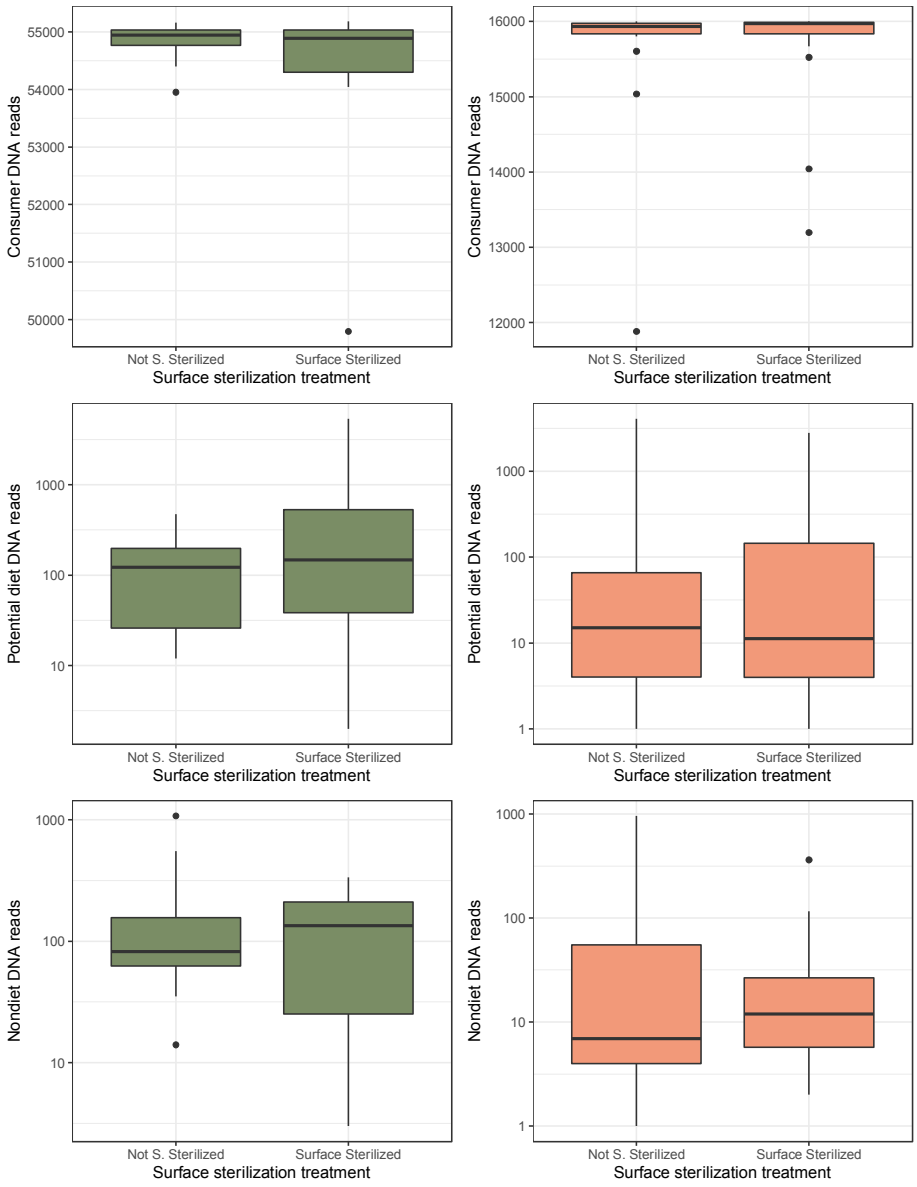
Appendix 2, Figure 13: Denoising Diagram

Denoising algorithms like UNOISE3 and DADA2 take into account DNA sequence abundance and error rates to assign groups of similar sequences to one amplicon sequence variant (ASV). In this process, reads with sequencing and PCR point error are identified and removed.



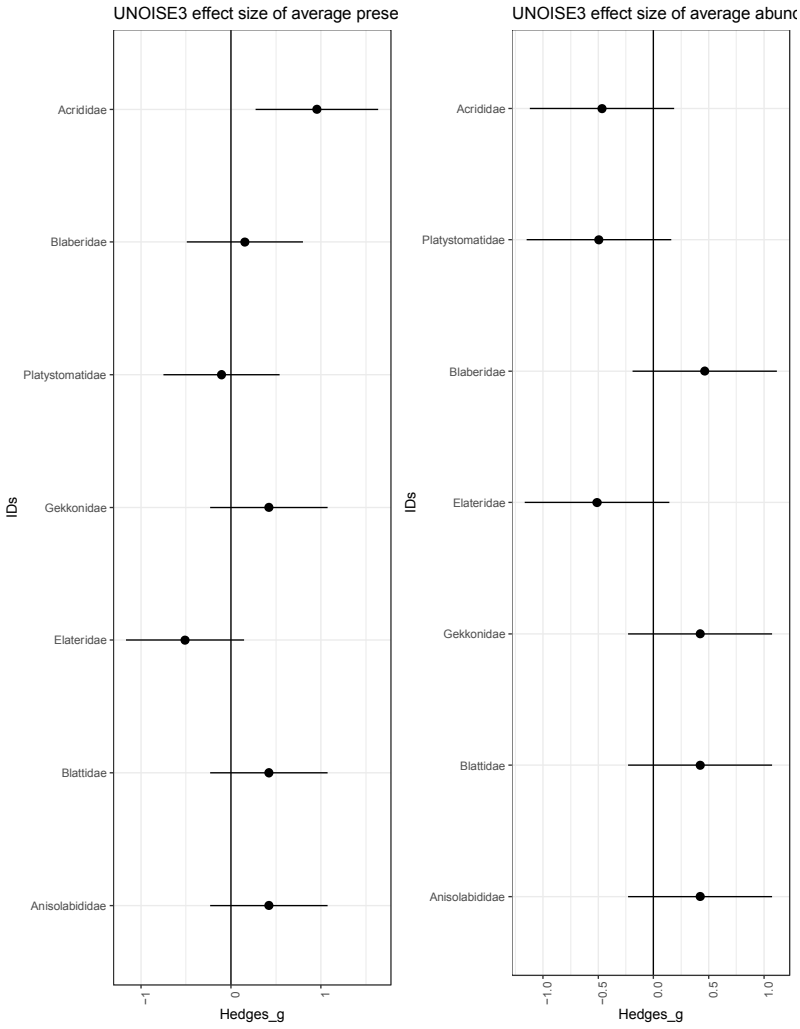
Appendix 2, Figure 14: Sample Sequencing Depth

Sequencing depth of a) mesocosm and b) natural environment consumers determined via interpolation and extrapolation in the iNEXT package in R. All samples were sequenced to 99-100% sequencing depth. Each colored line corresponds to a consumer individual and the dashed vertical line represents the sequencing depth to which all samples were rarefied prior to analyses for each set of consumers.



