

Assessing source-sink dynamics in invaded habitat using dietary metabarcoding

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Abstract

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Running title: Source-sink dynamics in invaded habitat

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Abstract

Earth systems are nearing a global tipping point, beyond which the dynamics of biological systems will become unstable. One major driver of instability is species invasion, especially by organisms that act as

“ecosystem engineers” through their modification of abiotic and biotic factors. In a mosaic landscape of non-invaded and invaded habitat, ecosystems modified through invasion may serve as “sink” habitat. To understand how native organisms respond to habitat that is becoming increasingly modified, it is essential to examine biological communities within invaded and non-invaded habitat, identifying compositional shifts between native and non-native taxa as well as measuring how modification has affected interactions among community members. Using dietary metabarcoding, our study examines the response of a native Hawaiian generalist predator to habitat modification by comparing biotic interactions across metapopulations of spiders collected in native forest and sites invaded by kahili ginger. Our study shows that, although there are shared components of the dietary community, spiders in invaded habitat are eating a less consistent and more diverse diet consisting of more non-native arthropods which are rarely or entirely undetected in spiders collected from native forest. Additionally, the frequency of novel interactions with parasites was significantly higher in invaded sites, reflected by the frequency and diversity of non-native Hymenoptera parasites and entomopathogenic fungi. The study highlights the role of habitat modification driven by an invasive plant in altering community structure and biotic interactions, appearing to create a “sink” for native arthropods and thereby threatening the stability of the ecosystem.

Keywords : source-sink dynamics, species invasion, metabarcoding, biotic interactions, *Hedychium gardnerianum* ,

Pagiopalus

Introduction

Over recent decades, global human transportation networks have led to the establishment of once geographically restricted species in new ecosystems (Hulme 2009; Hulme *et al.* 2008; Roderick & Navajas 2015; Sinclair *et al.* 2020). Introduced taxa may act as “ecosystem engineers” by changing the structure of an ecosystem through alteration of abiotic and biotic factors (Jones *et al.* 1994); such non-native taxa may then be called invasive because of their negative impact on ecosystem services and native species (Richardson *et al.* 2011). Invasive plants are particularly pervasive and can act as ecosystem engineers by changing habitat structure, soil chemistry (Ehrenfeld 2003), nutrient cycling (Weidenhamer & Callaway 2010) and microclimates (Ruckli *et al.* 2013). For example, the Hawaiian invasion of the firetree *Myrica faya* increases nitrogen availability in a generally nitrogen-limited system (Vitousek *et al.* 1987). One consequence of ecosystem engineers is invasional meltdown, in which modification driven by one invasive facilitates the establishment of other non-native taxa (Green *et al.* 2011; Simberloff & Von Holle 1999). Major compositional shifts in the community often occur following introduction of multiple non-native taxa, resulting in trophic cascades and shifts in biotic interactions (Borges *et al.* 2006; Cucherousset *et al.* 2012; Wainright *et al.* 2021). Through changed abiotic and biotic factors, invaded ecosystems may then function as a habitat “sink” for native taxa in a heterogenous landscape; in this case, endemic species would migrate from less-disturbed, productive “source” habitat into invaded “sink” habitat where reproductive output is diminished below population sustainability (Pulliam 1988). By studying source-sink dynamics across adjacent invaded and non-invaded habitat, we can start to quantify how alien invasion impacts ecosystem structure and function at a landscape scale.

To understand source-sink dynamics caused by invasion, it is important to go beyond single taxa and study entire biological communities. However, source-sink dynamics can produce misleading results when quantifying communities using classic biodiversity metrics. This may explain the contrasting results of studies examining how invasions impact biotic communities. For instance, the response of arthropod communities to plant invasion shows mixed effects, with many studies detecting abundance and richness decreases while others show increases (Litt *et al.* 2014). In a source-sink context, species presence and abundance values in a habitat would not indicate a productive system. Through changes in biotic interactions associated with invasion (Bezemer *et al.* 2014) such as increased predation risk, lack of the most nutritionally beneficial prey items or a higher interaction with parasites (Mattos & Orrock 2010; Suarez *et al.* 2000), invaded habitat may serve an ecological trap. Understanding the new network of biotic interactions between native and non-native taxa following invasion will provide insight into functional shifts that occur in ecosystems and indicate if invasion can induce an ecological trap for native taxa.

In our study, we use a recent invasion of kāhili ginger (*Hedychium gardnerianum*) in Hawai‘i to measure how the establishment of an invasive ecosystem engineer influences biotic interactions. Kāhili ginger is one of the world’s worst invasive species and has invaded the Azores, Madeira, Jamaica, Réunion, New Zealand and Hawai‘i, while also expanding in South and Central America, Australia and Southern Africa (Pereira *et al.* 2021). Native to the Himalayas, *H. gardnerianum* was brought to Hawai‘i in 1940 and is found in wet forest throughout the Hawaiian Islands, where it can form almost impenetrable stands up to 3 m in height (Santos *et al.* 1992; Vorsino *et al.* 2014). It is a very aggressive, shade-tolerant plant, and can invade and establish in intact native rain forest habitat, displacing native understory vegetation and altering composition of soil microbial decomposers (Kao-Kniffin & Balser 2008; Minden *et al.* 2010). Compositional differences between native forest and ginger-invaded sites have been noted using invertebrate communities in New Zealand, though the impact was context dependent (Yeates & Williams 2001). Changes in the abundance of fungivores and decomposers is the most consistent result of the invasion (Bassett 2014). The evidence of compositional shifts caused by ginger most certainly causes differences in the interactions between native and non-native taxa. Because of the global prevalence of ginger, understanding such impacts on biotic interactions are crucial to mitigate the costs associated with invasion.

