May 2021

Mosquito Distribution and Stoichiometric Analysis between Open and Closed Canopies in New Orleans Cemeteries

Rachel Rogers
Louisiana State University and Agricultural and Mechanical College

MOSQUITO DISTRIBUTION AND STOICHIOMETRIC ANALYSIS BETWEEN OPEN AND CLOSED CANOPIES IN NEW ORLEANS CEMETERIES

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Environmental Sciences

by
Rachel Rogers
B.S., The University of Southern Mississippi, 2018
August 2021

Acknowledgements

First, thank you to my amazing advisor, Dr. Rebeca de Jesús Crespo. I appreciate your expertise and support to finish this thesis. Also, a big thank you to my committee members, Dr. Linda Bùi and Dr. Michael Polito, for all your advice and encouragement. Thank you to Dr. Hayat Bennadji, who analyzed all my samples for stable isotopes/stoichiometry. Thank you to Emily Ford, who gave me access to Lafayette Cemetery No. 1. Thank you to Zachary Taylor, who went with me on almost all my sampling trips. Thank you to the two undergraduates, Matthew Mount and Hannah Orff, who helped me sample and process before COVID-19 closures. You two were awesome, and I wish that we had had more time to work together. Finally, thank you to all my friends and family who have supported me through this journey.

Table of Contents

List of Tables

List of Figures

Abstract

Cemetery vases represent an important container habitat for mosquito larvae. Some species, like, *Aedes albopictus* and *Aedes aegypti*, prefer container habitats, whereas others, like *Culex quinquefasciatus*, will opportunistically use containers. In New Orleans, these three medically important vector species (*Ae. albopictus Ae. aegypti*, and *Cx. quinquefasciatus*) co-occur, despite a demonstrated competitive advantage of *Ae. albopictus* to the other two.   Here we test the hypothesis that canopy cover from trees could be a mediating factor in driving mosquito assemblages in New Orleans, by influencing food sources, and the microclimate experienced by mosquito larvae. Samples from seven different cemeteries were divided between open and closed canopies. Abundance for larvae was analyzed for season and canopy. Larvae, particulate organic matter (POM) filtered from vases, and leaves were analyzed for elemental concentration (%C, %N, and C:N) and stable isotopes (δ^{13}C and δ^{15}N) and their relationships to season and canopy. The distribution of *Ae. aegypti* and *Ae. albopictus* under open and closed canopies could be explained by rainfall and nutrient availability. *Ae. aegypti* was more dominant under an open canopy early in peak mosquito season (summer) but switched to closed canopy vases late in the mosquito season (autumn). The opposite trend was observed for *Ae. albopictus*, and *Cx. quinquefasciatus* was only observed outside peak mosquito season. These dynamics suggest potential patterns of habitat segregation among these species over space and time. Open canopies had a significantly higher δ^{13}C for POM than closed canopy, whereas some significant effects concerning season and canopy existed among larvae δ^{13}C. δ^{15}N was consistent all year and between canopy types for both POM and larvae. Correlations in stable isotopes were found between larvae and the POM but not for larvae and leaves, suggesting that larvae are more likely feeding on POM and not directly on leaf litter. Our study helps inform the dynamics of important

mosquito vectors at the larval stage, which could aid in their management for the benefit of

human health.

Chapter 1. Introduction

1.1. Mosquito Biology and Importance

Mosquitoes are insects that form the family Culicidae in the fly order, Diptera. A mosquito's life cycle can make them difficult to study. They undergo a holometabolous, or complete, life cycle. They are terrestrial as adults, but their other life stages (egg, larvae, and pupae) are entirely aquatic (Clements, 2000). Life strategies among the >3,000 different species vary. In most species, the female mosquito requires a blood meal to provide nutrients for her eggs (Clements, 2000). This necessity of hematophagy in completing a mosquito's life cycle provides a chance for various diseases to be passed from one organism the mosquito feeds on to another. Because of this, mosquitoes can be competent vectors for a variety of diseases such as malaria, dengue, chikungunya, West Nile virus, Zika virus, etc. (Robert *et al*., 2016). Understanding the ecology of vector mosquitoes is important for mosquito control to protect human and veterinary health.

Some species of mosquito will utilize human-made container habitats to lay their eggs. Certain species, such as *Aedes* species, even prefer human-made containers to natural ones (Sota and Mogi, 1992, Yee *et al*., 2015). Examples of container habitats include, but are not limited to, abandoned tires, bird baths, gutters, and buckets.

Water-filled cemetery vases have been identified as an important habitat for some species of mosquito larvae (Vezzani, 2007). Grasses, leaves, flowers, and other vegetation contribute to the influx of nutrients into these vase habitats (Vezzani, 2007). In these habitats, mosquito larvae feed on the plant and animal detritus that has accumulated. Adult mosquitoes that eclose from the pupae in vases can then seek to blood-feed on cemetery visitors or even on residents that live

1

nearby (Vezzani, 2007). Because they can support the entire mosquito life cycle, cemeteries could harbor arboviral disease.

Three important vector species that can utilize the small cemetery vases are *Aedes albopictus*, *Aedes aegypti*, and *Culex quinquefasciatus*. Species in the genus *Aedes* are of global health importance, as they can vector serious diseases such as Zika virus, dengue, and chikungunya. *Cx. quinquefasciatus*, the southern house mosquito, is also of medical importance because it can vector West Nile virus.