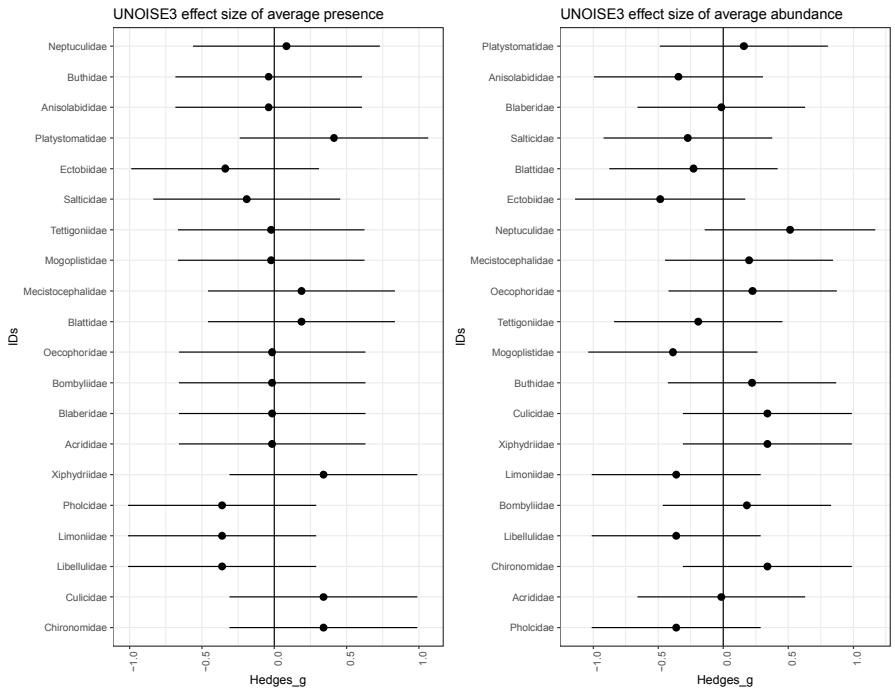
Appendix 2, Figure 15: Abundance of All Types of DNA

Consumer DNA read abundances from A) mesocosm consumers and B) natural-environment consumers, potential diet DNA reads from C) mesocosm consumers and D) natural-environment consumers, and non-diet DNA read abundance for E) mesocosm consumers and F) natural-environment consumers that were and were not surface sterilized. The surface sterilized/not surface sterilized treatment groups are not significantly different for any type of other DNA



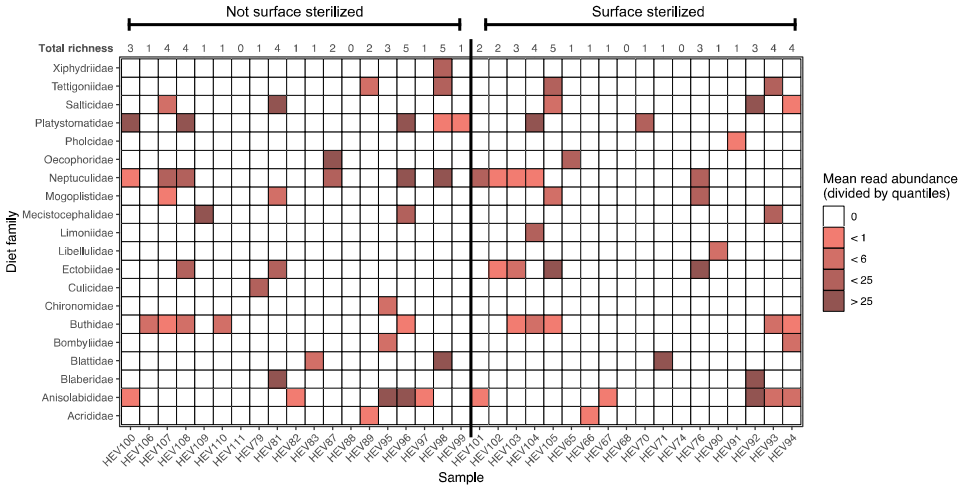
Appendix 2, Figure 16: Composition Effect Sizes Feeding Trials

The composition by-family of other diet in the mesocosm consumers, demonstrating that both presence- and abundance-based diet communities did not shift with surface sterilization treatment. (more positive means more present/abundant in unsterilized; more negative means more present/abundant in sterilized). Families are ranked by their overall presence in the population (A) or their overall abundance in the population (B) to demonstrate that there is no skew for relatively abundant or rare families.



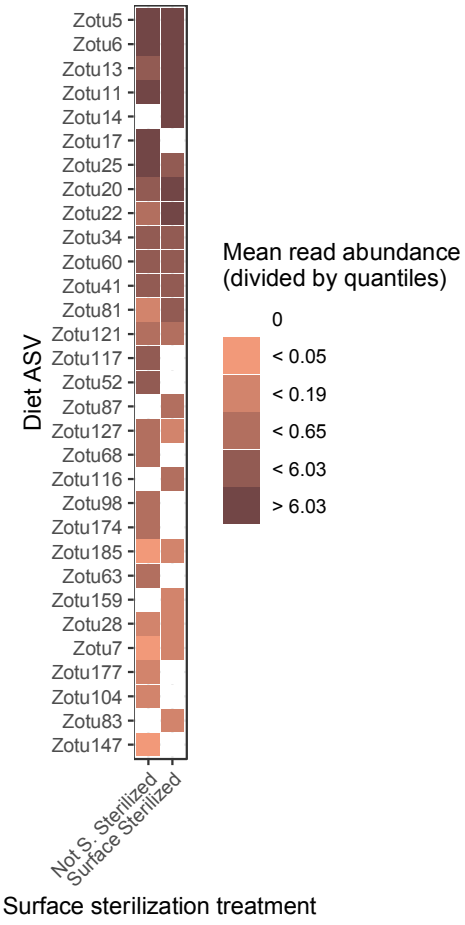
Appendix 2, Figure 17: Composition Effect Sizes Natural Consumers

The composition by-family of diet in the natural environment consumers, demonstrating that both presence- and abundance-based diet communities did not shift with surface sterilization treatment. More positive values mean more present/abundant in non-sterilized; more negative values mean more present/abundant in surface sterilized consumers). Families are ranked by their overall presence in the population (A) or their overall abundance in the population (B) to demonstrate that there is no skew for relatively abundant or rare families.