Our current study uses the generalist endemic spider (*Pagiopalus*, Philodromidae) (Suman 1967) to assess whether modified sites could serve as sink habitat. While detailed demographic studies are essential to confidently identify source-sink dynamic, this is logistically challenging in most systems. Instead, by assessing shifts in biotic interactions, we can ask if major shifts in ecological function occur for native taxa; because such changes will likely carry a cost, altered biotic interactions may then indicate suboptimal habitat. We utilize metabarcoding to compare dietary communities and parasite loads in spiders across sites in ginger-invaded habitat and native-forest. Through the diet of a generalist predator, we not only can assess biotic interactions between predator and prey, but we also obtain a window into the arthropod community composition. The study focuses on a site on the island of Maui, specifically the mesic forest of Waikamoi on East Maui. Here, a sharp boundary exists between the ginger invasion and the native forest due to the efforts of the Nature Conservancy of Hawai‘i in protecting their lands (The Nature Conservancy of Hawai‘i 2011). The adjacency of native forest and ginger-invaded sites creates the possibility of connectivity between metapopulations. This provides an optimal system to assess differences in the quality of neighboring habitat and ask if ginger habitat serves as a “sink” by changing the biotic interactions of an endemic generalist predator found in both habitats.

In pursuit of this question, we have four hypotheses. First, because *Pagiopalus* are generalist predators capable of eating a wide diversity of prey types, we expect to find establishment of the spider in ginger, reflected in similar abundances across sites. Second, the altered environmental conditions in ginger will result in arthropod communities differing from native forest sites; we expect, then, to detect compositional differences in the diets between spiders collected in ginger and native forest. Third, ginger sites will host more non-native prey items, and this will be reflected in the diet of *Pagiopalus* collected in ginger habitat while the diets of *Pagiopalus* in native forests will instead consist of mostly native prey. Lastly, given the hypothesis that the novel environments created by ginger will serve as sink habitat by increasing diversity of non-natives, we hypothesize a higher interaction with non-native parasites indicative of poor habitat quality.

Methods

Study system

The study site sits across two adjacent reserves on East Maui - The Nature Conservancy (TNC) of Hawai‘i’s Waikamoi Preserve and the Makawao Forest Reserve. This area was invaded by ginger in the early 1980s. The ginger has spread across the reserves, significantly increasing in density and coverage over the last decades (The Nature Conservancy of Hawaii 2011). The Waikamoi Preserve is actively managed by TNC, who regularly remove ginger seedlings throughout the preserve to maintain a largely native landscape within the fenced off area. In comparison, the Makawao Forest Reserve is maintained less frequently and thus a thick stand of ginger covers much of the reserve, meeting the fence line that separates the adjacent Waikamoi Preserve.

Sampling

To investigate the effects of invasive ginger, we laid 5 transects in the native mesic forest habitat of the Waikamoi Preserve and 5 transects in ginger-invaded habitat of the Makawao Forest Reserve (See S2 in Supplemental Information; see Data Accessibility for coordinates). Each transect ran 30 meters long and was 3 meters in width. Spiders were collected along transects between June 8 to June 21 in 2017 using vegetation beat sampling. Each transect was broken into 3 blocks, each 10 meters in length, creating a total of 30 sampling units. Vegetation along transects was sampled using a beat sheet for 5 seconds. Fifteen units of vegetation were beaten in each block of each transect. Combined transect and block are used as “sites” throughout the paper. Spiders were collected from the beat and preserved in 100% EtOH in individual 2mL vials. Samples were stored in a -20 freezer until further use.

Molecular procedures

To extract DNA from the gut of each spider, the opisthosoma (abdomen) was first cleaned using 70% ethanol and water then removed using a sterile scalpel blade and placed in Qiagen cell lysis solution. The opisthosoma was then ground using two 3 mm steel beads on a Genogrinder (Spex SamplePrep, Matuchen, NJ, USA) for 2 min at 1,200 hz. DNA from ground samples was extracted using the Qiagen Puregene kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. DNA was then amplified using primer sets for 28s, 18s, and 16s (Primers listed in S1 of Supplemental Information). 28s has been used for fungal identification (Xu 2016; Zhao *et al.* 2011) and was therefore appropriate for detecting both arthropod and fungi. Amplification was performed using the Qiagen multiplex PCR kit (Qiagen, Hilden, Germany) in 10 ul reactions with 1ul of template DNA, and 1ul 10uM primer dilutions for 35 PCR cycles at 46°C annealing temperature. A second round of PCR was performed to add 8bp indexing tails on 5’ end and Illumina (Illumina San Diego, CA, USA) TruSeq adapters. PCR products were visualized using a 1.5% agarose gel. Products were pooled in approximately equal amounts based on band strength. 1X AmpureBeads were used to clean the pooled products. The cleaned library was then sequenced on an Illumina Miseq using V3 chemistry. Negative controls were included on the sequencing run.

Bioinformatics

Demultiplexed sequences were batch processed using CutAdapt to remove primer sequences and perform preliminary quality filtering (Martin 2011). The denoising algorithm DADA2 was run in R to produce amplicon sequencing variants and remove chimeras (Callahan *et al.* 2016). Parameterization and length trimming was dependent on locus. Widespread contaminants were identified using package *decontam* in R using sequences identified in controls (Davis *et al.* 2018); the threshold argument was set to 0.5, which removes sequences more prevalent in controls than in true samples. Once contaminants were removed, ASVs were further curated using the LULU algorithm, which identifies NumTs and remaining artefactual sequences (Frøsvlev *et al.* 2017). Finally, sequences were filtered at the ASV and sample level by read counts. ASVs represented by less than 10 reads were removed. At the sample level, the number of reads found for an ASV that represented less than 0.01% of the total ASV reads or that represented less than 1% of the total reads for the sample were removed. Additionally, one sample had missing data and needed to be removed because it could not be confidently placed in ginger sites or native forest sites.

Sequences were then written to FASTA files and, using Geneious, assigned taxonomic identities through megablast. Full lineage information was assigned using a custom R script based on package *rentrez* (Winter 2017). The level of assigned taxonomic identity was dependent on percent identity match. Order-level identity required $\geq 85\%$, family $\geq 92\%$, genus $\geq 97\%$ and species $\geq 99\%$. Sequences that were not identifiable to order-level were removed. To construct the prey data set, arthropod reads were filtered to remove Philodromidae reads, which represented the spider itself, and sequences in order Hymenoptera, which belonged to parasitoid wasps rather than prey. Because the Hymenoptera with confident BLAST identifications were all parasitoids, the ASVs only identifiable to order were additionally removed as likely parasites. Prey reads were assigned native or non-native status based on publicly available literature. Sequences that likely belong to entomopathogenic fungi and parasitic wasps were similarly identified using publicly available literature.