Both *Ae. albopictus*, the Asian tiger mosquito, and *Ae. aegypti*, the yellow fever mosquito, are non-native to the United States. *Ae. albopictus* most likely was introduced through tire shipments from Asia (Hawley *et al.*, 1987), whereas *Ae. aegypti* was introduced from ships from Africa (Tabachnick, 1991). *Ae. albopictus* has been shown to be a superior competitor in areas where it occurs non-natively. *Ae. albopictus* is a superior competitor to *Cx. quinquefasciatus* as it can exclude *Cx. quinquefasciatus* especially in habitat with limited nutrients (Allgood and Yee, 2014). Interspecific competition between *Ae. albopictus* and *Ae. aegypti* is more important to the two species than intraspecific competition (Alto *et al.*, 2005). *Ae. albopictus* has been found to outcompete *Ae. aegypti*, and the overall survival rate of *Ae. albopictus* is higher than *Ae. aegypti* (Leisnham *et al.*, 2014; Yee *et al.*, 2015). However, in certain areas of the world especially tropical areas, *Ae. aegypti* has managed to displace *Ae. albopictus* (Lounibos and Juliano, 2018). Food quality is a factor in competition between the two species. Both species will coexist in containers with high food quality such as animal detritus, but in containers with low food quality such as leaves, *Ae. albopictus* outcompetes *Ae. aegypti* (Juliano, 2010). New Orleans is an unusual place because both *Ae. albopictus* and *Ae. aegypti* exist in close proximity to each other despite the competition.

1.2. Drivers of Habitat Quality for Container Breeding Mosquitos

Organic material and nutrient content within ecosystems help maintain a balance of chemicals known as ecological stoichiometry. Of the elements needed to maintain life, carbon, nitrogen, and phosphorus are the most limiting of them all (Sterner and Elser, 2002). Carbon, nitrogen, and phosphorus are often expressed as a ratio (C:N:P). It is important for all organisms to receive enough of these three elements, or they would not flourish. Lacking one of these important elements could even mean death for the organism (Sterner and Elser, 2002).

In container environments, such as cemetery vases, nitrogen seems to be the main limiting factor (Kaufman et $al.$, 2002). Carbon and nitrogen inputs into aquatic ecosystems can change depending on the vegetation surrounding it and any decomposing animals that may fall into it (e.g., arthropods in cemetery vases). For example, fresh leaves, that may contribute to a container, have a lower C:N than senescent leaves, thus fresh leaves would contribute more nitrogen to a system than already decaying leaves (Walker et $al.$, 1997). Both ecological stoichiometry and stable isotopes can help determine the detrital input (animal or plant based). Stable isotopes $^{13}C/^{12}C$ (expressed as $\delta^{13}C$ (‰)) and $^{15}N/^{14}N$ (expressed as $\delta^{15}N$ (‰)) can provide insights into trophic relationships. Typically, the fractionation between trophic levels of $\delta^{13}C$ is 0.4 ‰, while the fractionation of $\delta^{15}N$ is 3.4 ‰ (Post, 2002). Using stable isotope analysis, studies have estimated between 3-9 trophic levels between mosquitoes and leaves (Winters and Yee, 2012; Kaufman et $al.$, 2010). The influx of plant detritus into a container environment also seems to deplete ^{15}N in adult mosquitoes (Winters and Yee, 2012; Yee et $al.$, 2015).

Other driving factors of container environments can include temperature and rainfall. Rainfall is the source of water for container environments such as cemetery vases. However,

excess rainfall will overflow the container and can flush larvae out (Dieng *et al.*, 2011).

Humidity and temperature are important factors for *Aedes* egg survival (Juliano *et al.*, 2002).

Temperature can affect the mosquito's whole life cycle from the development of larvae, the

activity of adults, and the even the blood-feeding behavior of adult female mosquitoes (Reinhold

et al., 2018). If temperatures are high and there is little precipitation, containers may dry out.

Certain species, like *Ae. aegypti*, have more desiccation resistant eggs than other species, such as

Ae. albopictus, which may promote survival in dry weather (Costanzo *et al*., 2005).

1.3. Study Sites

The city of New Orleans, Louisiana, is located within Orleans Parish in southeast

Louisiana. New Orleans has a subtropical climate and is relatively warm year-round with the

temperatures in 2020 ranging from 1.67 °C to 37.22 °C (NOAA, 2020). Rainfall for New

Orleans in 2020 varied month to month with the lowest total monthly precipitation being 4.6 cm

and the highest being 38.6 cm (NOAA, 2020) The median household income for New Orleans is

$41,604 which is below the national median household income of $62,843 (U.S. Census Bureau,

2010).

Many of New Orleans cemeteries are surrounded by residential and commercial areas.

The proximity to people likely leads to adult mosquitoes dispersing from cemeteries to blood-

feed from residents and tourists alike. For this study we sampled from seven New Orleans

cemeteries (Figure 1, Table 1). Cemeteries were chosen based on accessibility, presence of

canopy cover, and proximity to residential areas (<150m in distance). Ideally, cemeteries that

were selected had both open and closed canopies, but we additionally selected two cemeteries

with no canopy cover due to previous studies collecting both *Aedes* species from them (Table 1)

(Deerman, 2018).

Most trees in the cemeteries are southern live oaks (*Quercus virgiana*). Other tree species include magnolia (*Magnolia grandiflora*), Chinese tallow (*Triadica sebifera*), camphor (*Cinnamomum camphora*), and various oak species (*Quercus* spp.). Leaves from these trees could contribute to the detrital composition of the vases.

Figure 1. Location of cemeteries within Orleans Parish and their proximity to each other.

Table 1. List of cemeteries included in the study with area, presence of canopy cover, number of samples per month, and the median household income of the surrounding area (ACS, 2017).
*Only a small portion of Metairie <150 m from residential areas was sampled.

Cemetery	Area (ha)	Canopy Cover	# Samples per Month	Median Household Income of Surrounding Area
Lafayette No. 1	2.29	Y	2	$132,750
Lafayette No. 2	4.26	Y	2	$10,096
St. Roch	2.46	N	1	$23,654
St. Vincent de Paul	3.35	N	1	$28,482
Carrollton	3.79	Y	2	$73,750
Holt	2.64	Y	2	$51,765
Metairie	119.38*	Y	6	$214,205

Chapter 2. Materials and Methods

2.1. Research Objectives and Hypotheses

Our two main objectives were to describe patterns of mosquito larvae assemblages in cemeteries of New Orleans and evaluate the role of vegetation on nutrient stoichiometry and stable isotopes of cemetery vases and mosquito larvae.

