INVESTIGATING FUNGI AS MODULATORS OF MOSQUITO LARVAL GROWTH AND DEVELOPMENT

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For MY GES COHORT FOR SUPPORTING ME ALONG THE WAY.

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ABSTRACT

As vectors of many arboviruses and diseases, mosquitoes greatly affect human and animal health. Mosquitoes' growth and development are influenced by their symbiotic microbes. Understanding the effects of these symbionts on mosquito growth and development, as well as mosquito microbiome dynamics as a whole, can inform studies and programs aimed at controlling mosquito populations. Here, I investigated the fungal communities present in *Aedes albopictus* mosquito larvae and their water environments. Microbe-free, lab-reared mosquitoes were given microbiota treatments from wild mosquito-associated or environmental sources, or both, along with one of two diet types (a lab diet or a more natural bamboo diet). I collected samples of the microbiota inocula, as well as water and late-stage mosquito larvae 5-8 days after inoculation, for DNA sequencing of the 18S SSU region, a molecular marker of eukaryotic communities. The remaining treated larvae were allowed to pupate for 40 days, and the total pupal biomass was collected. Consistent in water and larvae samples, fungal communities were more diverse in the bamboo diet than the lab diet, but community compositions differed by both diet and microbiota sources. No significant correlation between water or larvae fungal diversity and mosquito pupae biomass was found, indicating variation in biomass across treatments may be better explained by fungal community compositions or other unexamined microorganisms (e.g. bacteria). These findings highlight the importance of nutritional context when investigating the ecology of mosquito-fungi interactions.

Keywords: Mosquito, Fungi, *Aedes albopictus*, DNA sequencing

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1.0 INTRODUCTION

1.1 BACKGROUND

The fungal kingdom is highly diverse and plays critical roles in global ecosystems. Fungi can have various lifestyles in an ecosystem, ranging from parasitic or pathogenic to saprotrophic or mutualistic (Dube, 2013). As terrestrial decomposers, fungi transform nutrients and cycle them back into soil for other organisms to use (Talbot et al., 2008). As mycorrhizal mutualists, they help plants absorb water and nutrients from the soil, improving plant growth (Begum et al., 2019). Fungal fruiting bodies can also provide a food source for various organisms (Feeney et al., 2014; Inga, 2007). Although we are most familiar with larger mushrooms with fruiting bodies, the microscopic world of fungi is even more diverse and abundant (Debeljak and Baltar, 2023). Fungi can also parasitize organisms and cause various plant diseases that ultimately result in plant death (Fondevilla and Rubiales, 2011). Similarly, some species of fungi infect insects (entomopathogenic fungi), including various species of mosquitoes. Yet interactions between fungi and mosquitoes are not well characterized.

Due to anthropogenic impacts, mosquitoes have invaded ecosystems worldwide and are one of the most widespread vectors for deadly arboviruses and diseases (Gratz, 2004). To combat this global issue, many mosquito population control methods have been studied, including insecticides, genetic modifications, and natural ecosystem interactions (Chaudhry et al., 2019). Nevertheless, they each have their limitations, such as insect resistivity, high costs, and uncertain resulting ecosystem behaviors (Chaudhry et al., 2019). Research on microorganisms has been instrumental in such efforts, for instance bacteria *Bacillus thuringiensis israelensis* is a widely commercially available insecticide and the bacterial endosymbiont Wolbachia underlies the sterile insect technique (Ben-Dov, 2014; Yen and Barr, 1971).

The early development in aquatic habitats is a critical period during which microorganisms affect the proportion and rates of mosquitoes that reach adulthood. Mosquitoes lay their eggs in small bodies of water, where the eggs will hatch, and remain as larvae until pupation and subsequently adulthood. These water environments are where mosquito larvae can obtain beneficial bacterial and fungal symbionts to help them grow or encounter harmful species of microbes that can inhibit their development (Dube, 2013, ch 3 pg 32).

1.2 MOSQUITO-FUNGI INTERACTIONS

Recent research has found that the fungus *Purpureocillium lilacinum* is promising in disrupting the development of malaria and affecting transmission to adult mosquito *Aedes aegypti* (Niu et al., 2024), demonstrating how fungi can also potentially reduce the effectiveness of mosquitoes as vectors for harmful diseases. Conversely, fungi can break down complex substrates containing lignin and cellulose, which could provide nutrients for growing mosquito larvae (Baldrian and Valášková, 2008; Hammel, 1997). Learning more about which fungal taxa are present in the mosquito-associated mycobiome can create a better understanding of which may promote or inhibit mosquito success. This knowledge can then be applied to mosquito control operations to minimize the spread of deadly diseases.

Although many studies have examined the importance of bacteria, it is unclear whether fungal symbionts have a definitive role in mosquito development (Tawidian et al., 2019). However, some studies have identified that interactions between fungi and mosquito larvae can be important. For example, a species of yeast (*Saccharomyces cerevisiae*) has been found to induce hypoxia within the mosquito larval gut, an essential step in development (Valzania et al., 2018). This indicates that the presence of some fungi can be beneficial for the development of mosquitoes, but others can have negative impacts. Entomopathogenic fungi, such as the species belonging to the genera *Beauveria, Lagenidium, Coelomomyces, Entomophthora, Culicinomyces*, and Metarhizium can inhibit growth and yield lower survival rates of larvae (Scholte et al., 2004; Tawidian, 2023). More research is needed to better understand fungi-mosquito interactions, including how they may change under different ecological contexts.

1.3 STUDY OVERVIEW

In this study, I aimed to determine if fungal communities associated with mosquito larvae had an effect on the larval growth and development in order to further inform mosquito control operations. The mosquito I focus on, *Aedes albopictus*, is a vector for various diseases such as dengue and chikungunya viruses (Gratz, 2004) and is locally invasive in the Hawaiian Islands. Microbe-free *Ae. albopictus* larvae were provided microbiota from environmental, mosquito-associated, or both sources. To account for potential influence of diet on mosquito fitness and microbiome development, the microbiome treatments were crossed with one of two diets, where one was more standard for lab-rearing and one more natural. I used DNA sequencing to compare mosquito larvae-associated fungal diversity under these conditions and examine their correlations with mosquito fitness, as measured by the weight of pupae. I hypothesized that fungal diversity would have a negative correlation with the growth and development of mosquitoes due to higher chance of encountering the harmful entomopathogenic species, and that different microbiota treatments and diet types will affect the overall fungal community compositions. These results can help us more deeply understand putative connections between fungi and the health of mosquitoes.

2.0 METHODS

2.1 EXPERIMENTAL DESIGN

In 250 mL experimental mesocosms filled with 100 mL of sterile water, lab-reared, axenic *Ae. albopictus* mosquitoes were given one of three microbial treatments. Two of these treatments were natural sources of microbes collected from Lyon Arboretum (Mānoa, HI, USA): (1) environmental microbes from bromeliad *Neoregelia carolinae* pools (~30 mL water mixed from 10 plants) that lacked *Ae. albopictus* but contained other mosquito species or (2) mosquito-associated microbes collected from *Ae. albopictus* larvae (n = 58) residing in bamboo tree hole cavities, trunks from fallen bamboo trees that fill with rainwater. A third treatment received both of these natural microbe pools. To make the mosquito-associated microbiome inoculum, collected *Ae. albopictus* larvae were first sterilized to remove surface microbes (with a 5 min incubation in 500 µL of 70% ethanol and two washes of 500 µL of 1X phosphate-buffered saline (PBS)), homogenized with an autoclaved pestle, and diluted in ~30 mL of sterile water to match the collected volume of bromeliad water. To make the combined environmental and mosquito-associated microbiome inoculum, bromeliad water and mosquito larvae homogenate were passed through a 10 µm filter-capped syringe. Each experimental mesocosm's inoculum contained the microbiota sources (environmental or mosquito-associated or both) that were either live or heat-killed (via autoclaving) to control for inert materials across treatments (Table 1). Each inoculum was 300 µL total of microbiota sources (1:4 environmental:mosquito based on comparisons of cell density under a light microscope with Erythrosine B cell stain) that were pipetted into the mesocosms.