Appendix 2, Figure 18: By Sample Composition for Natural Environment Consumers

For natural environment consumers, per sample presence, abundance, and total richness of each diet family did not change with surface sterilization treatment.



Appendix 2, Figure 19: ZOTU Composition of Natural Environment Consumers

Abundance and presence of ZOTU taxonomic units as opposed to family-level diet, indicating that prey composition at the ZOTU level is also not influenced by surface sterilization treatment (both abundance and presence-based composition).

APPENDIX 3: Chapter 4

Appendix 3A: Supplementary and Expanded Methods and Results

3.A.1 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 et al. 2017, Macías-Hernández et al. 2018)) for DNA extraction following a modified CTAB extraction protocol (Fulton et al. 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 and Hajibabaei 2018) with general metazoan primers (mlCOIintF/Fol-degen-rev; (Yu et al. 2012, Leray et al. 2013, Krehenwinkel et al. 2017)). 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; (Toju et al. 2012, Clark et al. 2016, Apigo and Oono 2018)). 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 et al. 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 and 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 et al. 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. (Kartzinel et al. 2015, Brose et al. 2019, Eitzinger et al. 2019)) 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 et al. 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 4, 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 (Yaninek and Gnanvossou 1993, Sohlström et al. 2018, Su et al. 2020), Miller-ter Kuile *unpublished data*, McLaughlin et al. *unpublished data*). We fit a linear mixed effects model on $\log_{10}$-$\log_{10}$ 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.

3.A.2 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 10^7 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 10^7 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 ($\beta = 2.58$, p-value < 0.001, R2m = 0.69 and R2c = 0.95, SI Figure 9).

Species and sample sizes by sequencing run

Species	Samples	Run
Heteropoda venatoria	38	a
Neoscona theisi	22	a
Scytodes longipes	8	a
Mecistocephalus sp.	12	b
Phisis holdhausi	39	b
Smeringopus pallidus	12	b
Euborellia annulipes	17	c
Opopaea sp.	4	c
Pantala flavescens	8	c
Heteropoda venatoria	13	d

Appendix 3, Table 1: Samples by Species on Each Sequencing Run

Number of samples and interactions per species and traits						
species	hunting_mode	venom	webs	Class	samples	interactions
Mecistocephalus sp.	active	yes	no	Chilopoda	12	14
E. annulipes	active	no	no	Insecta	17	21
H. venatoria	active	yes	no	Arachnida	51	89
Opopaea sp.	active	yes	no	Arachnida	4	6
N. theisi	not_active	yes	yes	Arachnida	22	61
P. flavescens	active	no	no	Insecta	8	14
P. holdhausi	active	no	no	Insecta	39	63
S. longipes	not_active	yes	yes	Arachnida	8	16
S. pallidus	not_active	yes	yes	Arachnida	12	21

Appendix 3, Table 2: Predator Species, Traits, Sample Sizes, and Number of Total Interactions

Class	Order	Family
Arachnida	Araneae	Araneidae
Arachnida	Araneae	Oxyopidae
Arachnida	Araneae	Pholcidae
Arachnida	Araneae	Salticidae
Arachnida	Araneae	Theridiidae
Arachnida	Sarcoptiformes	Acaridae
Arachnida	Sarcoptiformes	Pyroglyphidae
Arachnida	Sarcoptiformes	Suidasiidae
Chilopoda	Geophilomorpha	Mecistocephalidae
Collembola	Entomobryomorpha	Entomobryidae
Collembola	Entomobryomorpha	Isotomidae
Insecta	Blattodea	Blaberidae
Insecta	Blattodea	Blattidae
Insecta	Blattodea	Ectobiidae
Insecta	Coleoptera	Coccinellidae
Insecta	Coleoptera	Curculionidae
Insecta	Coleoptera	Elateridae
Insecta	Coleoptera	Hydrophilidae
Insecta	Coleoptera	Staphylinidae
Insecta	Dermaptera	Anisolabididae
Insecta	Diptera	Agromyzidae
Insecta	Diptera	Cecidomyiidae
Insecta	Diptera	Ceratopogonidae
Insecta	Diptera	Chloropidae
Insecta	Diptera	Culicidae
Insecta	Diptera	Dolichopodidae
Insecta	Diptera	Limoniidae
Insecta	Diptera	Lonchaeidae
Insecta	Diptera	Phoridae
Insecta	Diptera	Platystomatidae
Insecta	Diptera	Sciaridae
Insecta	Diptera	Stratiomyidae
Insecta	Hemiptera	Aleyrodidae
Insecta	Hemiptera	Coccidae
Insecta	Hymenoptera	Eulophidae
Insecta	Hymenoptera	Evaniidae
Insecta	Hymenoptera	Formicidae

Insecta	Lepidoptera	Agonoxenidae
Insecta	Lepidoptera	Crambidae
Insecta	Lepidoptera	Erebidae
Insecta	Lepidoptera	Tineidae
Insecta	Odonata	Libellulidae
Insecta	Orthoptera	Acrididae
Insecta	Orthoptera	Mogoplistidae
Insecta	Orthoptera	Tettigoniidae
Insecta	Psocoptera	Ectopsocidae
Insecta	Psocoptera	Lepidopsocidae
Insecta	Psocoptera	Liposcelididae

Appendix 3, Table 3: Prey DNA Class, Order, and Family Identification

Model selection of number of individuals per sample model

No..Individuals	df	logLik	AICc	delta	weight
NA	3	−268.21	542.55	0.00	0.6356673
0.02	4	−267.72	543.66	1.11	0.3643327