We hypothesized that container environments, either closed canopy containers or open canopy containers, will yield different species and abundances of mosquito larvae due to differences in temperature and detrital food sources. We hypothesized that *Ae. albopictus* would prefer a closed canopy, while *Ae. aegypti* would prefer an open canopy. This is for several reasons.

First, *Ae. aegypti* eggs are more desiccation resistant than *Ae. albopictus* eggs (Costanzo *et al*., 2005). For *Ae. albopictus* humidity and lower temperature are more important to egg survival than *Ae. aegypti* (Juliano *et al.*, 2002). Assumably, vases under an open canopy would become warmer and drier than those under a closed canopy. This could give *Ae. aegypti* an advantage in open canopies.

Second, canopy cover provides a nutrient source of leaves and tree nuts. Lower quality food that decays slowly (such as leaves) can lead to a competitive advantage for *Ae. albopictus*, but the addition of higher quality food such as animal detritus can shift *Ae. albopictus* dominance to interspecific coexistence (Murrell and Juliano, 2008, Daugherty *et al.*, 2008). Thus, the majority of nutrient source for a vase under closed canopy cover may be mostly leaves and may lead to favoring *Ae. albopictus* dominance.

7

We hypothesized that because of different detrital influxes into closed canopy vs open canopy containers, elemental concentration among the water samples will be different and will be associated to larval stoichiometry and species assemblages, and that stable isotope signatures of larvae will be different in closed vs open canopy containers, revealing differences in detrital food sources.

We expected our observations to vary temporally, in accordance with different temperatures and detrital inputs. Because we should see more mosquito activity in warmer weather, we expected our hypotheses to be better supported in the summer months.

2.2. Mosquito Larvae Survey

Field collection from cemetery vases occurred monthly from January 2020 to December 2020, with the exception of March 2020, because of COVID-19 closures. We spaced samples at least 150 m apart, which is around the mean dispersal distance of both *Ae. albopictus* and *Ae. aegypti* (Harrington *et al*., 2005; Marini *et al*., 2010), and about 150 m from residential housing, in order to collect from the pool of mosquitoes that would be more likely to impact human health.

The samples collected from the cemeteries were separated into open and closed canopies. Canopy cover was measured with a spherical densiometer. We defined an open canopy as having a densiometer reading <60, and we defined closed canopies as having a densiometer reading >60. In cemeteries with trees (Lafayette No. 1, Lafayette No. 2, Holt, and Carrollton), one open canopy and one closed canopy sample were collected per sampling trip. In cemeteries with no trees (St. Roch and St. Vincent de Paul), only 1 open canopy sample was collected. The only exception to this was Metairie which was a large enough cemetery to collect a maximum of six

8

samples. On a perfect sampling trip, we would collect a total of 14 samples split between seven open canopy and seven closed canopy samples.

Vases were chosen based on the presence of larvae. However, we only collected from standard-sized vases. Buckets or extremely large vases were not considered even with larvae present. The temperature of the water before collection and the material of the vase were recorded. Leaves, in and around closed canopy vases, were identified to species and collected for stable isotope and elemental composition analysis. Larvae were collected from each vase and identified to species in the lab using the identification key by Darsie and Ward (2005). If only pupae were collected, they were retained in the lab until they eclosed into adults, and then identified to species (Darsie and Ward, 2005). Vase water and larvae were saved for stoichiometric and stable isotope analysis.

2.3. Elemental Concentration and Stable Isotopes

The collected vase water was transported back to LSU on ice and then kept cold until filtering which took place <24 hours after collection. To collect particulate organic matter (POM), water was filtered 50 mL at a time using Whatman grade GF/F glass microfiber filters until the filter was clogged. Examples of the POM on the filter can be seen in Figure 2. After identification, larvae were saved for further analysis if the sample contained ≥1 first instar larvae or ≥5 second or third instar larvae. This assured that the saved larvae would have enough mass to be analyzed by the mass spectrometer. If a sample contained >1 species, the species were separated.

Figure 2. Examples of POM accumulated on filters.

Larvae, filtered POM, and leaves were dried in an oven at 60°C for 48 hours before being

weighed to the nearest 0.001 mg and encapsulated into a tin capsule. Leaves were homogenized

to a fine powder using a mortar and pestle before encapsulation. Samples were analyzed for

elemental concentration and stable isotopes by the Louisiana State University Stable Isotope

Ecology Laboratory. Samples were combusted for C:N, ^{13}C, and ^{15}N analysis using a Costech

ECS4010 elemental analyzer coupled to a Thermo-Fisher Delta Plus XP continuous-flow stable

isotope ratio mass spectrometer. Standards used for analysis were USGS-40 and USGS-41.

Stable isotope values were calculated as $\delta X=[(R_{sample}/R_{standard})- 1] \times 1000$, where X is ^{13}C or ^{15}N

and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

2.4. Statistical Analysis

The data were analyzed using R (R Core Team, 2020). The Shapiro-Wilk test was used to

test the normality of variables. For abundance, elemental concentration (%C, %N, and C:N) and

stable isotopes (δ^{13}C and δ^{15}N), some values were not normally distributed according to Shapiro-

Wilk tests. These values were transformed, as necessary. Raw data from elemental concentration and stable isotopes can be seen in Appendix B.