All microbiota treatments were crossed with two autoclave-sterilized diets: a standard lab-rearing diet of 1:1 beef liver powder:powdered yeast extract (lab diet), or a diet of ground bamboo leaves, mimicking detritus in bamboo tree hole cavities (bamboo powder diet). These six microbe by diet combinations were each replicated five times for a total of 30 mesocosms. Twenty-five 1-2 day old L1 larvae, hatched from surface-sterilized eggs from a lab-reared colony (Kriefall et al., 2024), were placed into each mesocosm (n = 750 larvae total). For indicators of mosquito fitness, pupae were collected daily, dried in a drying oven at 50˚C for 11 days, and their individual weights were recorded. The experiment was terminated on day 40.

Table 1. Experimental mesocosm inoculation contents: live (+) or heat-killed (-) microbes from either environmental or mosquito-associated sources.

	Environmental microbes treatment	**Mosquito-associated microbes treatment**	**Combined environmental and mosquito-associated microbes treatment**
Environmental microbes	+	-	+
Mosquito-associated microbes	-	+	+

2.2 SAMPLE COLLECTION AND SEQUENCING

A volume of 500 µL of each of the stock solutions of environmental microorganisms and mosquito-associated microorganisms were collected on the day the experiment began to characterize the microbial communities in the inocula (n = 2). On experimental day five, 500 µL of mesocosm liquid (containing water and microbial treatments) were collected from each mesocosm (n = 30) to characterize microbial communities prior to pupation as mosquitoes tend to purge microbiota during metamorphosis (Moll et al., 2001). On the first day of pupation (experimental day eight), five larvae per mesocosm were pooled to characterize late-stage larval communities (n = 30 pools total).

DNA from all aforementioned samples and six additional negative controls with only kit reagents (n = 68 total) were extracted with the Macherey-Nagel NucleoMag Tissue kit following manufacturer instructions and with a Kingfisher Flex. The DNA extracts were sent to the Microbial Genomics and Analytical Laboratory (MGAL) at University of Hawaiʻi (UH) at Mānoa (HI, USA) for library preparation. There, Polymerase Chain Reaction (PCR) amplification of the fungal SSU rRNA gene was conducted using the following primers: 18S-82F: 5′-GAAACTGCGAATGGCTC-3′ (Lopez-Garcia et al., 2003and Euk-516R: 5′-ACCAGACTTGCCCTCC-3′ (. Each PCR mix contained 6.3 µL of molecular-grade water, 12.5 µL of 2X KAPA 3G Buffer, 1.5 µL $MgCl_2$ (25 mM), 0.2 µL KAPA 3G Taq, 0.75 µL of the forward primer (10 µM), 0.75 µL of the reverse primer (10 µM), and 3 µL of DNA template (total reaction volume of 25 µL). The reactions were held for 3 minutes at 95 ˚C before cycling 35 times through 20 seconds at 95 ˚C, 15 seconds at 55 ˚C, and 30 seconds at 72 ˚C, and a final hold for 3 minutes at 72 ˚C. Three additional PCR positives (Zymo) and PCR negatives (only kit reagents) were also generated during these steps. Amplicons were barcoded using a NEXTERA kit (Illumina Inc., CA, USA). Libraries were then submitted to the Advanced Studies in Genomics, Proteomics and Bioinformatics (ASGPB) at UH Mānoa (HI, USA) for 2 x 300 paired-end sequencing on a MiSeq (Illumina).

2.3 BIOINFORMATICS AND STATISTICAL ANALYSES

I analyzed the sequenced data, including quality control and clustering of reads into amplicon sequence variants (ASVs), using the QIIME2 pipeline (Bolyen et al., 2019). For taxonomic assignment, I used the SILVA version 138 non-redundant (99% identity criterion) reference database (Quast et al., 3013) reformatted for QIIME2 with RESCRIPt (Robeson et al., 2021). All further processing took place in R (v.4.4.1; R Core Team, 2024) and R studio (v4.4.1; Posit Team, 2024) unless otherwise noted. The package decontam (Davis et al., 2017) removed putative contaminant ASVs (n = 8 removed). Non-eukaryotic phyla were removed from further analyses and undetermined Eukarya phyla were cross-referenced with the NCBI BLAST database to ensure all the fungal phyla were included. The data were then analyzed with the phyloseq package (McMurdie and Holmes, 2013) to visualize and compare alpha diversity metrics (Shannon's index) across samples and treatments. Shannon's index was statistically compared across diet × microbial treatment combinations with a generalized linear model using package stats (R Core Team, 2024), with a Gaussian distribution. *Post hoc* analyses were conducted using the emmeans package (Lenth 2025). I also correlated the Shannon indices of the microbial communities in water and mosquito samples with mosquito pupal biomass (a proxy for fitness). For these correlations, I used generalized linear mixed models from the glmmTMB package (Brooks et al., 2017) and examined whether there was significant diet $\times$ diversity interactive effect on mosquito pupal biomass, with microbial treatment as a random effect. Nested models with and without terms of interest assessed statistical inference using log-likelihood ratio tests assuming a χ^2-distribution. To compare overall community compositions, principal coordinate analysis (PCoA) was performed within phyloseq using the Bray-Curtis dissimilarity metric, while a PERMANOVA (adonis2 in the vegan package; Oksanen et al., 2024) assessed statistical significance. Relative abundances of fungal taxa at the order and genus levels were characterized in these mosquito and water samples.

3.0 RESULTS

3.1 MOSQUITO PUPAL BIOMASS DIFFERED BY EXPERIMENTAL TREATMENTS

Total pupal biomass per mesocosm was influenced by interactions between diet and microbiota treatment ($P < 0.05$; Figure 1). Specifically, the lab diet (larval food) yielded higher pupal biomass than the bamboo powder diet overall, except for the combined environmental + mosquito microbial treatment which produced approximately equivalent biomass to the bamboo diet groups (Figure 1). Within the bamboo powder diet, none of the microbiota treatments were distinguishable from each other with *post hoc* comparisons (Figure 1). Within the lab diet, biomass accumulated from the mosquito-associated microbiota treatment did not differ from the environmental microbiota treatment, but it was statistically significant and higher than the combination environmental + mosquito-associated microbiota treatment (Figure 1). Biomass from the environmental microbiota treatment did not differ from the combination treatment within the lab diet (Figure 1).