Statistical analyses

Community data frames were constructed using individuals as “sites” against ASV, family or order identifications. Incidence and Hellinger-transformed relative read abundances were used as values. Incidence data alone outweighs rare taxa and can significantly alter detected ecological patterns. While read abundances are not directly translatable to taxon abundances in a community, there is utility in transformed abundances to detect otherwise obscured ecological patterns. Hill numbers (Alberdi & Gilbert 2019) were calculated to quantify prey diversity for all three community data frames. Welch’s t-test was used to test the hypothesis of no differences in dietary prey diversity between spiders in ginger and native forest. Additionally, Welch’s t-test was used to test the hypothesis of no differences in read abundances between both groups.

To assess overall compositional differences between the diets of spiders in ginger sites versus native forests, community matrices were constructed using block number combined with transect number as the site, against ASV or order. An order-level food web grid was constructed. Family, genus or species was not used because of overall low BLAST matches; these taxonomic levels provided less compositional information as well as the data was biased towards taxa well-represented in GenBank. Order-level provided the coarsest view of diets while ASV provided the finest grain view. Beta diversity was calculated based on Jaccard distances on both Hellinger-transformed reads and incidence data. Produced values were plotted against distance between sites to assess level of spatial autocorrelation (see S5 in Supplemental Information). Because of very weak correlation between distance and beta diversity and our interest in only a single explanatory variable distinctly split across sites, spatial autocorrelation was not identified as a concern. Non-metric dimensional scaling (NMDS) was performed using beta diversity values, with $k = 2$ and using a maximum of 1,000 random starts to achieve convergence. Permutational multivariate analysis of variance (PERMANOVA) was performed based on beta diversity values to test the hypothesis that the center and spread of dietary communities across ginger and native forest sites are equivalent.

To assess compositional differences within the two most prevalent orders (Hemiptera and Lepidoptera), 16s sequences were aligned using MUSCLE and a neighbor joining phylogenetic tree using the Jukes-Cantor model of evolution was constructed in Geneious (See S8 and S9 in Supplemental Information). This tree was then used to calculate phylogenetic beta diversity. Phylogenetic beta diversity was used here to allow assessment of relationships between taxa within each order, as family, genus or species identifications were not obtainable using BLAST. Again, NMDS was used to visually assess community similarity in ordination space and differences tested using PERMANOVA.

The lack of confident BLAST identifications resulted in a lower number of identifiable native/non-native sequences; more identifiable sequences were detected in the spiders in ginger because of the higher proportion of introduced taxa. Parasites were identifiable across ginger and native forest sites using BLAST. Parasite frequency was assessed using both the number of ASVs within ginger and native forest sites that were identified as parasitic, and the relative number of parasitic reads in individual spiders. Welch’s t-test was used to test the hypothesis of no differences in parasitic load between ginger and native forest sites.

BLAST assignment, alignment and phylogenetic reconstruction was completed in Geneious Prime v. 2022.0.2. Analyses were conducted in R version 4.1.2. Analyses and figures were produced using the following packages in R: vegan, BAT, ape, tidyverse, reshape2, ggplot2, ggpubr, ggvenn, formattable, gapminder, bipartite, and ComplexHeatmap. Code for analysis and data is available on GitHub and Dryad (see Data Accessibility).

Results

Summary of collections

168 *Pagiopalus* sp. were collected in total; abundances were nearly equal across ginger and native forest with 82 specimens collected from ginger sites and 86 specimens collected from native forest. There was on average 5.79 ± 0.55 spiders collected per site (block and transect) and 16.8 ± 2.4 spiders collected per transect.

Summary of molecular findings

Of the 1,370,873 reads retained following DADA2 and additional filtering, 1,059,644 reads were not prey reads; this reduction came largely from sequences of the spiders themselves (679,534 or 64.1% Philodromidae reads) followed by fungi (234,630 reads, 22.1%). Parasitoid wasps represented an additional 60,060 (5.7%) of the non-prey reads (see S3 in Supplemental Information). Following prey read curation, there was an average of $2,288.4 \pm 288.1$ reads and 2.55 ± 0.13 ASVs per individual when combining across loci. 16s returned the highest number of ASVs at 110 ASVs, followed by 28s at 44 ASVs and 18s at 10 ASVs. This was reflected in the number of specimens retained following filtering procedures as well; 112 specimens with 16s reads, 76 specimens with 28s reads and 17 specimens with 18s reads.

Summary of taxonomy

There were 23 species, 32 genera, and 31 families belonging to 9 orders detected across ginger and native forest sites. Only 23.2% and 37.8% of ASVs returned a confident species or genus ID respectively. This was improved at the family-level; 61.6% of ASVs returned family IDs. 16s sequences returned the lowest matches at 91.58% pairwise identity on average. While returning the lowest ASVs and retained specimens, 18s had the highest matches at 98.27%; 28s was near with an average match of 97.72%. The low percent identity matches for many ASVs biased taxonomic results at species and genera level towards well-known taxa present in GenBank; because of this, family- and order-level were used along with ASV identity.

Prey diversity and abundance

Of the total 164 ASVs in the dataset, only 29 ASVs were shared across ginger and native-forest sites. Dietary richness was higher in ginger than in native forest, with 66 total ASVs found in native forest compared to 127 total ASVs found in dietary communities in ginger sites (Figure 1a). Taxonomic composition showed similar trends; 11 of 31 families, 10 of 32 genera, and 5 of 23 species were shared across ginger and native forest sites, with ginger sites containing higher overall taxonomic richness (Figure 1b).

Hill numbers were used to quantify prey diversity within spiders between ginger and native forest sites based on ASV and taxonomic identity. Values were significantly different between ginger and native forest sites (Welch t-test, p-value < 0.005; Table 1), with spiders in ginger sites having higher values on average, representing a wider niche breadth than spiders in native forest. Spiders in ginger had higher reads as well; 3468.9 ± 448.98 prey reads were retained from spiders in ginger sites compared to 700.9 ± 133.19 reads from spiders in native forest (Welch t-test, p-value < 0.005; Figure 2). While reads are not tightly correlated with abundance, the general distribution of reads support differences in prey abundances in the diets of spiders between ginger and native forest sites.