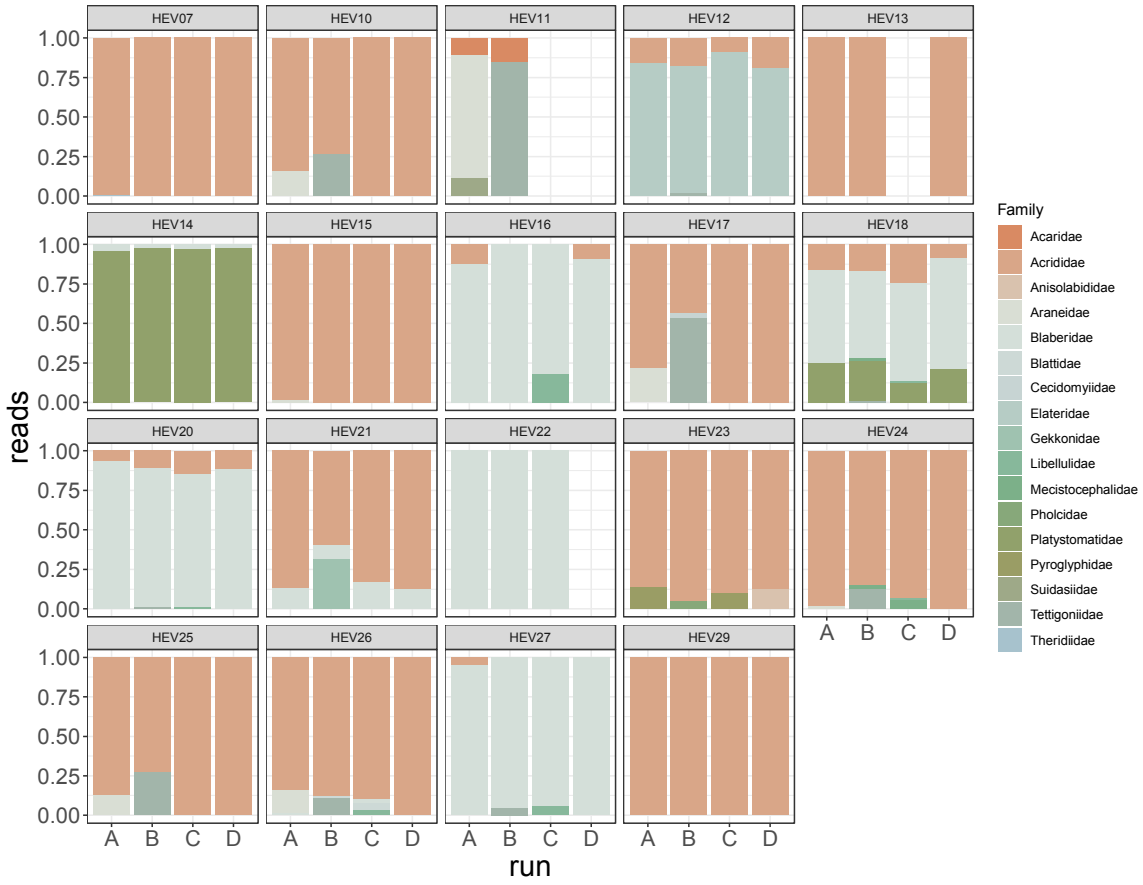
Appendix 3, Table 4: Model Selection for Number of Individuals Per Sample

Model selection of predator-prey size linear model						
log10 Predator mass	Predator species	log10 Predator mass*Predator species	df	logLik	AICc	delta
0.32	+	NA	12	−379.20	783.46	0.00
−0.40	+	+	20	−373.85	790.66	7.20
NA	+	NA	11	−384.34	791.58	8.12
0.21	NA	NA	4	−430.57	869.27	85.80
NA	NA	NA	3	−433.60	873.28	89.82

Appendix 3, Table 5: Model Selection for Predator-Prey Size and Species Model

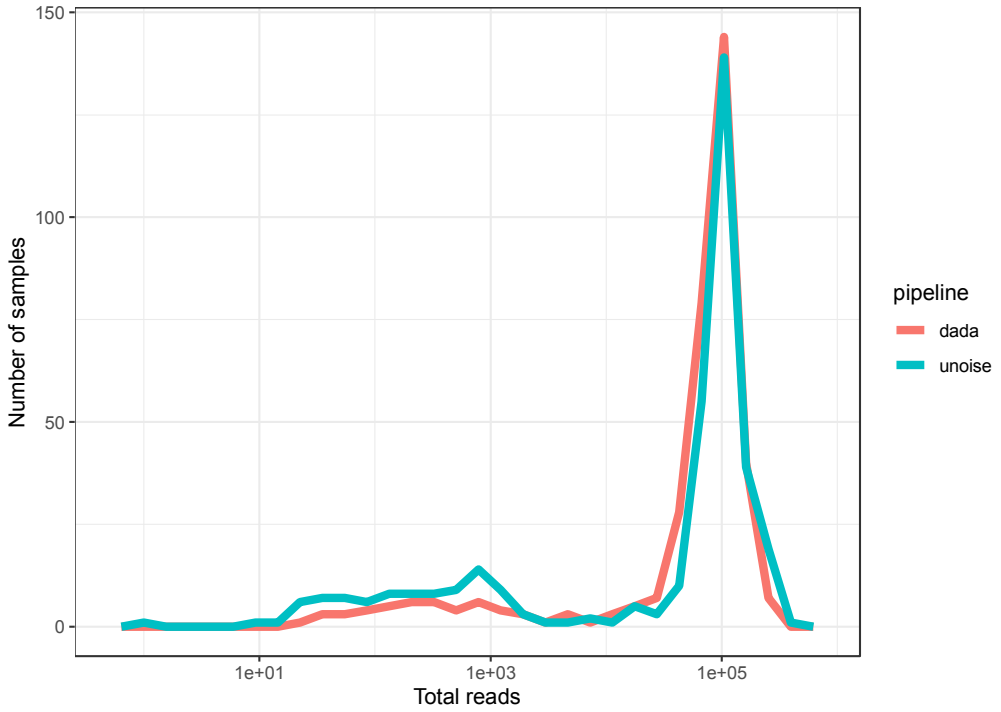
Model selection of predator trait models			
model	df	AICc	delta
m_species	11.00	826.79	0.00
m_class	6.00	850.58	23.79
m_null	4.00	852.06	25.27
m_venom	5.00	853.22	26.43
m_webs	5.00	853.48	26.70

Appendix 3, Table 6: Model Selection for Trait and Phylogeny Predator:Prey Ratio Models