A one-way analysis of variance (ANOVA) was used to determine whether larval abundance varied among species. Once that was determined, abundance of all larvae was analyzed using a two-way ANOVA and a post-hoc Tukey's Honestly Significant Difference (HSD) test to evaluate the effects of canopy cover and season

Two-way ANOVAs and post-hoc Tukey's HSD tests were used to evaluate if elemental concentration and stable isotopes varied among seasons, between canopy types, or with an interaction of both. Combined larvae of all species and filters were assessed in this way. A one-way ANOVA was used to determine if the elemental stoichiometry and stable isotope values varied among species. Regressions were used to determine if larvae stable isotope signatures were more closely related to POM or leaf isotope samples. Paired T-Tests were used to quantify the mean difference between larvae and POM for $\delta^{13}C$ and $\delta^{15}N$.

Chapter 3. Results

3.1. Patterns of Mosquito Larvae Assemblages in Cemeteries of New Orleans

Only four mosquito species were collected in the cemeteries over the year timespan. These species were *Ae. albopictus*, *Ae. aegypti*, *Cx. quinquefasciatus*, and *Cx. restuans* (Figure 3). In total, 1,615 larvae were collected during 2020 (Appendix A). The most abundant species was *Cx. quinquefasciatus* with a total of 748 individuals collected. However, *Cx. quinquefasciatus* was not present in the cemeteries after early summer (June) and did not appear again even as the weather became colder at the end of the year. *Ae. albopictus* and *Ae. aegypti* are present all year throughout the cemeteries and become more abundant as the weather warms. *Ae. albopictus* and *Ae. aegypti* were found co-occurring in 7 vases. Of these vases, 3 were under closed canopy and 4 were in the open canopy. *Cx. restuans* was only found on the final sampling trip in December where 4 individuals were collected.
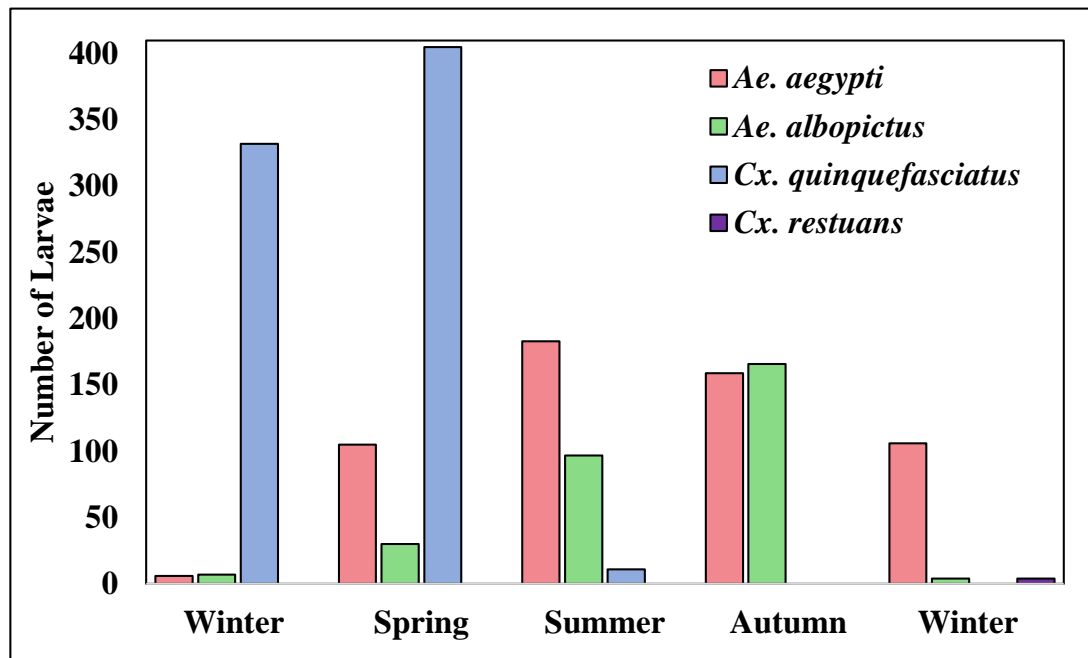
Figure 3. The abundance of larvae divided into species across seasons.

Abundance did vary among species (ANOVA; $F_{3, 90} = 5.012$; $p = 0.003$). A Tukey's HSD post-hoc test revealed significant differences between *Cx. quinquefasciatus* and both *Ae. albopictus* ($p = 0.002$) and *Ae. aegypti* ($p = 0.014$), but no significant differences in abundance between *Ae. albopictus* and *Ae. aegypti* ($p = 0.722$). Season (ANOVA; $F_{4, 21} = 1.135$; $p = 0.367$), canopy cover (ANOVA; $F_{1, 21} = 0.864$; $p = 0.363$), and the interaction between season and canopy cover (ANOVA; $F_{2, 21} = 1.241$; $p = 0.309$) did not significantly affect *Ae. albopictus*. The interaction between season and canopy was significant in *Ae. aegypti* (ANOVA; $F_{4, 34} = 4.789$; $p = 0.004$) with autumn closed canopy having significantly higher abundance than autumn open canopy (Tukey's HSD; $p = 0.01161$) (Figure 4). In *Cx. quinquefasciatus*, the interaction between season and canopy is approaching significance with more *Cx. quinquefasciatus* appearing under the open canopy than the closed canopy (ANOVA; $F_{1, 15} = 3.434$; $p = 0.084$).