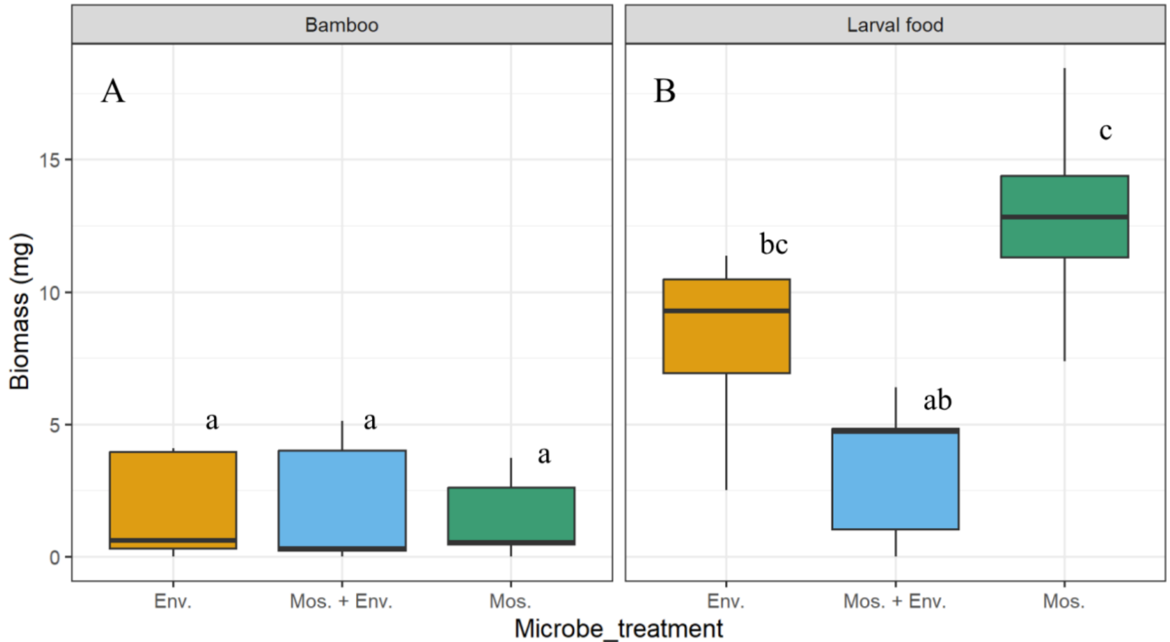

Figure 1. Box plots showing the total mosquito pupae biomass per mesocosm (mg) for (A) bamboo powder diet and (B) larval food (lab diet) across all the microbiota treatments. "Env." (colored in orange) has environmental microbes only, "Mos. + Env." (colored in blue) has both mosquito and environmental microbes, and "Mos." (colored in green) has mosquito microbes only. The black lines in the boxes indicate the median, and the colored boxes indicate the interquartile range from the 25th percentile (beneath the median) to the 75th percentile (above the median) for n = 5 mesocosms per diet by microbial treatment combination. The whiskers show the range of data excluding outliers. No outliers are present in this plot. Significant differences between the microbiota treatments are indicated by different lowercase letters.

3.2 FUNGAL TAXA IN MICROBIAL STOCKS, WATER, AND LARVAE SAMPLES

The environmental microbiota stock sampled on experimental day zero had a higher fungal diversity than the mosquito-associated microbiota stock (Figure 2). The genus *Saturnispora* was prevalent in the environmental microbiota stock, but not in the mosquito-associated microbiota stock (Figure 2). In the mosquito-associated microbiota stock, the most prevalent genus was *Malassezia* (which was also found in the environmental microbiota stock) (Figure 2). About half of each of the microbiota stocks were made up of ASVs that did not fall into the four most abundant ASV orders, as indicated by the grey-scale colors (Figure 2).

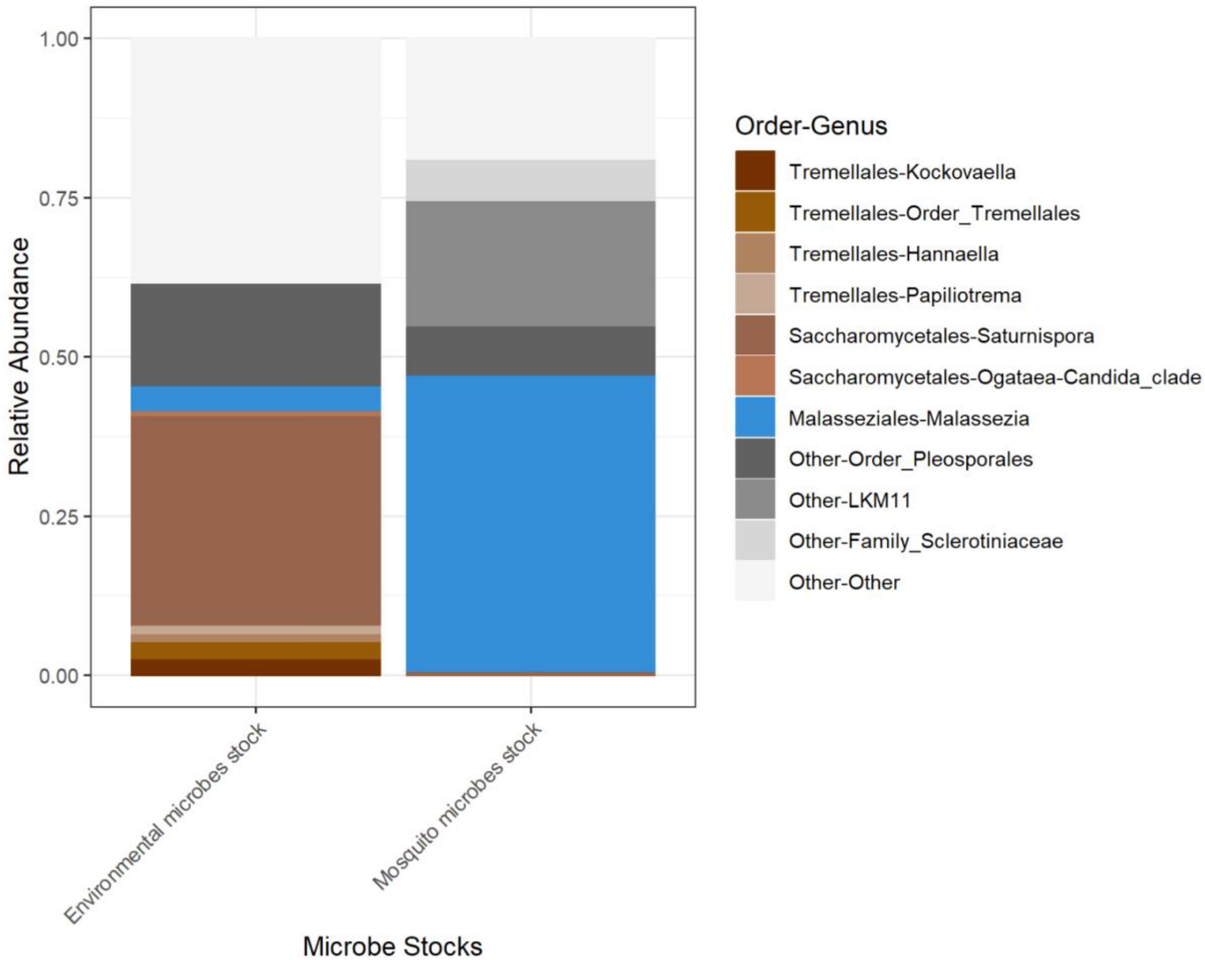

Figure 2. Bar plots showing the relative abundance of fungal orders and genera in the two microbiota stocks given to mesocosms on experimental day zero. Different colors indicate different genera (or the next lowest taxonomic level identified), with the colored scales indicating the four most abundant genera within the four most abundant orders, and the grayscale indicating other taxa that did not fall under the most abundant orders.

Malasseziales and *Saccharomycetales*, two of the most abundant orders found in the microbiota stocks from day zero, were also found to be the most abundant orders in the experimental samples taken on days five and eight (Figures 2 and 3). Both of these orders were found in all diet × microbiota treatment samples, with the relative abundances largely corresponding between larvae and water samples (Figure 3). Due to lack of fungi, seven larvae samples and two water samples did not amplify during PCR. These samples were left out of the analyses, and our total number of 60 samples (30 larvae and 30 water) was reduced to 51 total samples.