Appendix 3, Figure 1: Cross-Run Sample Compositions

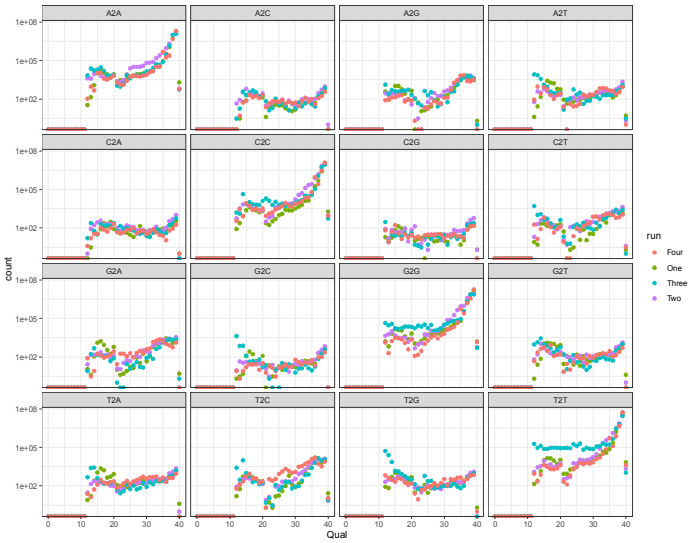
There was no clear statistical difference in the number of reads assigned to different diet families across sequencing runs for the 19 samples re-run across all four sequencing runs (PERMANOVA model results: run A-run B: $\beta = 0.07$, p-value = 0.82, run A-run C: $\beta = 0.13$, p-value = 0.70; run A-run D: $\beta = 0.17$, p-value = 0.48).



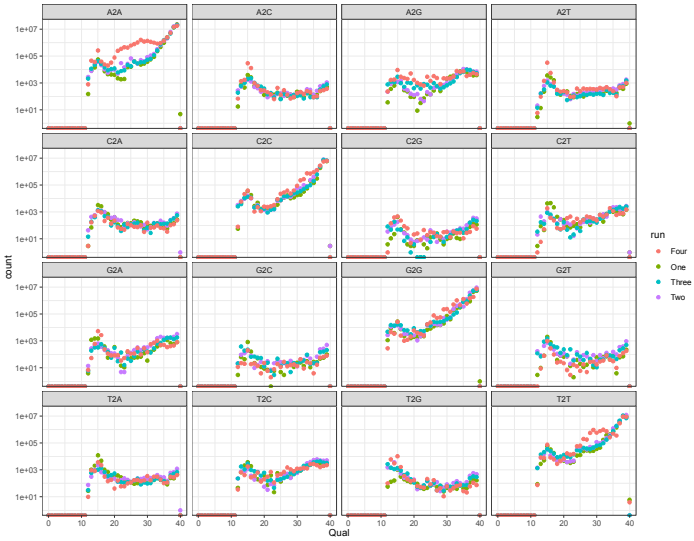
Appendix 3, Figure 2: DADA2 and UNOISE Sample Sequencing Depth

We compared the reads assigned using both the DADA2 and UNOISE3 algorithms. DADA2 produced more samples with high read abundances than UNOISE3 (slightly higher peak of the number of samples with high read abundances) and so we used this denoising algorithm for this study.

A:

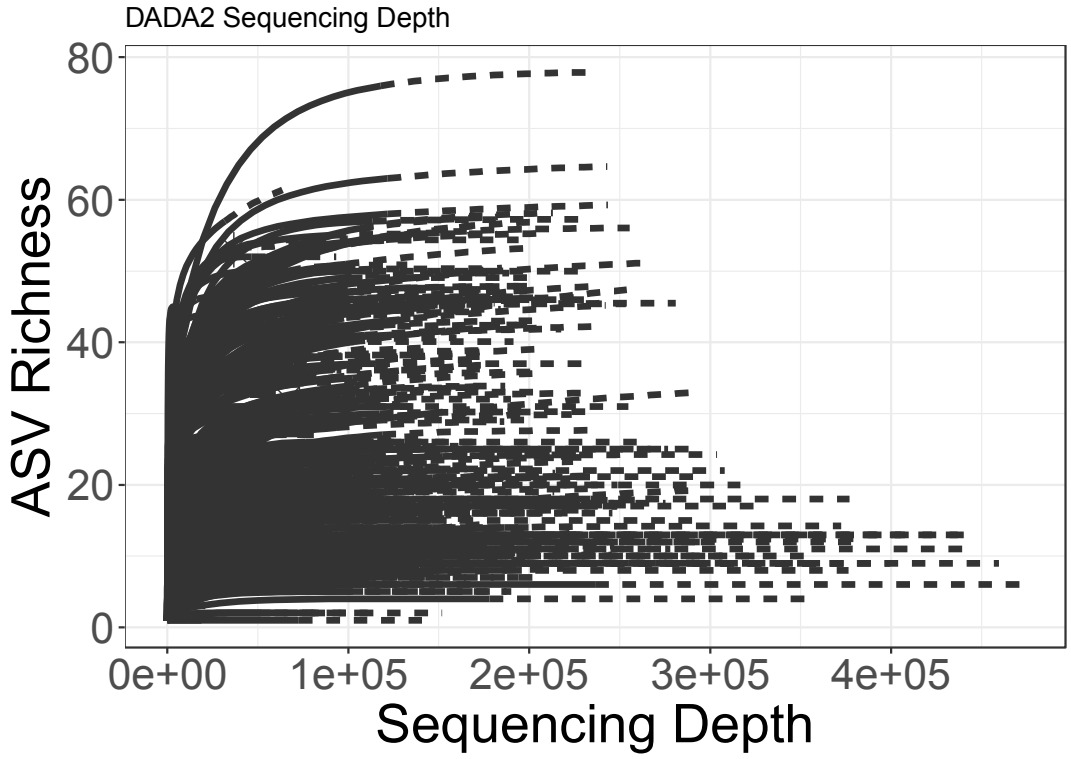


B:



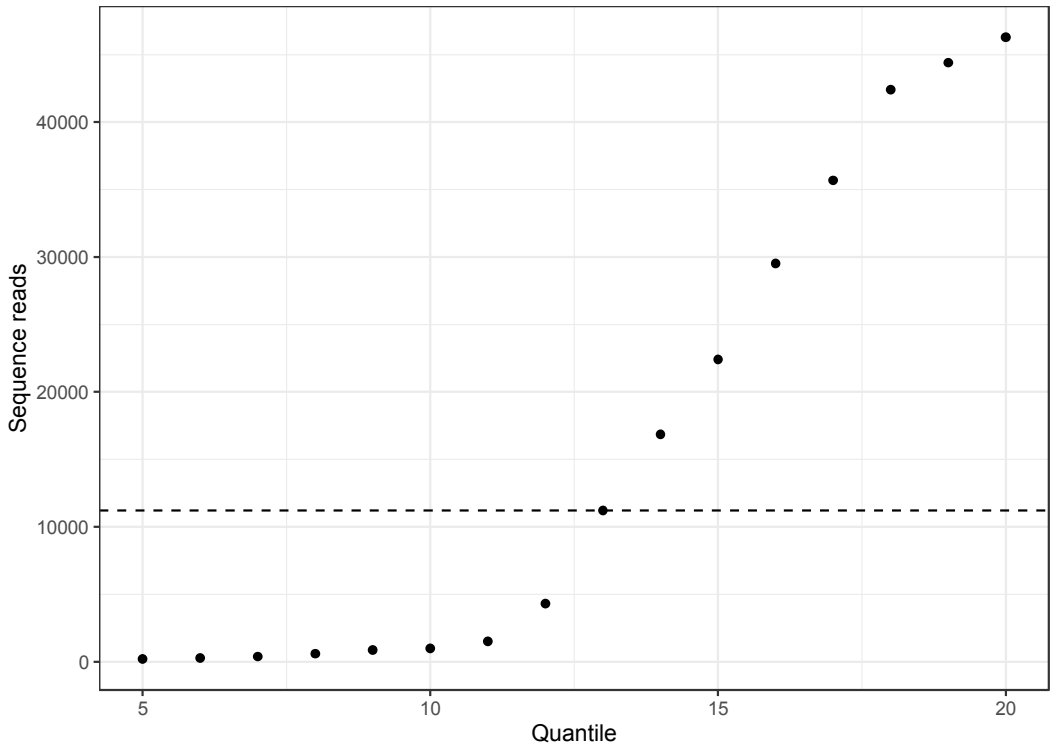
Appendix 3, Figure 3: DADA2 Error Rates by Sequencing Run

Error rates for each of the sequencing runs (A: forward reads, B: reverse reads). Based on recommendations from the developers of DADA2, we ran the DADA2 algorithm on all samples from all runs combined after verifying that the error rates were similar across runs before doing so.



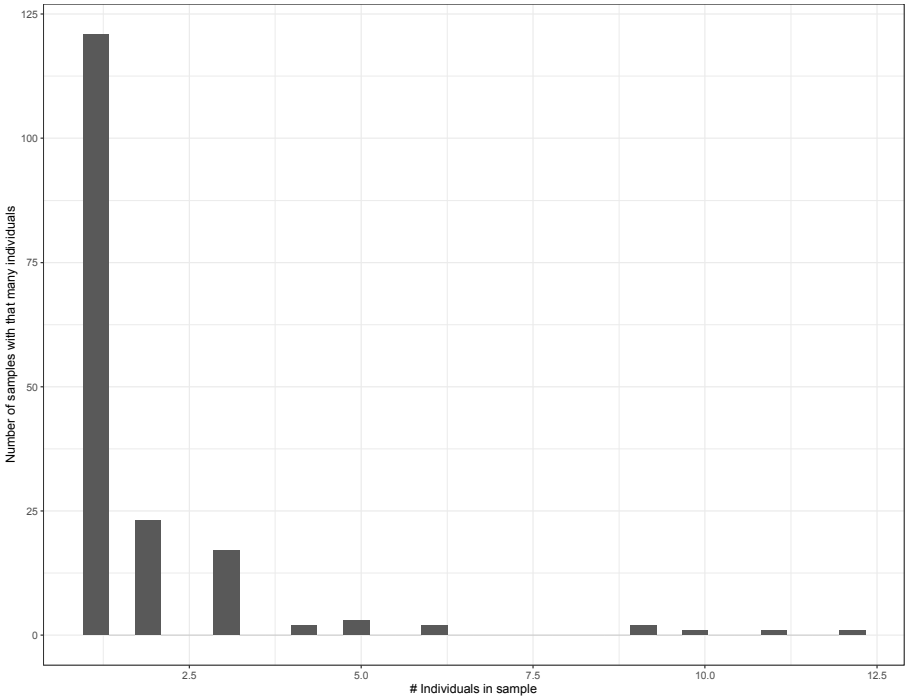
Appendix 3, Figure 4: Sequencing Depth of All Predators

Sequencing depths across all samples in our study, demonstrating the wide range in depths (10,000 – 100,000 for the current study) that can occur in DNA sequencing studies.



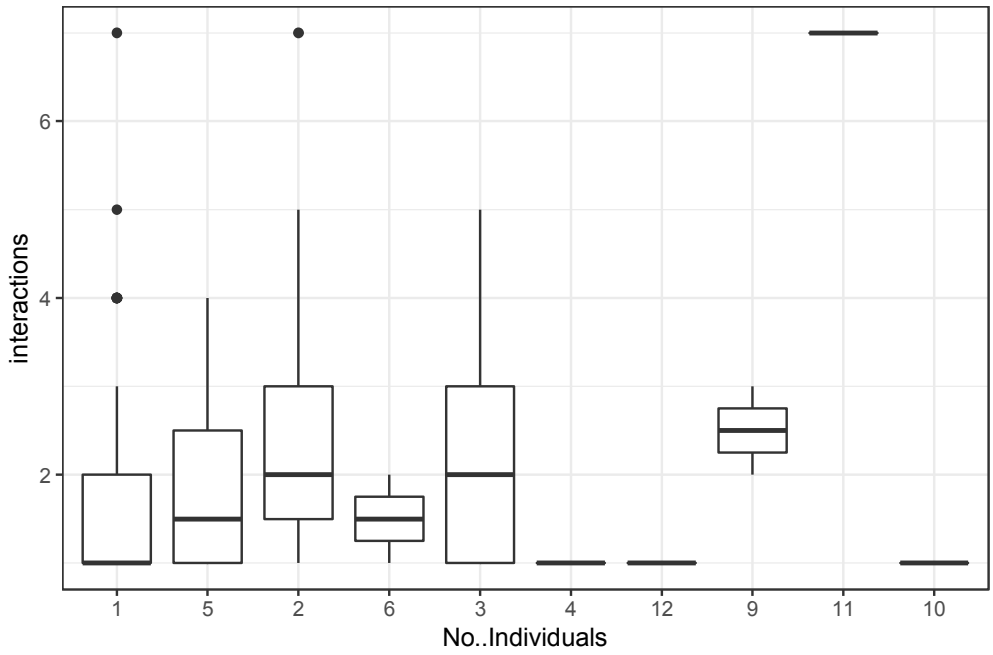
Appendix 3, Figure 5: Sequencing Depth Cutoff

We chose to remove samples from analyses that had been sequenced below a certain threshold. We determined the quantiles at 0.01 increments within the dataset and found the inflection point where a 0.01 increase in the quantile led to the greatest increase in read abundance. We determined that any samples below this sequencing depth were likely sequenced at too low a level to be comparable to the other samples in our study. Through this process, this was determined to be the 0.13 quantile and a sequencing depth below 11,211. All samples with sequencing depths below this threshold were removed from further analyses.