Dietary composition

To further explore dietary differences between ginger and native forest, samples were assessed by sampling event, defining a site using the block and transect number, rather than the diet of each individual spider. Incidence and Hellinger-transformed relative read abundances were used to calculate beta diversity using ASV, and order-level identities. Beta diversity values were highest between the sites in ginger and sites in native forest when using incidence data, indicating compositional differences (Figure 3). Ordination space overlapped between ginger and native forest sites, most noticeably when using ASVs (Figure 4); using ASVs, high dietary dissimilarities existed across all sites, both within habitat types and between habitat types (Figure 3), which resulted in less clear differentiation. Hellinger transformed read abundance produced a significant difference between ginger sites and native forest sites when using ASVs but not when using orders. This is likely because dietary communities across ginger and native forest sites showed more similarity at the order level while other sites in ginger habitat were extremely different. However, using beta diversity calculated from incidence data, there was a significant difference between ginger and native forest sites across both ASVs and order (PERMANOVA, p-value < 0.05; Figure 4b, 4d). This provides interesting implications for the different ecological patterns detected with and without abundance information. In our case, a high similarity in a handful of sites across both habitat types is detected when using relative read abundances because certain orders are being eaten in similar quantities; without abundance, rarer orders are having more of an influence.

Another interesting finding is that there is high compositional turnover in the diets of spiders within ginger habitat when we look at order-level diversity; this is reflected in the beta-diversity values (Figure 3) and in the NMDS plots, where the polygon encompassing ginger sites occupies a larger portion of the ordination space (Figure 4). The compositional differences in the diets of spiders from ginger sites are due to the higher diversity of prey consumed, with four orders (Hemiptera, Lepidoptera, Diptera and Entomobryomorpha) detected most commonly (Figure 5). The diets of spiders in ginger are not dominated by any one order, with the most common prey (Hemiptera) detected in the diet of 43.6% of ginger spiders followed by Lepidoptera in 42.3% of spiders and Diptera in 37.1% of spiders; the non-consistency of the diet across spiders in ginger is the cause of increased beta diversity and wider polygons. In contrast, spiders in native forest are consuming more similar diets consisting predominantly of Hemiptera, with 72.4% of spider diets containing Hemiptera. The second most common order, Lepidoptera, only occurs in 36.2% of spider diets. Spiders in native forest are not preying on Entomobryomorpha (Collembola) or Coleoptera at the same rate as ginger spiders. In fact, Entomobryomorpha was the fourth most common prey group in ginger, detected in 27 spiders (34.6%) while Entomobryomorpha were only detected in 1 spider (1.7%) in native forest sites (Figure 5).

To further understand diets between native forest and ginger forest, we examined phylogenetic beta diversity within the most common orders, Hemiptera and Lepidoptera. Here, we were interested to see if spiders were eating the same taxa within commonly detected orders. Because of low BLAST identification, phylogenetic beta diversity using 16S within orders was used to allow us to detect any finer scale taxonomic similarity that may exist. We find large overlap between the Hemipteran and Lepidopteran prey in both native forest and ginger (see S6 in Supplemental Information). Hemipteran in the family Cicadellidae and Lepidoptera in the families Geometridae and Hesperidae were most detected across both habitats. In native forest, more spiders were detected consuming family Cicadellidae and family Hesperidae (See S7 in Supplemental Information). There were three Hemipteran families and six Lepidopteran families detected in ginger that are were found in native forest; however, these were detected in the diets of very few spiders.

Native versus introduced prey items

BLAST matches were low for most ASVs, resulting in lack of confident identification. Because of this, only 53 of 164 ASVs were identifiable as native versus non-native prey taxa. Therefore, most ASVs in the diets of spiders in ginger or native forest were not assigned an endemism status. With those that were identifiable, we find more non-native prey ASVs in ginger forest than in native forest (Figure 6). Additionally, we find less identifiable ASVs in spiders collected from native forest. Because widely spread introduced taxa are usually well-studied, the fewer identifiable sequences found in spiders in native forest could indicate more native prey taxa not yet sequenced.

Looking at the diets of individual spiders, we find that more spiders in ginger-invaded habitat are consuming diets consisting entirely of non-native prey than diets consisting of native prey (Figure 7). Moreover, the prey orders not found in high abundance in native forest but eaten commonly in ginger are largely non-native. In particular, all Entomobryomorpha detected in the diets of spiders are non-native. Spiders in ginger were additionally eating more non-native hemipterans than the spiders in native forest. More unidentifiable sequences were detected in native forest; the prey status in the diets of 33 of the 58 spiders from native forest were unknown. This could relate to lack of presence in GenBank and indicate higher concentration of native prey taxa, while adventive or introduced taxa are more well-represented resulting in a higher level of ASV identification in ginger sites.

Parasitism

61 spiders had reads from entomopathogenic fungi or parasitic wasps (Figure 8); these likely are from parasitized prey eaten by the spiders, rather than parasitism of the spider itself. However, the latter cannot be ruled out. Using both number of ASVs associated with parasites in individual spiders and relative read abundances, there is a significant difference between ginger and native forest sites (Welch t-test; p-value < 0.005). 49 of the 61 spiders had sequences from parasitic wasps, covering 29 ASVs. All but one of the parasites were identified as non-native. 31 spiders in ginger had parasitic wasp reads compared to 18 in native forest.

This results in a significant difference, albeit weak, between invaded habitat and native forest (Welch t-test; p-value < 0.05). Braconid wasps, predominantly *Rhopalophorus* and *Cotesia*, were most common among forest types. This was followed by ichneumonid wasps, predominantly *Ichneumon*. Three families of parasitic wasps were detected only in ginger sites; however, these were detected in very few specimens.

Entomopathogenic fungi were identified in 22 spiders in ginger sites and only 1 spider in native forest. There was a highly significant difference between sites (Welch t-test; p-value < 0.001). *Beauveria* was identified in 16 spiders in ginger, followed by *Ophiocordyceps* in 7 spiders. Most spiders (20 of 22) had a single fungal type. However, *Beauveria* and *Ophiocordyceps* were found co-occurring in two spiders in ginger forest. The single spider in native forest was identified with *Gibellula*, a known arachnid parasitic fungus. This genus was not detected in ginger sites, although it falls in the same family as *Beauveria*. Infections of spiders with *Beauveria* have been reported, but this is uncommon (Evans & Samson 1987).