Average vase temperatures, average ambient temperatures, and total rainfall can be seen on Table 2. Temperatures under closed and open canopy differed on average by 1.4°C, with open canopy as expected having warmer temperatures, except for the early winter, where temperatures where slightly colder in the open canopy. As expected, the warmest temperatures in vase and ambient temperatures were observed in the summer months, and the coldest in the winter. *Ae. albopictus* and *Ae. aegypti* were most abundant in the summer and autumn. The most rainfall occurred in the summer with a total of 74.7 cm. Total precipitation in 2020 for the city was 179.6 cm, which is wetter than the average yearly recorded precipitation of 144.3 cm (1981-2010) (NOAA, 2010).
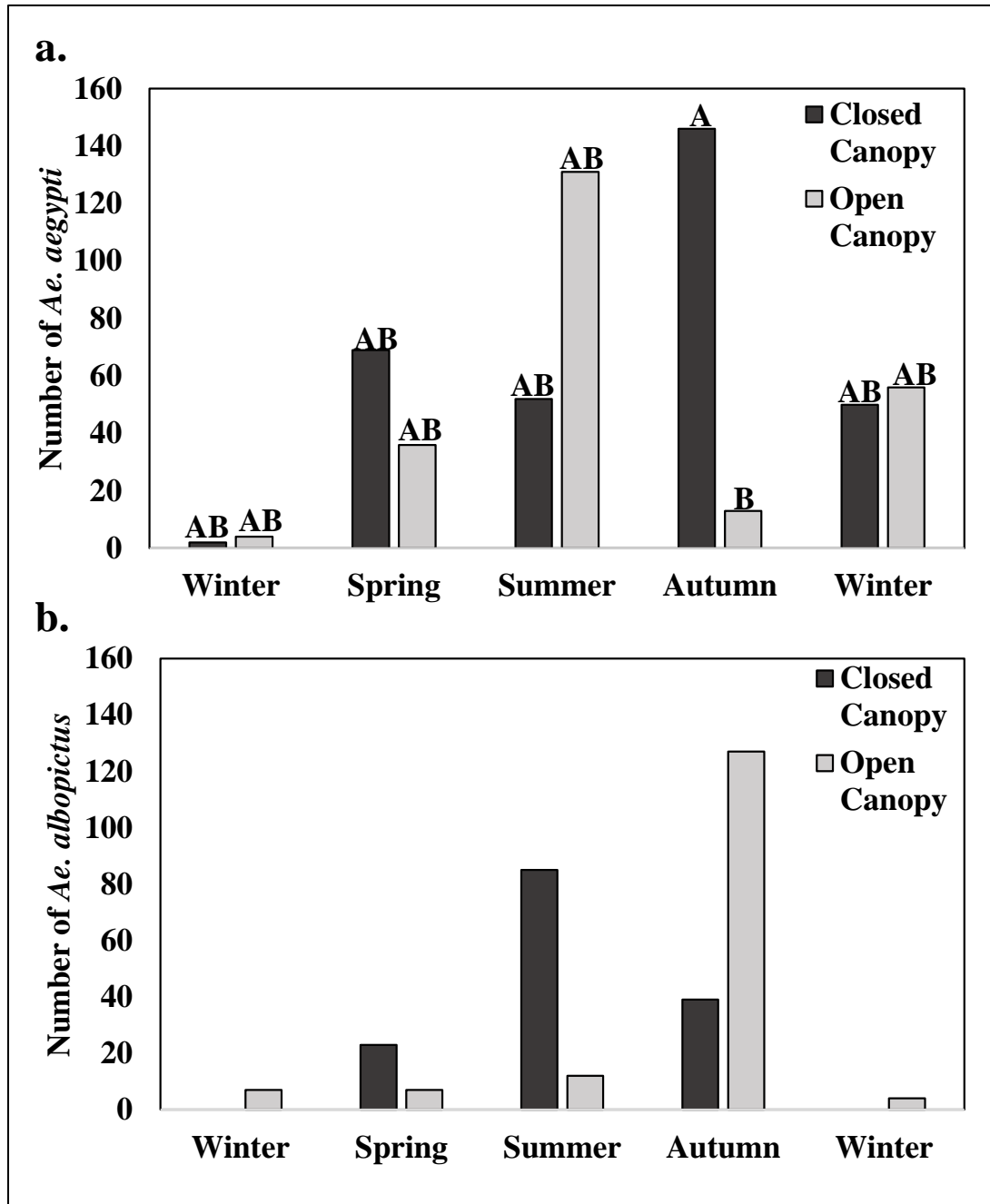
Figure 4. Abundances regarding season and canopy for a.) *Ae. aegypti* with significant interactions in season × canopy labeled, and for b.) *Ae. albopictus*. Bars that do not share a letter are significantly different.

Table 2. Average vase temperature, average ambient temperature, and total rainfall in New Orleans throughout the year 2020 (NOAA, 2020).

Variables Measured	Sampling Period				
Avg Vase Temperature (°C)	**Winter (Jan/Feb)**	**Spring (Apr/May)**	**Summer (Jun/Jul)**	**Autumn (Aug/Sep/Oct)**	**Winter (Nov/Dec)**
Closed Canopy	15.9	25.6	27.7	25.3	14.55
Open Canopy	15.3	26.7	29.2	26.7	17
Avg Ambient Temperature (°C)	15.8	24	29.2	26.3	16.6
Total Rainfall (cm)	21.3	35.1	64.5	32.5	26.7

3.2. The Role of Vegetation on Elemental Concentration and Stable Isotopes

For elemental concentration and stable isotope values, we focused our analysis on the samples from January to October 2020. November and December data were not available at the time of analysis. This omission is not expected to bias our results, because as shown in Figure 3 these months were outside of the peak mosquito season. For the POM samples, %C and %N were not significantly different temporally, between open or closed canopy, or with the interaction between season and canopy cover (Table 3). There are no clear trends in season or canopy for POM %C and %N. However, the C:N for POM samples was significant for the interaction between season and canopy (ANOVA; $F_{3, 69} = 3.600$; $p = 0.018$) (Figure 5) (Table 3). The autumn closed canopy sample had the significantly lower C:N compared to winter open canopy and both open and closed canopy in the summer. The overall trend was for C:N to be higher in the open canopy, with the exception of the autumn period.

Table 3. Results of two-way ANOVAs for elemental concentration of POM when compared to season, canopy cover, and the interaction between season and canopy cover.