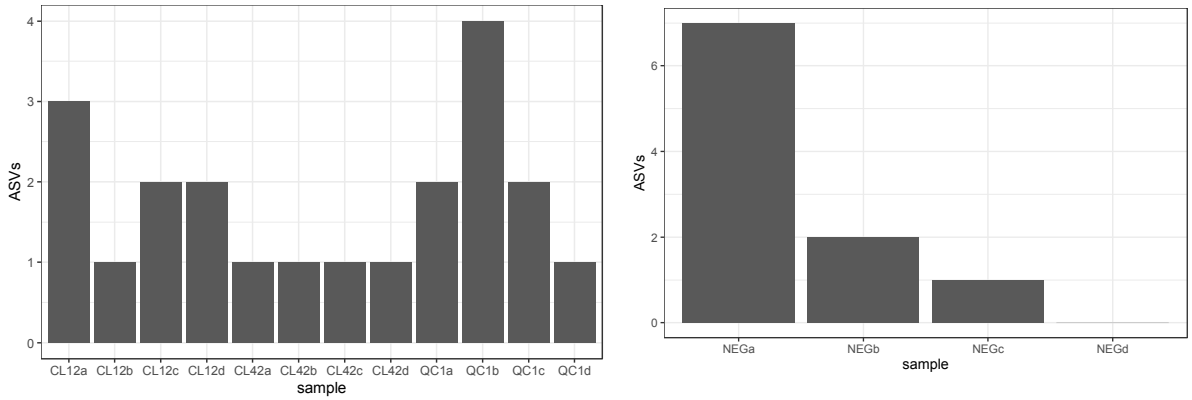
Appendix 3, Figure 6: Individuals per Sample

A distribution of the number of individuals in each sample in this study. Individuals were combined for some samples to obtain ample DNA product for DNA cleaning and PCR protocols. We only combined individuals from shared species, size (± 0.5 mm), and sampling sessions. Seventy percent (n = 121/173) of samples came from individual predators, and the remaining samples comprised one or more individuals with a maximum of 12.



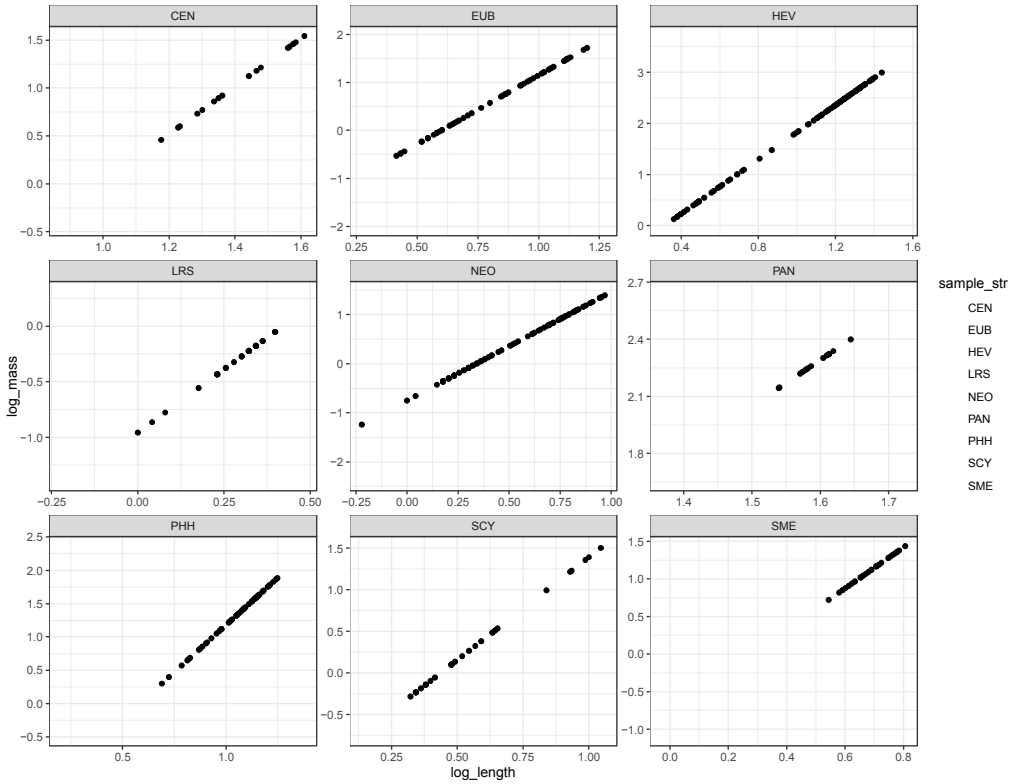
Appendix 3, Figure 7: Interactions per Sample

The number of interactions (measured as number of prey families) per number of individuals in a sample. The number of individuals in a sample did not influence over all interactions per sample.