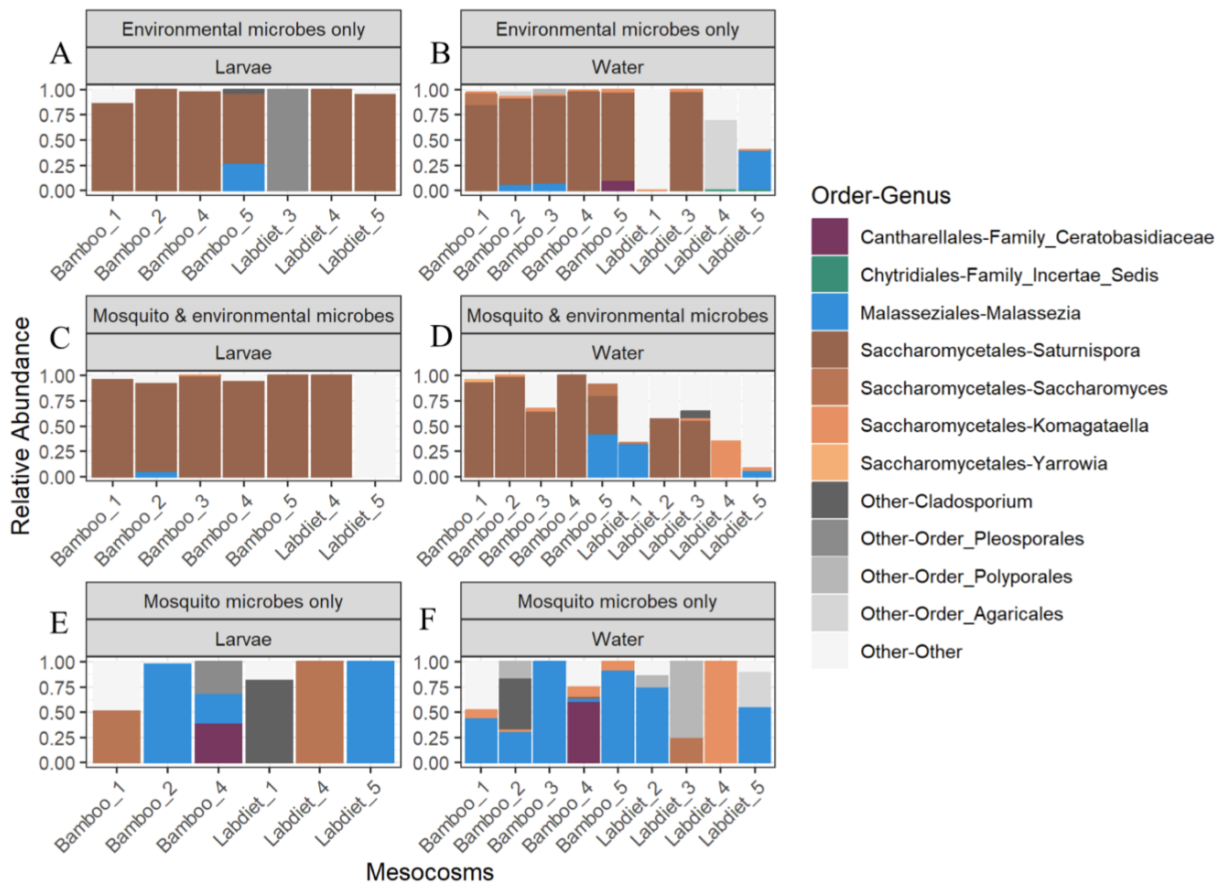

Figure 3. Bar plots showing the relative abundance of fungal orders and genera (or the next lowest classification identified) of (A, C, and E) larvae samples taken on experimental day eight, and (B, D, and F) mesocosm liquid (water) samples taken on experimental day five. Each microbiota treatment is displayed in separate rows. The same colors represent different fungal orders as in Figure 2 and are indicated in the legend on the right. While we began with 5 replicates per diet-microbiota combination, some samples failed to amplify during sequencing library preparation, indicating a lack of fungal DNA.

Common genera that were found in the water and larvae samples across microbiota treatments included *Malassezia* and *Komagataella*. *Saturnispora* was found in both the environmental microbiota samples and the combination of environmental + mosquito-associated microbiota samples, but it was not found (or found at a very low relative abundance) in the samples only given the mosquito-associated microbiota (Figure 3). *Saccharomyces* was found in each water sample across the microbiota treatments, but at a much lower relative abundance than the other three genera listed above. For both the water and larvae samples, the mosquito-associated microbiota treatments had a relatively low abundance of *Saturnispora*, despite the rest of the microbiota treatments having a high relative abundance of this genus (Figure 3). Instead, the mosquito-associated microbiota treatments for water and larvae both had a higher relative abundance of *Malassezia* compared to the other microbiota treatments (Figure 3). The family *Ceratobasidiaceae* was present in three of the samples, two from mosquito-associated microbiota treatments in water and larvae samples, and the third in a water sample from an environmental microbiota treatment.

3.3 FUNGAL DIVERSITY ACROSS TREATMENTS

Shannon diversity analyses showed there was no significant main effect of microbiota treatment or interactive effect of diet and microbiota treatments on the diversity of fungi from mosquito larvae samples ($P = 0.11$ for microbiota treatment, $P = 0.62$ for interaction; Figure 4). However, larvae reared on the bamboo diet had overall higher Shannon diversity than those reared on the lab diet ($P < 0.05$; Figure 4). The fungal Shannon diversity of water samples were marginally affected by the interaction between diet and microbiota treatments ($P = 0.079$; Figure 4), and not by the main effects of microbiota treatments or diet ($P = 0.15$ for microbiota treatment, $P = 0.89$ for diet; Figure 4).

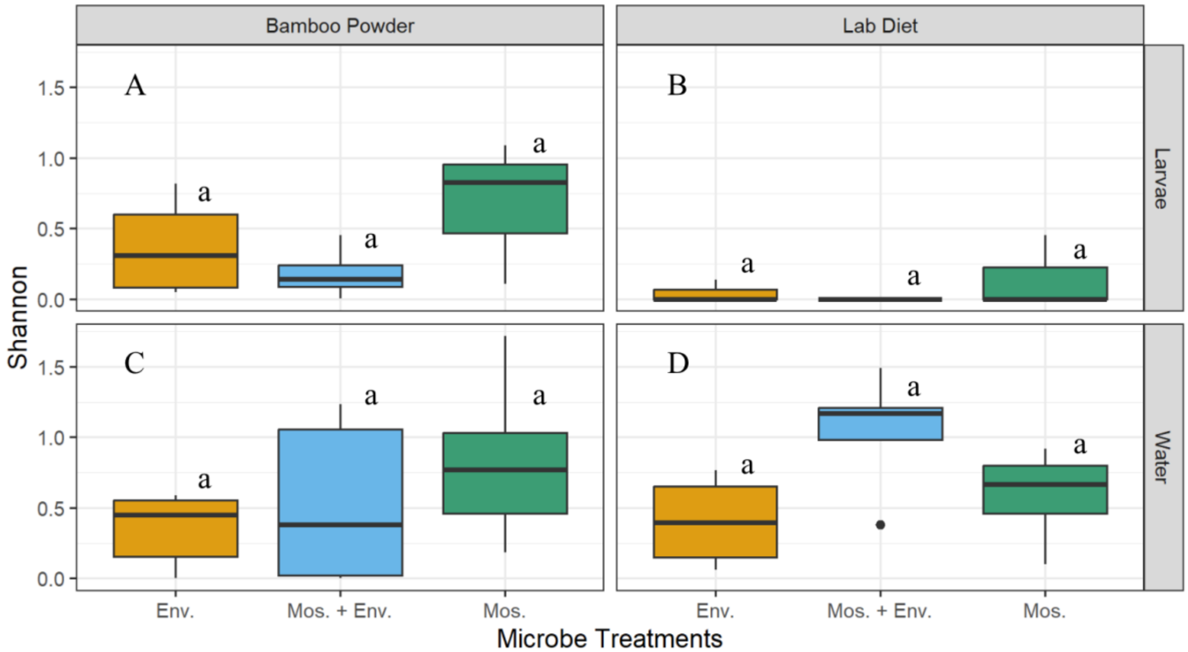

Figure 4. Box plots showing the fungal diversity, based on the Shannon diversity index, across the different microbiota treatments and diet types in mosquito larvae samples (collected on experimental day eight; A and B) and water samples (collected on experimental day five; C and D). Each color corresponds to a different microbiota treatment, orange being environmental microbiota "Env.", blue being mosquito-associated + environmental microbiota "Mos + Env.", and green being only mosquito-associated microbiota "Mos". Outliers are indicated by black dots. There were no statistically significant *post hoc* differences between treatments, as indicated by the letter 'a' above all groups.