Discussion

Our study set out to examine if invasion by a plant which drastically alters the environment would create a sink habitat through changes in biotic interactions, attracting endemic taxa from native forest into habitat containing conditions that may diminish fitness (Pulliam 1988). Our first goal was to determine whether endemic natural predators maintain their natural interactions with prey, despite the marked modification in habitat; this would suggest that ginger-invaded sites can serve as a habitat refuge for native arthropods and support natural biotic interactions. Alternatively, the diet of the spiders in invaded sites could differ significantly from spiders in native forest and consist of less native prey taxa, which may indicate suboptimal habitat. Our second goal was to assess how interactions with parasites may change, in which there may be a higher prevalence of non-native parasites in ginger-invaded sites, imparting a fitness cost on native prey or on spiders themselves. Using both prey and parasitism, our results support the idea that ginger-invaded sites may serve as a sink for *Pagiopalus* spiders.

Spiders are present in similar abundances across habitat types

Despite the drastically different habitat in ginger-invaded sites and native forest, we found *Pagiopalus* in nearly identical quantities. This is in line with our first hypothesis, in which the generalist ecological strategies of *Pagiopalus* would enable establishment in altered habitat.

Previous findings showed the invasion of guava (*Psidium* spp.) in lowland forest of Hawaii is associated with an almost entirely endemic spider community (Gillespie 1991; Gillespie *et al.* 1998), with similar results from ginger-invasion. Similarly, native land snails have been found to prefer invasive ginger to native plant species, suggesting no negative effect when the understory plant assemblage shifts (Meyer 2012). This may then suggest that the vegetation may not constitute a major perturbation to generalist arthropods (Gillespie 1999). However, as we have discussed, single taxon studies are inadequate to assess the true impact of invasion on native taxa; an equal abundance of *Pagiopalus* in ginger does not eliminate the possibility that invaded sites serve as sinks.

Arthropod communities in the spider diet differ between native and invaded sites,

Our results show that in areas modified by invasive ginger, spiders are consuming a very different, but partially overlapping, spectrum of prey items compared to spiders in native forest (Figure 4). Spiders in native forest are preying predominantly on Hemipterans and show a narrow selection of prey while the diets of spiders in ginger are more varied, and consist of prey orders uncommonly found in the diets of spiders collected from native forest (Figure 5). In invaded sites, a more diverse prey community was detected in totality, reflected in the individual diets of spiders as well which showed wider breadth than spiders from native sites. Trophic dispersion has been detected in other studies following invasion, which can be followed by destabilization of the food network (Wainright *et al.* 2021).

Spiders in ginger also appear to be consuming more prey, when using sequencing reads as a proxy measurement for abundance (Figure 2). Invasive plants have been found to increase the abundance of available arthropods that can serve as prey in some studies. For example, a study in the Iberian Peninsula found a

much higher abundance of generalist herbivore species on nonnative plants (Rodríguez *et al.* 2019). This increase may partially be due to the provision of shelter (Landsman *et al.* 2020). A similar result was found for arthropods associated with invasive tamarisk in Colorado (Uhey *et al.* 2020). Spiders make site settlement decisions based on multiple factors, predominantly microhabitat and prey availability (Riechert & Gillespie 1986). If there is abundant potential prey and if ginger provides additional protection through the dense plant structure, spiders may perceive the habitat to be highly suitable (Gillespie & Tabashnik 1994). However, unpublished data from arthropod sampling in this invasion of ginger did not detect higher densities of arthropod prey. The higher reads in the diets of spiders in ginger may then point towards suboptimal prey, where the spiders must consume more prey to meet their nutritional requirements.

The diets of spiders in ginger sites contain more non-native taxa

The metabarcoding approach used in our study provided an avenue for assignment of arthropod taxa to native versus invasive status for any taxa included in the GenBank database. For those not included, identifications were only to genus, family, or order. However, assignment to native versus nonnative status is simplified for the Hawaiian arthropods because many taxa are endemic at the genus level (Eldredge & Miller 1995) and many insect families and orders do not occur in the native arthropod biota, allowing classification at a higher taxonomic level (Howarth 1990).

Our results showed that native taxa were found in the diet of the endemic *Pagiopalus* in both native forest and ginger-invaded sites: *Campsicnemus* (Dolichopodidae) in 10 spiders from ginger sites and 3 from native forest, *Limonia* (Limoniidae) in 10 spiders from ginger sites and 4 from native forest, and *Nesophrosyne* (Cicadellidae) from 7 spiders from ginger sites and 13 spiders from native forest. These taxa are included within some of the largest insect radiations in the islands. The hemipterans in family Cicadellidae are specific to native plant species (Bennett, pers comm, Bennett & O'Grady 2012). As the dominant prey family in the diets of spiders collected in native forest (detected in 25 of the 58 spiders), and as endemic taxa associated with native flora, these hemipterans likely represent an important prey source with which *Pagiopalus* evolved. Because the host plants are absent from heavily invaded ginger sites, access to host-specific native prey is likely limited for spiders in ginger forest.

Differences between the diets of *Pagiopalus* in ginger and native-forest sites were driven largely by the high numbers of nonnative taxa (Figure 7), specifically Entomobryomorpha (Collembola) which was detected in the diets of 28 spiders from ginger while being almost entirely absent in the diets of spiders from the native sites (Figure 5). Collembolans have been found to greatly outnumber other arthropods in ginger-invaded habitat (unpublished data). The collembolan *Salina celebensis* was the most prevalent in ginger (detected in 18 spiders) and was not detected in native forest. This species, introduced from Asia, is characteristic of moist understory vegetation, with its extraordinary abundance noted in previous studies (Christiansen & Bellinger 1994; Gruner & Taylor 2006; Gruner *et al.* 2005). The other species of Collembola was *Tomocerus* sp ; although not identifiable to species, *T. minor* , introduced from Europe has been known from Hawaii, for > 50 years (Christiansen & Bellinger 1992). *Tomocerus* was found in a single spider in native forest contrasting its detection in 15 spiders from ginger sites.