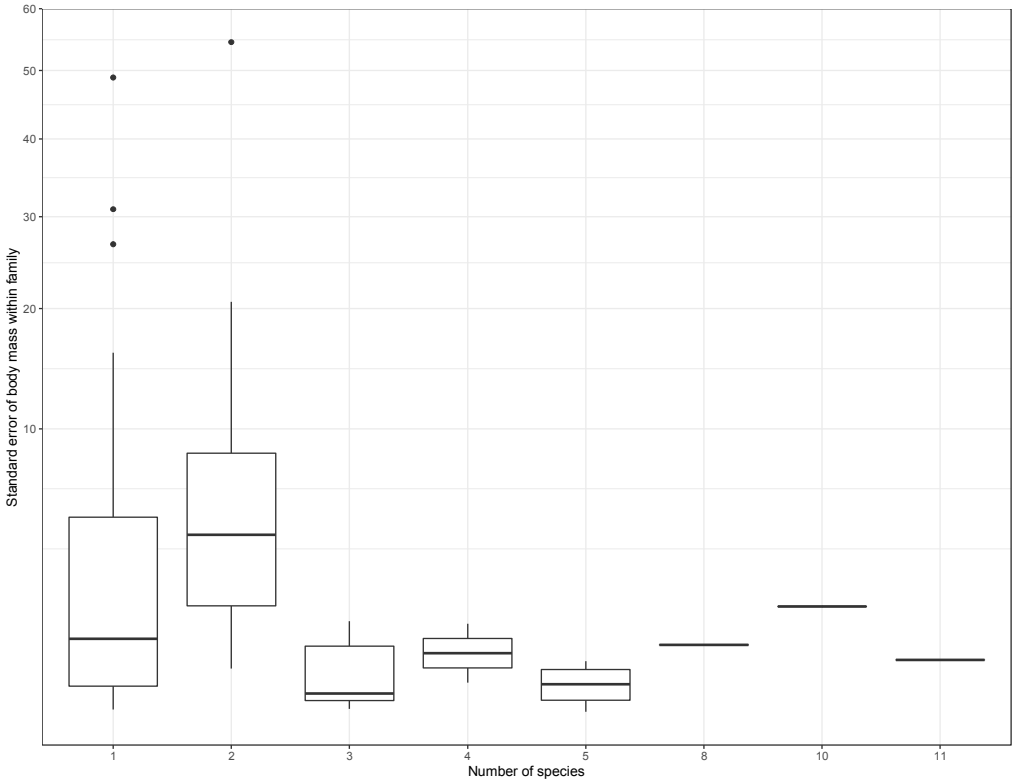
Appendix 3, Figure 8: Positive and Negative Controls

The number of ASVs assigned to both positive (A) and negative (B) controls. The low number of ASVs assigned per positive control suggests high assignment specificity from the DADA2 algorithm and all ASVs from positive controls matched to the fungal clonal sequence in GenBank. The low number of ASVs assigned to negative controls suggests low rates of sequence jumping. The total read number in each of these samples after denoising was: Run A: 314; Run B: 30; Run C: 22; Run D: 0.



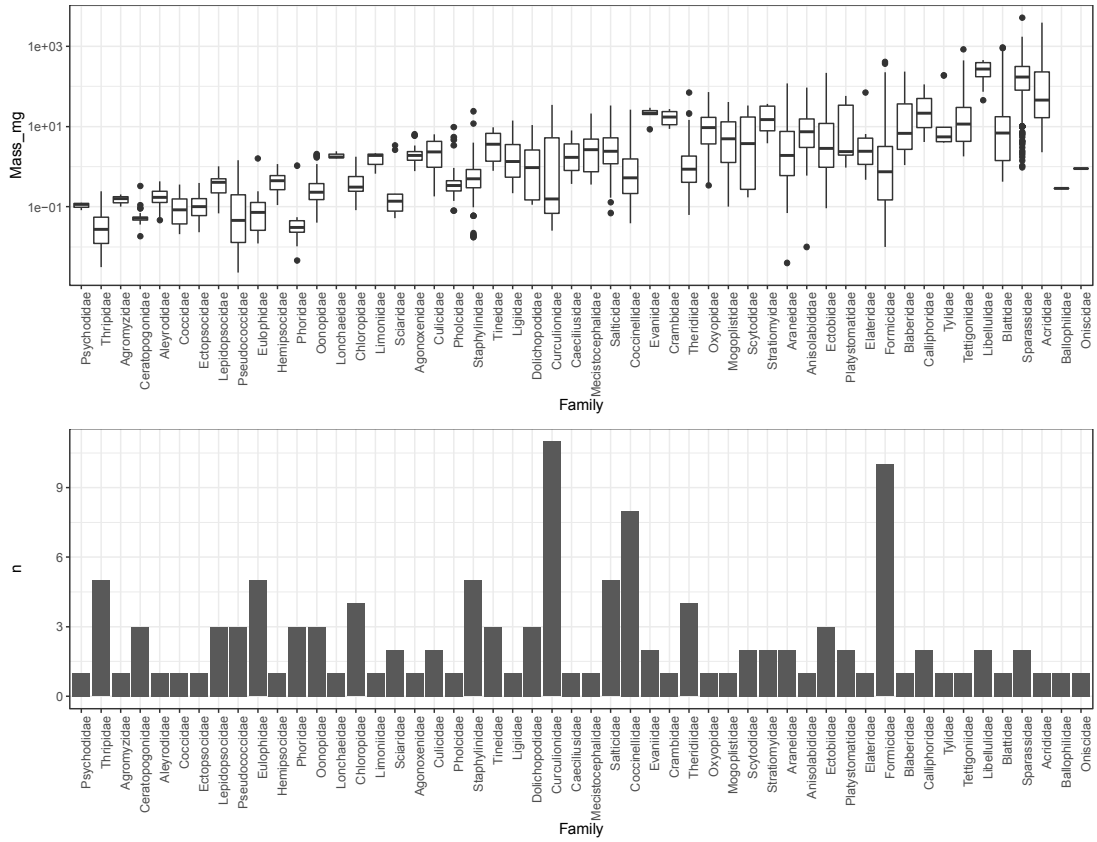
Appendix 3, Figure 9: Predator Mass-Length Relationships

We predicted the mass of predators in this study based on mass-length relationships from predators from Palmyra Atoll and the literature. Plotted are each species' $\log_{10}$-$\log_{10}$ mass-length relationships, with the lines and black dots indicating predicted values for predator individuals in this study and the grey background dots the distributions of those predators used to build those models. We predicted predator mass with a linear mixed effects model of predator mass predicted by predator length with a random slope and intercept of predator species by size, such that the per-species slope could vary. This model had a significant by-species length-mass relationship ($\beta = 2.58$, p-value < 0.001, $R^2_m = 0.69$ and $R^2_c = 0.95$).



Appendix 3, Figure 10: Body Mass by Species in Families

The relationship between the number of species in a family and the size range of individuals measured in that family, quantified as standard error, demonstrating that beyond family-level assignments representing, on average, very few species each (1.9 ± SE 0.1 species), families that consist of more species do not have wider size ranges on average.



Appendix 3, Figure 11: Body Size by Prey Families and Species Counts

The body size variation of individuals measured in each family compared to the number of species in each family in the Palmyra arthropod community.