Variable Tested	F-value	df	p-value
POM %C			
Canopy	0.724	3, 69	0.541
Season	0.552	1, 69	0.460
Season × Canopy	1.835	3, 69	0.149
POM %N			
Season	1.553	3, 69	0.209
Canopy	0.948	1, 69	0.334
Season × Canopy	0.855	3, 69	0.469
POM C:N			
Season	4.850	3, 69	**0.004**
Canopy	0.098	1, 69	0.755
Season × Canopy	3.600	3, 69	**0.018**
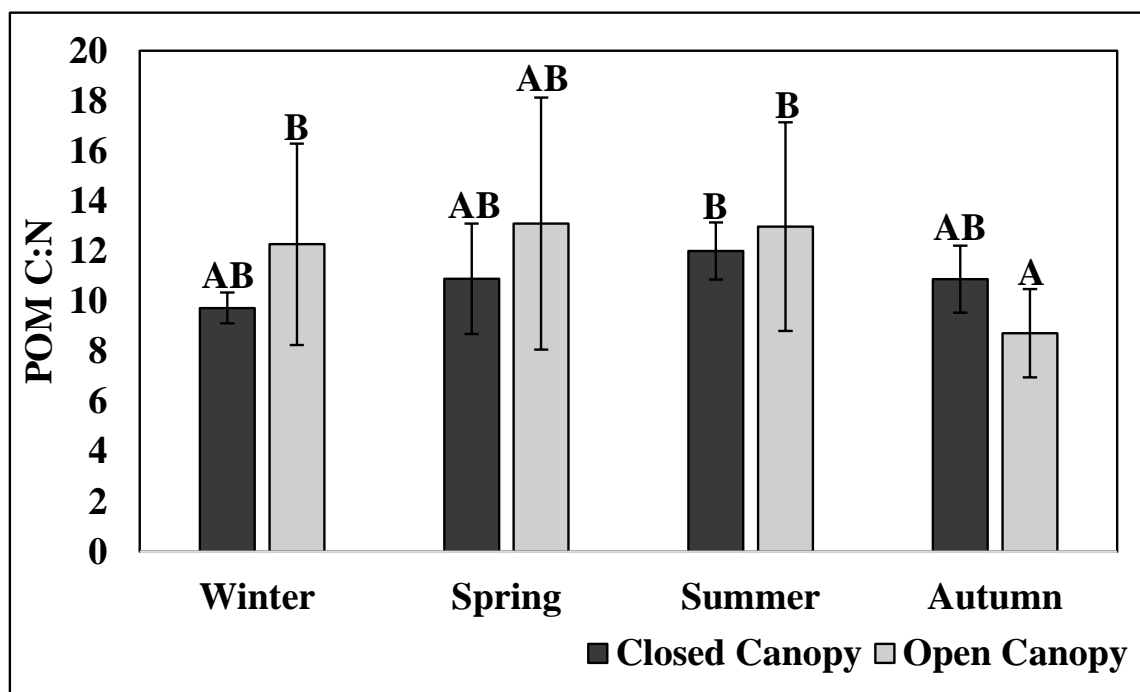
Figure 5. C:N for POM samples when compared to season and canopy cover. Means that do not share a letter are significantly different.

Elemental concentration did not vary among species for %N (ANOVA; $F_{2, 50} = 0.309$; $p = 0.736$), %C (ANOVA; $F_{2, 50} = 0.466$; $p = 0.631$), or C:N (ANOVA; $F_{2, 50} = 0.031$; $p = 0.969$). For all species combined, larvae %C was significantly different temporally with %C higher in autumn and lowest in spring and summer (Table 4). %N and C:N in larvae were significant temporally and between open and closed canopy cover, but there were no significant interactions between canopy cover and season (Table 4). Larval %N trended lower in the spring and summer and was lower in open canopy than closed. Larval C:N is higher in spring and summer than in winter and is lower in closed canopy than in open.

Table 4. Results of two-way ANOVAs for elemental concentration of larvae when compared to season, canopy cover, and the interaction between season and canopy cover.

Variables Tested	F-value	df	p-value
Larvae %C			
Season	5.279	3, 42	**0.004**
Canopy	1.614	1, 42	0.211
Season × Canopy	0.298	3, 42	0.827
Larvae %N			
Season	10.129	3, 42	**< 0.0001**
Canopy	4.285	1, 42	**0.045**
Season × Canopy	0.331	3, 42	0.803
Larvae C:N			
Season	4.737	3, 42	**0.006**
Canopy	6.239	1, 42	**0.017**
Season × Canopy	0.196	3, 42	0.898

POM $\delta^{13}C$ varied significantly between open and closed canopy (ANOVA; $F_{1, 69} = 27.124$; $p < 0.0001$) (Figure 6) and was approaching significance for the interaction between canopy and season (ANOVA; $F_{3, 69} = 2.354$; $p = 0.0796$) (Table 5). The open canopy POM was more enriched in ^{13}C than the closed canopy POM year-round. POM $\delta^{15}N$ was approaching significance for the interaction between season and canopy ($F_{3, 69} = 2.487$; $p = 0.068$) (Table 5).