The much higher numbers of Collembola in invaded habitat may enhance the survival of native spiders such as *Pagiopalus* , simply because of their abundance; alternatively, they may serve to detract from their survival if they do not support the nutritional needs of the predator. Previous work has shown that, while being a common source of prey in cursorial spiders (Birkhofer & Wolters 2012), Collembola have mixed nutritional benefits. Studies have found inclusion of certain species of collembolans (*Tomocerus bidentatus*, *Isotoma anglicana*) in the diets of spiders increases reproductive output or survival while other species (*Folsomia candida* , *Folsomia fimetaria*, *Isotoma trispinata*) drastically decrease reproductive output or result in high mortality (Møller Marcussen *et al.* 1999; Toft & Wise 1999; Rickers *et al.* 2006). These studies additionally note the possible toxicity of certain collembolans, resulting in a significant fitness cost (Toft & Wise 1999). Generalist predators are well documented to be dietarily selective when given a diverse set of prey, preferentially eating prey items which have the highest nutritional benefit (Michalko *et al.* 2019; Rendon *et al.* 2019; Toft 1999). In order to obtain nutritional balance, however, a mixed diet consisting

of nutritionally imbalanced or even toxic prey may be consumed when optimal prey is in low abundance. Supplementation of Collembola and other prey not found in native forest may be a necessity for spiders in ginger because of the lack of access to host-plant specific taxa. The higher prey diversity and higher reads in spiders from ginger sites supports this, showing a mixed dietary strategy not adopted by spiders in native forest. While prey may be available in ginger, the prey itself may be less suitable than native insects found predominantly in native forest; high prevalence of some prey items, in particular Collembola, may create an ecological trap by attracting spiders while requiring spiders to predate more taxa to accommodate nutritional inefficiencies.

Higher parasitic load was detected in the spiders in ginger

Entomopathogenic fungus and hymenopteran taxa were detected in spiders collected from both ginger and native forest (Figure 8). While these reads likely originate from parasitized prey rather than parasitized spiders, the high incidence of parasitism within arthropod taxa across both ginger and native forest, but especially in ginger, is apparent. This is yet another benefit of high throughput sequencing approaches; not only can prey communities be detected, but parasites of prey themselves may be detected, supported in other studies (Traugott & Symondson 2008).

We can identify some well-known lepidopteran parasitoids that have been accidentally or purposefully introduced, including *Cotesia vestalis*, *Ichneumon xanthorius*, *Meteorus laphygmae*, and the scale parasite *Aphytis chrysomphali*; these parasitoids are found in both native forest and ginger-invaded sites. The major hymenopteran parasitoids were braconids, in particular *Microctonus* (*Rhopalophorus*) which is a well-known adventive species across the islands (Nishida 2002) and appears to parasitize beetles, notably chrysomelids (Beardsley 1961). Infiltration of native forest by nonnative parasitoids, in particular parasitoids of Lepidoptera, has been well documented (Henneman & Memmott 2001). Their prevalence in spiders from native forest, then, is not surprising. However, we do show that the overall prevalence of Hymenoptera was higher in ginger-invaded sites than native sites. A wasp, in the non-native family Pteromalidae (Beardsley 1961; Nishida 2002) was found exclusively on ginger; Pteromalidae are mostly parasitoids of Lepidoptera and Coleoptera, but can also attack larvae and pupae of Diptera and the eggs of Coccidae. Spiders from the ginger-invaded sites also showed a higher diversity of other parasitoids, including *Oraesema* sp., in which the one species known from Hawaii is a parasite of another invasive species in Hawaii, the little fire ant (Heraty *et al.* 2021).

Entomopathogenic fungal reads were detected predominantly using 28S; while not the most widely used marker for fungal DBA barcoding, 28S has been shown to be relatively effective (Xu 2016; Zhao *et al.* 2011). For the sequences detected in the spiders in our study, their high percent identity matches and known presence on Hawaii provides more support for our finding. Entomopathogenic fungi were detected in 22 spiders in ginger sites while only found in 1 spider from native forest. This result is consistent with previous work in New Zealand which has shown that fungivores are much more abundant in sites that have been invaded by ginger (Bassett 2014). The entomopathogenic fungi *Gibellula*, the most common spider fungal pathogen (Shrestha *et al.* 2019), was detected in only one spider in native forest. *Beauveria* was the most commonly detected fungi in spiders from ginger-invaded sites, followed by *Ophiocordyceps*, a known parasite of beetle larvae (Wang *et al.* 2021). *Beauveria* is genus of cosmopolitan fungal pathogens, found associated with arthropods and the surrounding habitat, including in the soil and on vegetation. It has a wide host range over 17 arthropod orders which includes spiders (Shrestha *et al.* 2019; Zimmermann 2007), although infections have been seldomly documented (Meyling & Eilenberg 2007). In Hawai'i, *B. bassiana* is used as a component of integrated pest management strategies in coffee plantations to control the coffee berry borer (*Hypothenemus hampei*) first detected in Hawai'i in 2014 (Greco *et al.* 2018; Hollingsworth *et al.* 2020). Multiple indigenous strains of *B. bassiana* are now found in Hawai'i and detected in crops where there were no previous mycoinsecticide treatment (Castrillo *et al.* 2020; Hollingsworth *et al.* 2011).

While we cannot comment on the particular strain or on the arthropod host with which *Beauveria* was carried, its prevalence, along with presence of other parasites, demonstrates a higher parasitic load found in the arthropods from ginger sites. Because entomopathogenic strains such as *Beauveria* and *Ophiocordyceps*

are known to infect spiders, interaction with infected prey may then increase the risk of infection of the spider itself, unlike prey infected by hymenopteran parasitoids. Additionally, parasitism can impart indirect effects by altering prey density or prey behavior. The higher detection of parasites, then, supports that ginger-invaded habitat may produce suboptimal habitat for native arthropods by introducing new biotic interactions with potentially harmful taxa.

Conclusion

The combination of major dietary shifts driven by non-native taxa and the high prevalence of parasite reads from spiders in ginger sites, gives support to the idea that heavily invaded habitat could function as a sink, rather than a source, environment for endemic taxa. Because spider diets tend to reflect not only the diversity of insect prey in a given habitat but also the choice of the spider (Cuff *et al.* 2021), the spiders are showing not only how the arthropod composition changes with ginger invasion, but also how spiders are responding to environmental shifts. The high density of non-native taxa and the increases in both parasitoid wasps and entomopathogenic fungi clearly demonstrates that the sites modified by plant invasion are associated with a transformation of the arthropod community. The importance of this work is in highlighting how entire communities, and the associated interactions, are modified by a single invasive species. These introduced species do not function as reservoirs – or “safe havens” – for native taxa. Rather, they may act as “sinks”, drawing native taxa into the transformed environment lacking native prey and exposing species to higher levels of parasitism. Cascading effects of ecosystem alteration and the restructuring of biotic interactions may contribute to extinction debt in invaded systems, where the full consequences of invasion do not become evident for many years (Kuussaari *et al.* 2009).