3.4 NO CORRELATION BETWEEN SHANNON DIVERSITY AND MOSQUITO BIOMASS

No correlation was found between fungal diversity (Shannon's index) and pupae biomass for both larvae and water samples ($P = 0.21$, $P = 0.12$, respectively; Figure 5). Microbiota treatment included as a random intercept explained almost no variation in biomass (variance = 1.38e-8). The diet type did have a significant influence on biomass, with higher biomass in the lab diet, which corresponds to what we found previously ($P < 0.001$; Figures 1 and 5A). We also examined biomass correlations with the Shannon index within each diet type separately and found that correlations were not significant for either the bamboo diet or the lab diet ($P = 0.21$, $P = 0.45$, respectively; Figure 5A). Similar results were found for the water samples, as diversity did not correlate with biomass within each diet type individually ($P = 0.87$ for bamboo diet, $P = 0.25$ for larval food; Figure 5B).

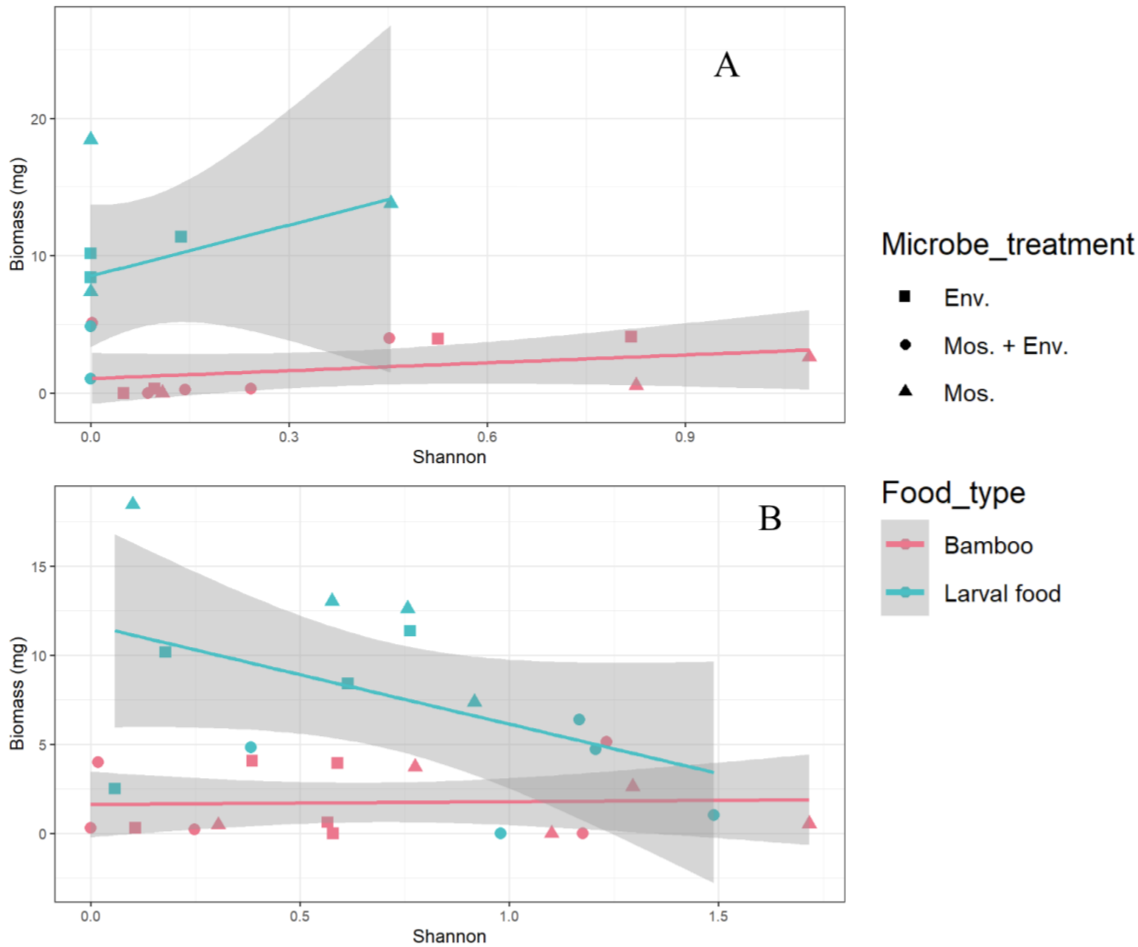

Figure 5. Scatter plots showing fungal Shannon's diversity and pupae biomass summed per mesocosm (mg). A shows Shannon's diversity of fungi associated with larvae, and B shows Shannon's diversity of fungi in the water samples. Shapes (microbiota treatments) and colors (diet) for both A and B are according to the legend on the right. Regression lines show the linear trends between Shannon's diversity and mosquito pupae biomass, with shaded regions around the lines representing the 95% confidence intervals.

3.5 COMMUNITY COMPOSITIONS DIFFER BY EXPERIMENTAL TREATMENTS

The interaction between microbiota treatments and diet had a significant impact on fungal community composition ($P < 0.05$, $R^2 = 0.056$; Figure 6). Sample type, meaning either larvae or water, did not have a significant impact on microbial community composition ($P = 0.60$; Figure 6). Together, these factors explained approximately 29.7% of the variation in community composition, while the remaining 70.3% was unexplained.

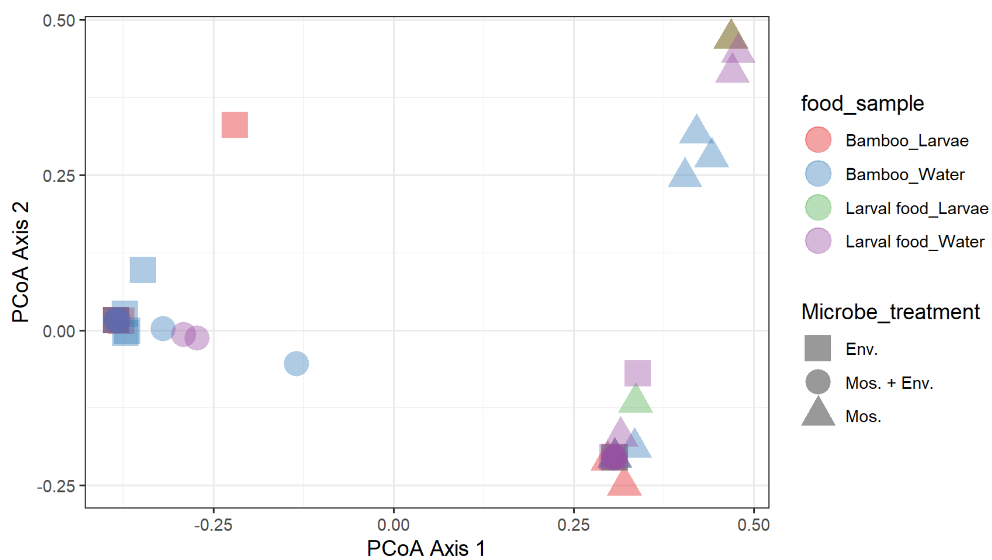

Figure 6. Principal Coordinates Analysis (PCoA) plot to visualize Bray-Curtis dissimilarities between samples. Samples are colored by food type (bamboo vs. larval food) crossed with sample type (larvae vs. water) and shaped by microbiota treatment (environmental microbiota, mosquito-associated microbiota, and mosquito-associated + environmental microbiota). It is important to note that many points with highly similar communities may be overlapping.