Open canopy $\delta^{15}N$ for POM trended upwards in the summer, while closed canopy $\delta^{15}N$ was at its highest in the winter and trended downwards throughout the year.
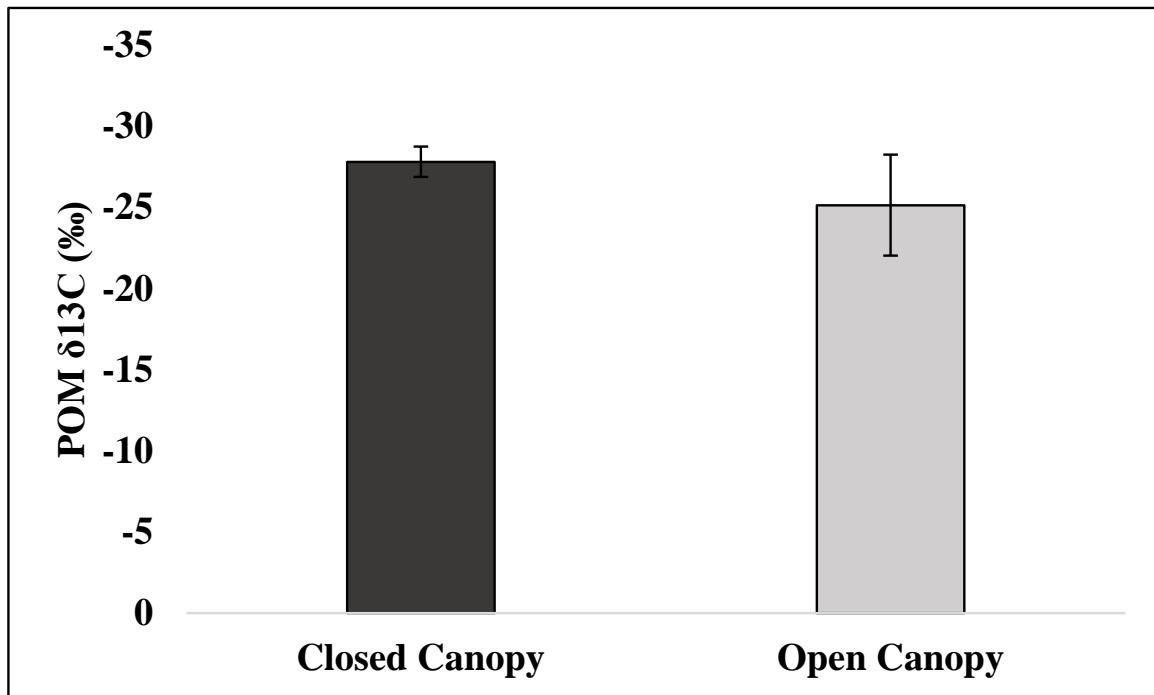
Figure 6. Significant difference between open and closed canopy for $\delta^{13}C$ in POM.

No significant differences were found among the species for $\delta^{13}C$ (ANOVA; $F_{2, 42}$ = 0.788; p = 0.461) and $\delta^{15}N$ (ANOVA; $F_{2, 42}$ = 1.314; p = 0.278). For larvae in general, there were no significant differences in $\delta^{15}N$, but there were significant differences in the interaction of season and canopy in $\delta^{13}C$ (Figure 7) (Table 5). Autumn under closed canopy was significantly less enriched in ^{13}C than summer and winter under an open canopy for the larvae.
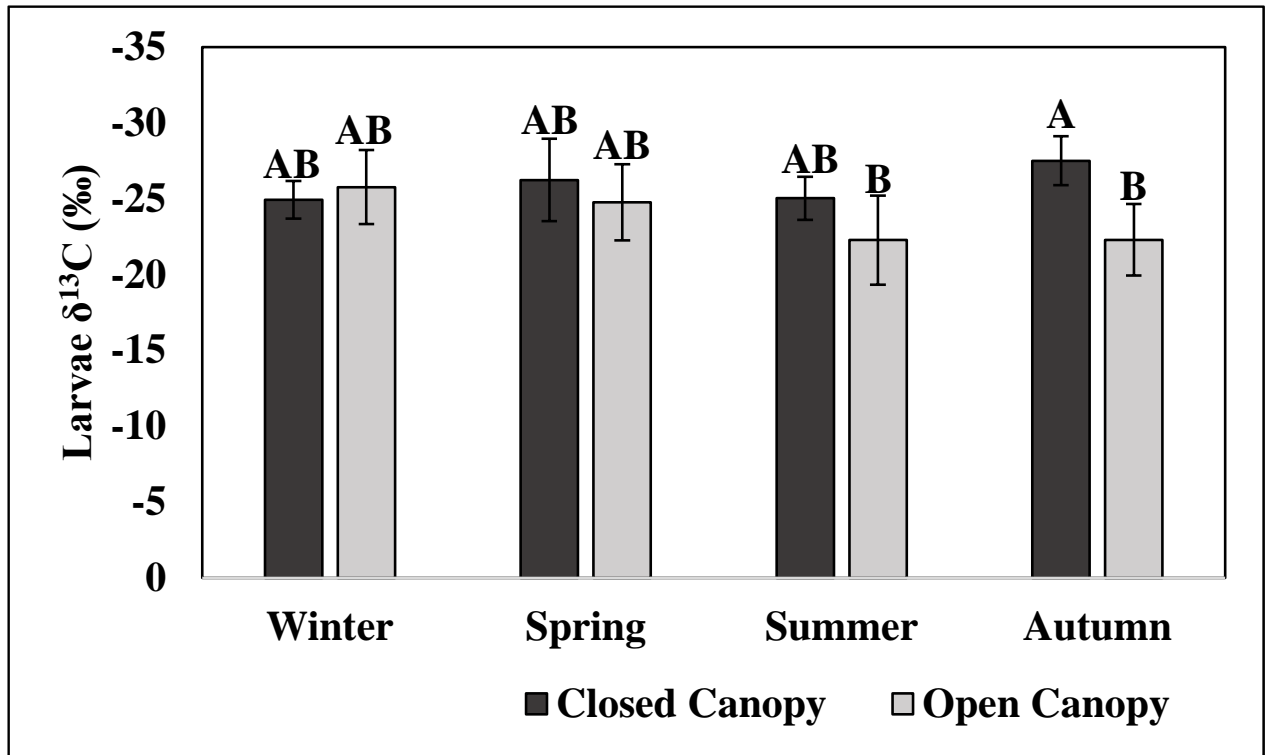
Figure 7. Larvae δ^{13}C among seasons and between closed and open canopy. Means that do not share a letter are significantly different.