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Data Accessibility and Benefit-Sharing

The data and code used to perform analyses are publicly available on GitHub at [ajholmqu/pagiopalusinger](https://github.com/ajholmqu/pagiopalusinger). This includes (a) FASTA files containing all sequences, (b) CSV files with associated metadata, and (c) reproducible code to generate analyses. Benefits from this research include accessible data and code as outlined above.

Author Contributions

S.A.A. and R.G.G. designed the study. S.A.A. collected and processed samples. A.J.H. performed bioinformatic procedures and statistical analyses. A.J.H. and R.G.G. wrote the manuscript with input from S.A.A.

	Ginger sites - mean values	Native sites - mean values	P-value
ASV			
q = 0	2.88	2.10	1.45×10^{-3}
q = 1	2.52	1.87	1.12×10^{-3}
q = 2	2.31	1.73	1.04×10^{-3}
Family			
q = 0	1.81	1.26	1.57×10^{-5}
q = 1	1.66	1.19	1.14×10^{-5}
q = 2	1.59	1.15	9.42×10^{-6}
Order			
q = 0	1.85	1.41	3.96×10^{-4}
q = 1	1.67	1.33	9.49×10^{-4}
q = 2	1.58	1.28	1.46×10^{-3}

Table 1. Hill numbers (q = 0, 1, 2) using ASV, family or order diversity. Values were significantly different at all values and taxonomic levels.

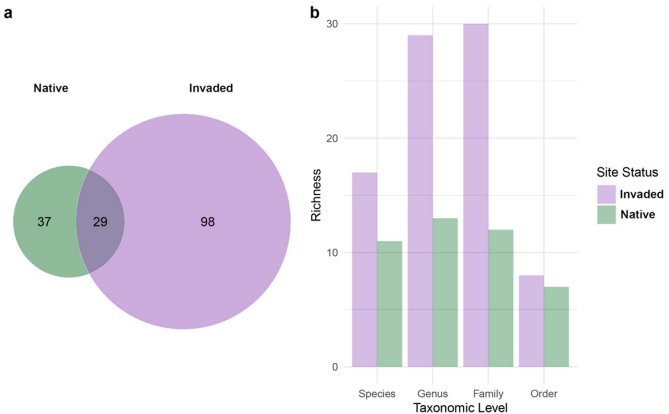


Figure 1. ASV and taxonomic richness between dietary communities in ginger sites (purple) and native forest sites (green). Diets of spiders in ginger sites had more ASVs (a) and additionally had higher taxonomic richness when examining species, genus and family (b).

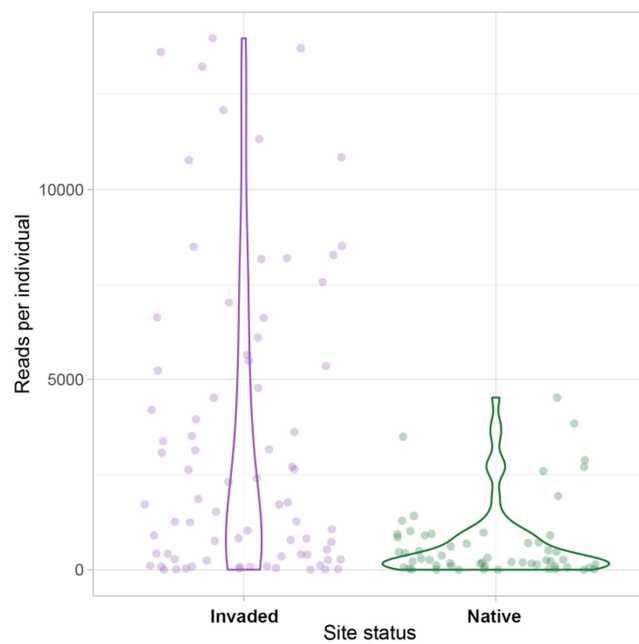


Figure 2. Number of prey reads in individual spiders across ginger sites (purple) and native forest sites (green). Spiders from ginger sites had significantly higher prey read counts as well as higher variation in prey reads.

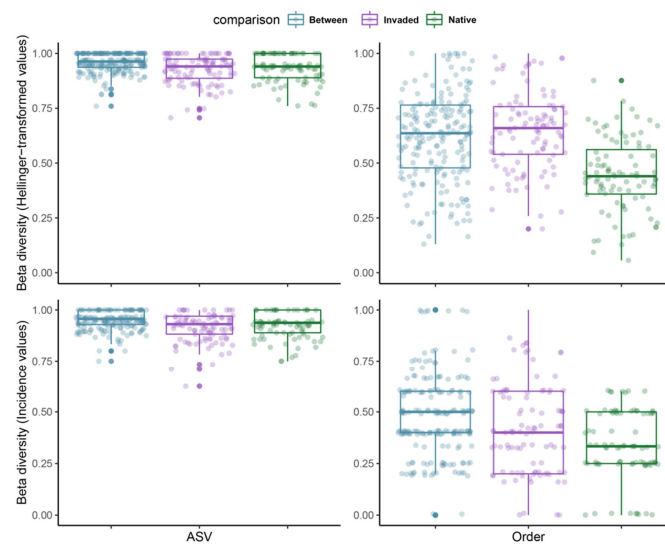


Figure 3. Beta diversity values generated by comparing the diets of spiders between ginger sites and native forest sites (blue), the diets of spiders within ginger sites (purple) and the diets of spiders within native forest sites (green). Using ASVs, median beta diversity values across all comparisons were above 0.9, indicating high dissimilarity. When using order-level diversity, the diets of spiders within native forests were most similar to one another. The diets of spiders between ginger sites and native forest sites as well as the diets of spiders within ginger sites show greater dissimilarity.