4.0 DISCUSSION

4.1 SUMMARY

The focus of this study was to further understand mosquito-fungi interactions. The data showed an overall variability in fungal taxa among microbiota treatments (Figures 2, 3). Fungal genera detected in both mosquito larvae and water samples after five to eight days largely corresponded to the microbiota stocks given to the mesocosms initially (Figures 2, 3). Similar to other research (Luis et al., 2019), the dominant orders found in *Ae. albopictus* mosquito larvae were *Saccharomyces* and *Malasseziales*. Fungal diversity did not statistically correlate with mosquito fitness, as measured by pupal biomass (Figure 5), and community compositions were largely impacted by the interaction between diet type and microbiota treatments (Figure 6).

4.2 MOSQUITO PUPAL BIOMASS DIFFERED BY EXPERIMENTAL TREATMENTS

The interaction between diet and microbiota treatments impacted mosquito pupal biomass, largely due to overall higher biomass found with the lab diet compared to the bamboo diet, and variation in biomass among microbiota treatments within the lab diet (Figure 1). Diet plays an important role in nutritional health in mosquito larvae, which in turn sets up the individual for later growth as pupa and adult (Wilkerson et al., 2021). Various studies have also shown how microbiota communities in mosquito larvae guts are necessary for development (Coon et al., 2014). As such, it is not surprising that both diet and microbiota influenced pupal biomass. Here, the lab diet, which was easier for the mosquito larvae to digest and contained high nutritional value, resulted in the two single-sourced microbiota treatments each producing significantly higher pupal biomass when compared to the bamboo powder diet. Sourced from either conspecific mosquito larvae collected from bamboo cavities or bromeliad pools in which heterospecific mosquito larvae lived, both mosquito-associated and environmental microbial treatments offered microbiomes where members co-exist and have adapted to mosquito-related environments (Seabourn et al., 2023). These microbial communities are likely stable in their adapted mosquito-related environments because of well-established niche partition and/or synergistic interactions among themselves, or between them and host mosquito (Seabourn et al., 2023). This could explain why these two microbiota treatments yielded the highest host mosquito pupal biomass when a nutritious lab diet was provided. In contrast, a microbiome with combined sources where members did not go through natural adaptation together showed less benefit to the host even with ample nutrients. One possible explanation could be the introduction of competition between microbes, or between the microbes and the larvae, which could negatively affect the growth of the mosquitoes (Lucas-Barbosa et al., 2023). Pupal biomass was consistently low across microbiota treatments in the bamboo diet, likely due to the difficulty of accessing the nutrition in the plant leaves (Martinson et al., 2021). This finding signifies the importance of nutritional context of microbiome influence on mosquito development.

4.3 FUNGAL TAXA IN MICROBIAL STOCKS, WATER, AND LARVAE SAMPLES

The environmental microbiota stock was found to have the highest fungal diversity. This is likely due to bromeliad water providing more niches for diverse fungal taxa than the narrower niche inside the mosquito larvae, consistent with what other studies have found (Caragata et al., 2021; Tawidian et al., 2021). Similar fungal genera were found within the microbiota stocks, water samples, and larvae samples. This is consistent with other research findings that mosquito microbiomes are largely influenced by host-environment and host species microbial communities (Tawidian et al., 2021; Hegde et al., 2024). A high relative abundance of yeasts such as *Saccharomyces* and *Komagataella* in both the water and larvae samples can be explained by associations between yeasts, tank bromeliads, and mosquitoes (Lúcia dos Santos et al., 2023; Malassigné et al., 2020).

Saccharomyces cerevisiae was the yeast species in the lab diet provided to half of the mesocosms. Because it was present in the diet, we expected to find the genus *Saccharomyces* in all the samples fed the lab diet. However, *Saccharomyces* was only detected in three of the lab diet samples (two water samples and one larvae sample). It is possible that in these mesocosms that *Saccharomyces* was not detected, at the time of sampling, the food was already broken down by the mosquitoes, resulting in relatively low abundance of *Saccharomyces* that failed to be picked up by sequencing. This indicates that *Saccharomyces* from the lab diet can fail to establish in the mesocosm environment. Additionally, *Saccharomyces* was found in mesocosms fed the bamboo powder diet, which could be because these yeasts are commonly found in bamboo environments (Lúcia dos Santos et al., 2023).

Malassezia has been found in association with mosquitoes in previous studies (Hedge et al., 2024) and in a wide variety of other habitats (Amend, 2014). In our experiment, its high abundance and presence throughout all types of samples and treatments still lack an explanation. Since *Malassezia* is commonly found on human skin and scalps (Ianiri et al., 2022), we cannot rule out the possibility that it was introduced from human sources during field sampling, stock preparation, mosquito rearing, sampling, or molecular analysis, although standard laboratory personal protective equipment (e.g. gloves, sterile materials) were used to protect the samples from human contact.

4.4 FUNGAL DIVERSITY ACROSS DIET AND MICROBIOTA TREATMENTS

In this study, larvae reared with the bamboo diet had a significantly higher fungal diversity (Figure 4) compared to those fed a lab diet. The bamboo diet likely contained a greater diversity of complex substrates, such as lignin and cellulose, which may preferentially select for some groups of fungi capable of utilizing them (Baldrian and Valášková, 2008; Hammel, 1997). The bamboo powder diet mimics a more natural diet that mosquitoes might encounter in the wild, which could have yielded a higher fungal diversity because it more closely resembles the environment in which fungal communities can thrive (Brown, 2005).

4.5 NO CORRELATION BETWEEN FUNGAL DIVERSITY AND MOSQUITO BIOMASS

Contrary to expectations, there was no statistically significant correlation between mosquito pupal biomass and the diversity of fungi in both larvae and water samples (Figure 5). The exact reason for this lack of correlation is unknown, but when these community compositions are compared to other studies, key fungal species that might have had negative impacts on mosquitoes appear absent from our study. Species like *Beauveria bassiana* and *Metarhizium anisopliae* have been found to reduce *Ae. albopictus* and *Ae. aegypti* larval and pupal survival, or delay development (Tawidian, 2023; Scholte, 2007; Accoti et al., 2021). My study did not find *B. bassiana* or *M. anisopliae* in either the larvae or water samples, which along with other entomopathogenic species, might have caused a negative correlation, had they been present.

The lack of correlation between fungal diversity and mosquito pupal biomass in this study could also be uncaptured influence of other microbial groups associated with mosquito microbiome. Bacterial communities present in mosquito larvae (*Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus*) can have positive effects on mosquito fitness through adulthood and in some cases have been found to be essential for mosquito development (Giraud et al., 2021; Coon et al., 2016). Since bacterial communities were not analyzed in this study, whether their influence on mosquito fitness is stronger than those from fungal communities warrants attention in future studies.

4.6 COMMUNITY COMPOSITIONS DIFFER BY EXPERIMENTAL TREATMENTS

Multivariate analyses showed significant effects of diet and microbiota treatment on fungal community compositions (Figure 6), consistent with findings from previous studies (Hegde, 2024; Zouache et al., 2022). Similar findings have been shown in related studies which investigated the effects of protein-high and natural diets, as well as microbial inocula on how fast mosquitoes reached adulthood, and their sizes (Santos Souza et al., 2019; Martinson and Strand, 2021). Unlike what has been observed in mosquito bacterial communities (Wang et al., 2018; Dada et al., 2014), sample type (water or larvae) did not have a significant impact on fungal community compositions, likely because mosquito fungal communities are largely adopted from their environment.

5.0 CONCLUSION

This study analyzed the fungal communities present in mosquito *Ae. albopictus* larvae as well as its water environment when given different microbiota sources and diets. Contrary to my initial hypothesis, fungal diversity and mosquito fitness, as measured by pupal biomass, were not correlated in this study. Including simpler communities, for example, excluding bacteria, or even individual fungi in future microbiota treatments (especially entomopathogenic species and yeasts) could help to better understand the roles fungi play in mosquito fitness. Additionally, measuring fitness through other metrics such as speed of pupation and adult reproductive success would also benefit future work. Specifically isolating the fungal communities within mosquito larvae guts could also give better insights into whether fungi add nutritional benefits to the mosquitoes. Furthermore, studying the mosquitoes at multiple life stages would broaden the understanding of fungal community interactions with mosquitoes as a whole. It is important to understand mosquito-microbiota associations to better inform mosquito control operations. Mosquitoes are important vectors for diseases that have the potential to harm native Hawaiian bird populations, as well as humans, which is why novel methods of population control are under investigation.

LITERATURE CITED

Accoti, A., Engdahl, C.S., and Dimopoulos, G. (2021). Discovery of novel entomopathogenic fungi for mosquito-borne disease control. Frontiers in Fungal Biology. 2. https://doi.org/10.3389/ffunb.2021.637234

Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA (1990) Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl Environ Microbiol 56:1919–1925

Amend, A. (2014). From dandruff to deep-sea vents: Malassezia-like fungi are ecologically hyper-diverse. Public Library of Science Pathogens. 10(8). doi: 10.1371/journal.ppat.1004277

Baldrian, P. and Valášková, V. (2008). Degradation of cellulose by basidiomycetous fungi. FEMS Microbiology Reviews. 32(3). 501-521. https://doi.org/10.1111/j.1574-6976.2008.00106.x

Begum, N., Qin, C., Abass Ahanger, M., Raza, S., Ishfaq Khan, M., Ashraf, M., Ahmed, N., and Zhang, L. (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Frontiers in Plant Science. 10. https://doi.org/10.3389/fpls.2019.01068

Ben-Dov, E. (2014). Bacillus thuringiensis subsp. israelensis and its dipteran-specific toxins. Multidisciplinary Digital Publishing Institute. Toxins. 6(4). 1222-1243. https://doi.org/10.3390/toxins6041222

Billingsley Tobias, T. (2023). Freshwater fungal diversity and ecological interactions with mosquitoes and plant communities. Western Illinois University ProQuest Dissertations & Theses. https://www.proquest.com/dissertations-theses/freshwater-fungal-diversity-ecological/docview/2910100826/se-2

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, and Caporaso JG. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37: 852–857. https://doi.org/10.1038/s41587-019-0209-9

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM (2017). "glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling." The R Journal, 9(2), 378–400. doi:10.32614/RJ-2017-066.

Brown, C. (2005). Effects of detritus abundance and diversity on the composition of detritivore communities within tank bromeliads, May 2005. Monteverde Institute: Tropical Ecology and Conservation. 115. https://digitalcommons.usf.edu/tropical_ecology/115

Caragata, E.P., Otero, L.M., Tikhe, C.V., Barrera, R., and Dimopoulos, G. (2022). Microbial diversity of adult Aedes aegypti and water collected from different mosquito aquatic habitats in Puerto Rico. Microbial Ecology. 83. 182-201. https://doi.org/10.1007/s00248-021-01743-6

Coon, K.L., Vogel, K.J., Brown, M.R., and Strand, M.R. (2014). Mosquitoes rely on their gut microbiota for development. Molecular Ecology. 23(11). 2727-2739. https://doi-org.eres.library.manoa.hawaii.edu/10.1111/mec.12771

Dada, N., Jumas-Bilak, E., Manguin, S., Seidu, R., Stenström, T., and Overgaard, H.J. (2014). Comparative assessment of the bacterial communities associated with Aedes aegypti larvae and water from domestic water storage containers. Parasites & Vectors. 7(391). https://doi.org/10.1186/1756-3305-7-391

Davis NM, Proctor D, Holmes SP, Relman DA, Callahan BJ (2017). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. bioRxiv, 221499. doi:10.1101/221499.

Debeljak, P. and Baltar F. (2023). Fungal diversity and community composition across ecosystems. Multidisciplinary Digital Publishing Institute. 9(5). 510. https://doi.org/10.3390/jof9050510

Dos Santos, V.L., Silva, U.C., Santos, E.H., Resende, A.A., Dias, M.F., Cuadros-Orellana, S., and Marques, A.R. (2023). Exploring the mycobiota of bromeliads phytotelmata in Brazilian Campos Rupestres. Braz J Microbiol 54, 1885–1897. https://doi.org/10.1007/s42770-023-00977-5

Dube, H.C. (2013). Fungi - General Characteristics. An introduction to Fungi 4th Revised Edition. (pp.32-56). Scientific Publishers.

Feeney, M.J., Miller A.M., and Roupas P. (2014). Mushrooms-biologically distinct and nutritionally unique exploring a “third food kingdom”. Nutrition Today. 49(6). 301-307. DOI: 10.1097/NT.0000000000000063

Fondevilla, S. and Rubiales D. (2011). Powdery mildew control in pea. A review. Agronomy for Sustainable Development. 32. 401-409. https://doi.org/10.1007/s13593-011-0033-1

Giraud, É., Varet, H., Legendre, R., Sismeiro, O., Aubry, F., Dabo, S., Dickson, L.B., Valiente Moro, C., Lambrechts, L. (2021). Mosquito-bacteria interactions during larval development trigger metabolic changes with carry-over effects on adult fitness. Molecular Ecology. 31(5). 1444-1460. https://doi.org/10.1111/mec.16327

Gratz, N.G. (2004). Critical review of the vector status of *Aedes albopictus.* Medical and Veterinary Entomology. 18(3). 215-227. https://doi.org/10.1111/j.0269-283X.2004.00513.x

Hammel, K.E. (1997). Fungal Degradation of Lignin. CAB INTERNATIONAL Driven by Nature: Plant Litter Quality and Decomposition. 33-45. https://www.fpl.fs.usda.gov/documnts/pdf1997/hamme97a.pdf

Hegde, S., Khanipov, K., Hornett, E.A., Nilyanimit, P., Pimenova, M., Saldaña, M.A., de Bekker, C., Golovko, G., and Hughes G.L. (2024). Interkingdom interactions shape the fungal microbiome of mosquitoes. Animal Microbiome. 6(11). https://doi.org/10.1186/s42523-024-00298-4

Hossain, S., Raihan, E., Hossain, S., Syeed, M., Rashid, H., and Reza, S. (2022). Aedes larva detection using ensemble learning to prevent Dengue endemic. BioMedInformatics. 2(3). 405-423. https://doi.org/10.3390/biomedinformatics2030026

Ianiri, G., LeibundGut-Landmann, S., and Dawson, T.L. (2022). Malassezia: a commensal, pathogen, and mutualist of human and animal skin. Annual Review of Microbiology. 76. 757-782. https://www.annualreviews.org/content/journals/10.1146/annurev-micro-040820-010114

Inga, B. (2007). Reindeer (Rangifer tarandus tarandus) feeding on lichens and mushrooms: traditional ecological knowledge among reindeer-herding Sami in northern Sweden. Rangifer, 27(2), 93–106. https://doi.org/10.7557/2.27.2.163

Kriefall, N.G., Seabourn, P.S., Yoneishi, N.M., Davis, K., Nakayama, K.K., Weber, D.E., Hynson, N.A., and Medeiros, M.C.I. (2024). Abiotic factors shape mosquito microbes that enhance host development. The ISME Journal: Multidisciplinary Journal of Microbial Ecology. 18(1). https://doi.org/10.1093/ismejo/wrae181

Lenth, R. (2025). _emmeans: Estimated Marginal Means, aka Least-Squares Means_.R package version 1.10.7, https://CRAN.R-project.org/package=emmeans.