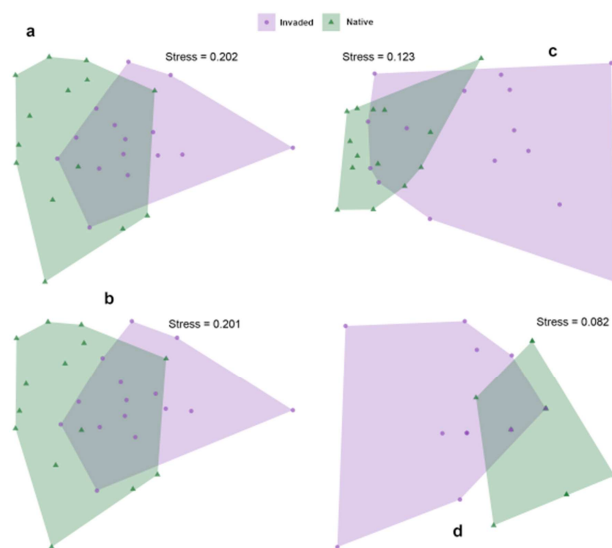


Figure 4. NMDS plots at each site using ASV and order-level community data, based on Hellinger-transformed read abundance and incidence values. More ordination space overlaps when using ASV-level community data (a, b). Order-level community plots overlap less (c, d) and particularly when using incidence values (d).

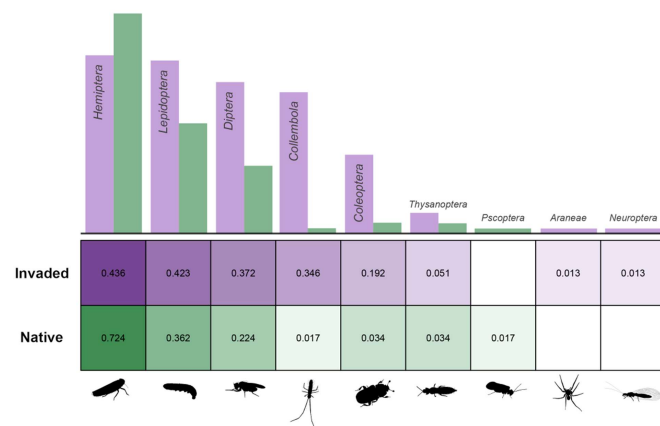


Figure 5. Proportion of spiders (shown in grid) and number of spiders (shown in bars) eating each order of arthropod prey in ginger sites (purple) and native forest sites (green). Hemiptera was the dominant prey order in spiders from native forest, followed by Lepidoptera. The diets of spiders in ginger sites were less consistent, with diets consisting of Hemiptera, Lepidoptera, Diptera and Collembola. Collembola was detected in many more spiders from ginger sites than in spiders from native forests.

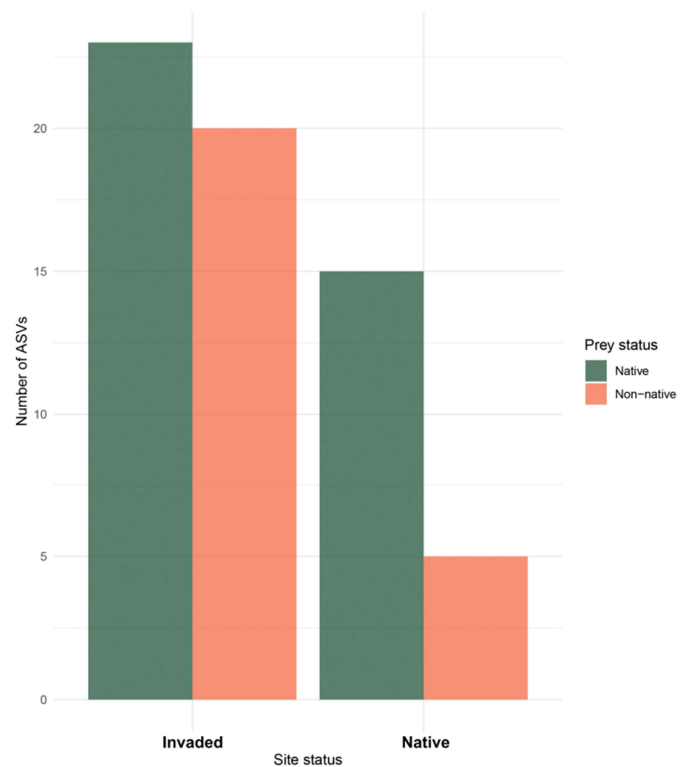


Figure 6. Number of ASVs detected in the dietary communities in ginger sites (left) and native sites (right) that were identified as native species or non-native species. More non-native ASVs were detected in ginger sites than in native forest sites.

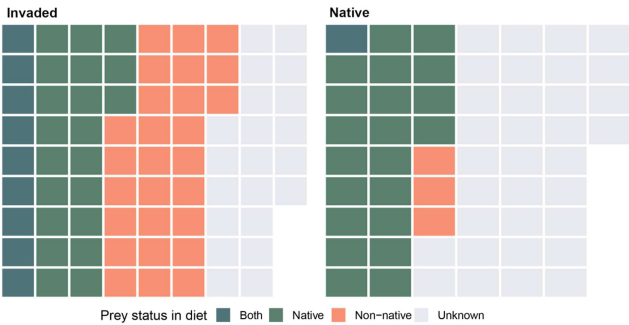


Figure 7. Dietary composition of each spider (represented by a single square) and whether prey in diet was entirely native, entirely non-native, or mixed in origins. More spiders were eating an entirely non-native diet or a mixed diet in ginger sites. More spiders in native forest were consuming prey that was not identifiable.

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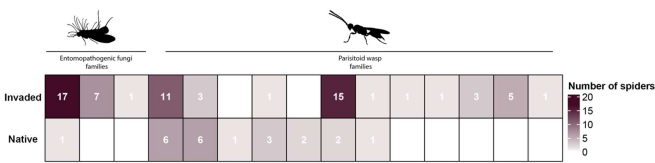


Figure 8. Number of spiders with parasite reads, where each cell represents a different family of Fungi or Hymenoptera. More spiders in ginger sites produced parasite reads for both Fungi and for Hymenoptera parasites.