Lopez-Garcia P, Philippe H, Gail F, Moreira D (2003) Autochthonous eukaryotic diversity in hydrothermal sediment and experimental microcolonizers at the Mid-Atlantic Ridge. Proc Natl Acad Sci U S A 100:697–702

Lucas-Barbosa, D., Balvers, C., Bellantuono, A.J., Castillo, J.S., Costa-da-Silva, A.L., De Moraes, C.M., DeGennaro, M., and Verhulst, N.O. (2023). Competition matters: using in vitro community models to study the impact of human skin bacteria on mosquito attraction. Frontiers in Ecology and Evolution. 11. https://doi.org/10.3389/fevo.2023.1156311

Luis, P., Vallon, L., Tran, F., Hugoni, M., Tran-Van, V., Mavingui, P., Minard, G., and Valiente Moro, C. (2019). *Aedes albopictus* mosquitoes host a locally structured mycobiota with evidence of reduced fungal diversity in invasive populations. Fungal Ecology. 39. 257-266. https://doi.org/10.1016/j.funeco.2019.02.004

Martinson, V.G. and Strand, M.R. (2021). Diet-microbiota interactions alter mosquito development. Frontiers in Microbiology.12. https://doi.org/10.3389/fmicb.2021.650743

McMurdie and Holmes (2013) phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE. 8(4):e61217

Moll, R.M., Romoser, W.S., Modrakowski, M.C., Moncayo, A.C., Lerdthusnee, K. 2001). Meconial peritrophic membranes and the fate of midgut bacteria during mosquito (Diptera: Culicidae) metamorphosis. Journal of Medical Entomology. 38(1). 29-32. https://doi.org/10.1603/0022-2585-38.1.29

Niu G., Wang, X., Gao W., Cui L., and Li J. (2024). Leucinostatins from fungal extracts block malaria transmission to mosquitoes. Parasites & Vectors. 17(401). https://doi.org/10.1186/s13071-024-06450-y

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro, C.E., Smith, T., Stier, A., Ter Braak, C., Weedon, J. (2024). _vegan: Community Ecology Package_.R package version 2.6-8, https://CRAN.R-project.org/package=vegan.

Posit team (2024). RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. URL http://www.posit.co/.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data a processing and web-based tools. DOI: 10.1093/nar/gks1219

R Core Team (2024). R: A Language and Environment for Statistical Computing_.R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Robeson, M.S. II, O'Rourke, D.R., Kaehler, B.D., Ziemski, M., Dillon, M.R., Foster, J.T., Bokulich, N.A. (2021). RESCRIPt: Reproducible sequence taxonomy reference database management. Public Library of Science Computational Biology. 17(11): e1009581. https://doi.org/10.1371/journal.pcbi.1009581

Samuel, M.D., Hobbelen, P.H.F., DeCastro, F., Ahumada, J.A., LaPointe, D.A., Atkinson, C.T., Woodworth, B.L., Hart, P.J., and Duffy, D.C. (2011). The dynamics, transmission, and population impacts of avian malaria in native Hawaiian birds: a modeling approach. Ecological Applications. 21(8). 2960-2973. https://doi-org.eres.library.manoa.hawaii.edu/10.1890/10-1311.1

Santos Souza, R., Virginio, F., Souza Riback, T.I., Suesdek, L., Barufi, J.B., and Genta, F.A. (2019). Microorganism-based larval diets affect mosquito development, size and nutritional reserves in the yellow fever mosquito Aedes aegypti (Diptera: Culicidae). Frontiers in Physiology. 10. https://doi.org/10.3389/fphys.2019.00152

Schmidt, O., Wei, D.S., Tang, T.K.H., and Liese, W. (2013). Bamboo and fungi. Journal of Bamboo and Rattan. 12(1-4). 1-14. https://www.researchgate.net/profile/Olaf-Schmidt/publication/272073553_Bamboo_and_fungi/links/5a7d6d11a6fdccc013f5a712/Bamboo-and-fungi.pdf

Scholte, E., Knols, B.G.J., Samson, R.A., and Takken W. (2004). Entomopathogenic fungi for mosquito control: A review. Journal of Insect Science. 4(1). https://doi.org/10.1093/jis/4.1.19

Scholte, E., Takken, W., and Knols, B.G.J. (2007). Infection of adult *Aedes aegypti* and *Ae. albopictus* mosquitoes with the entomopathogenic fungus Metarhizium anisopliae. Acta Tropica. 102(3). 151-158. https://doi.org/10.1016/j.actatropica.2007.04.011

Seabourn, P.S., Weber, D.E., Spafford, H., Medeiros, M.C.I. (2023). *Aedes albopictus* microbiome derives from environmental sources and partitions across distinct host tissues. Microbiology Open. 12(3). https://doi.org/10.1002/mbo3.1364

Talbot, J.M., Allison, S.D., and Treseder, K.K. (2008). Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. Functional Ecology. 22(6). 955-963. https://doi.org/10.1111/j.1365-2435.2008.01402.x

Tawidian, P., Coon, K.L., Jumpponen, A., Cohnstaedt, L.W., and Michel, K. (2021). Host-environment interplay shapes fungal diversity in mosquitoes. mSphere. https://doi.org/10.1128/msphere.00646-21

Valzania, L., Martinson, V.G., Harrison, R.E., Boyd, B.M., Coon, K.L., Brown, M.R., and Strand, M.R. (2018). Both living bacteria and eukaryotes in the mosquito gut promote growth of larvae. PLOS Neglected Tropical Diseases. https://doi.org/10.1371/journal.pntd.0006638

Wang, X., Liu, T., Wu, Y., Zhong, D., Zhou, G., Su, X., Xu, J., Sotero, C.F., Sadruddin, A.A., Wu, K., Chen, X., and Yan, G. (2018). Bacterial microbiota assemblage in *Aedes albopictus* mosquitoes and its impacts on larval development. Molecular Ecology. 27(14). 2972-2985. https://doi.org/10.1111/mec.14732

Wilkerson, R.C., Linton, Y., and Strickman, D. (2021). Mosquitoes of the World. Johns Hopkins University Press. 1. ISBN 9781421438153 (ebook).

Yen, J.H. and Barr, A.R. (1971). New hypothesis of the cause of cytoplasmic incompatibility in Culex pipiens L. Nature. 232. 657-658. https://doi-org.eres.library.manoa.hawaii.edu/10.1038/232657a0

Zouache, K., Martin, E., Rahola, N., Gangue, M.F., Minard, G., Dubost, A., Van, V.T., Dickson, L., Ayala, D., Lambrechts, L., and Valiente Moro, C. (2022). Larval habitat determines the bacterial and fungal microbiota of the mosquito vector Aedes aegypti. FEMS Microbiology Ecology. 98(1). https://doi.org/10.1093/femsec/fiac016