Table 5. Results of two-way ANOVAs for stable isotopes of POM and larvae when compared to season, canopy cover, and the interaction between season and canopy cover.

Variable Tested	F-value	df	p-value
POM δ^{13}C			
Season	1.137	3, 69	0.340
Canopy	27.124	1, 69	**<0.0001**
Season × Canopy	2.354	3, 69	0.080
POM δ^{15}N			
Season	0.902	3, 69	0.448
Canopy	0.687	1, 69	0.412
Season × Canopy	2.487	3, 69	0.068

table cont'd.

Variable Tested	F-value	df	p-value
Larvae $\delta^{13}C$			
Season	2.672	3, 42	0.060
Canopy	15.912	1, 42	**0.0003**
Season × Canopy	3.002	3, 42	**0.041**
Larvae $\delta^{15}N$			
Season	1.707	3, 42	0.180
Canopy	0.342	1, 42	0.562
Season × Canopy	1.324	3, 42	0.279

Regressions for the relationship between larvae and filter stable isotopes were split between open and closed canopies. The correlation of POM $\delta^{13}C$ and larvae $\delta^{13}C$ for closed canopy ($R^2 = 0.3062$; $F_{1, 22} = 9.71$; $p = 0.00503$) and open canopy ($R^2 = 0.7239$, $F_{1, 24} = 62.93$; $p < 0.0001$) was significant (Figure 8). The correlation of larvae $\delta^{15}N$ and POM $\delta^{15}N$ for closed canopy ($R^2 = 0.6765$; $F_{1, 22} = 46.01$; $p < 0.0001$) and open canopy ($R^2 = 0.593$, $F_{1, 24} = 34.97$; $p < 0.0001$) was also significant (Figure 9). Therefore, larvae stable isotopes are correlated to POM stable isotopes.
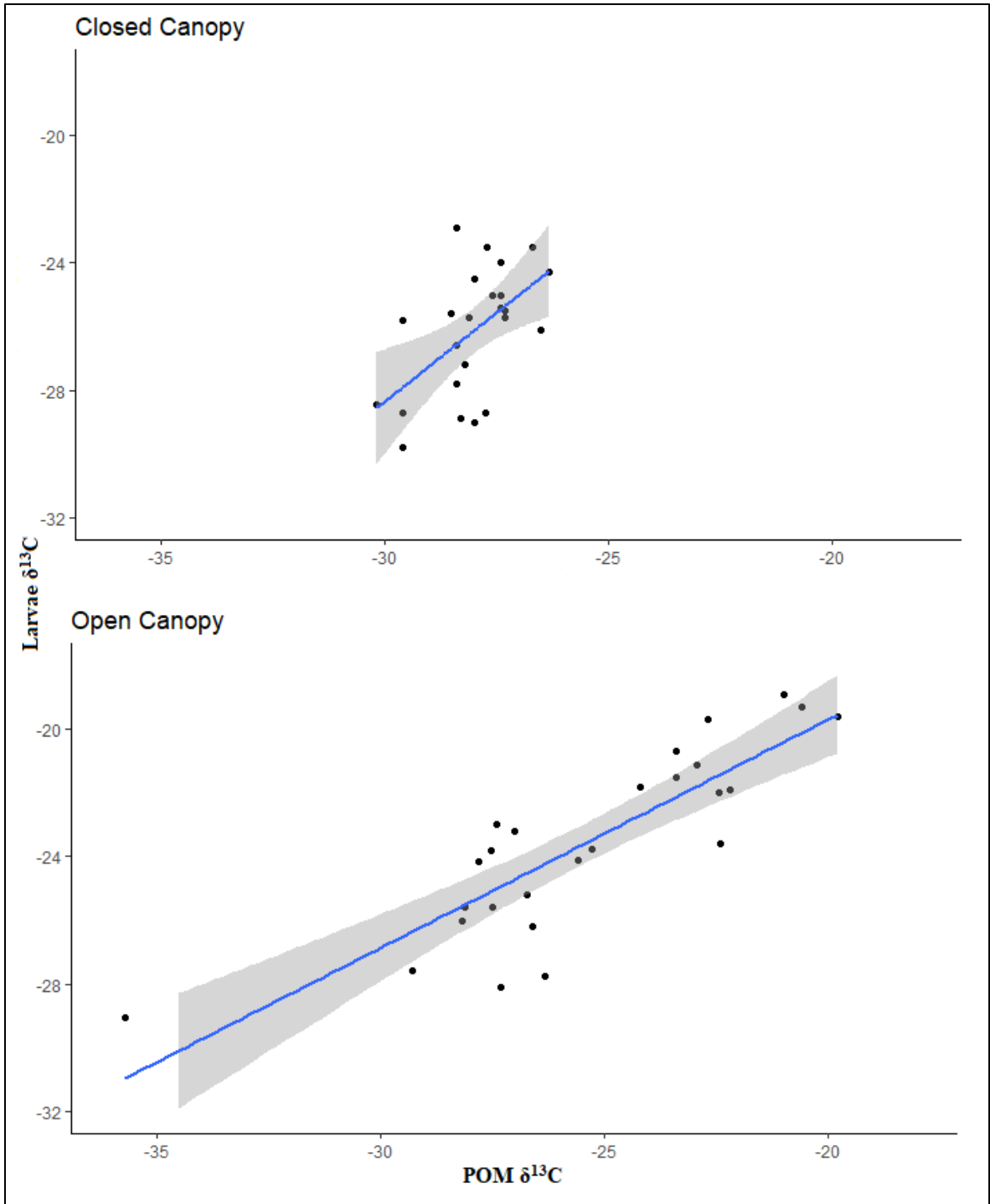
Figure 8. Regressions from closed and open canopy for the relationship between larvae and filter $\delta^{13}C$.
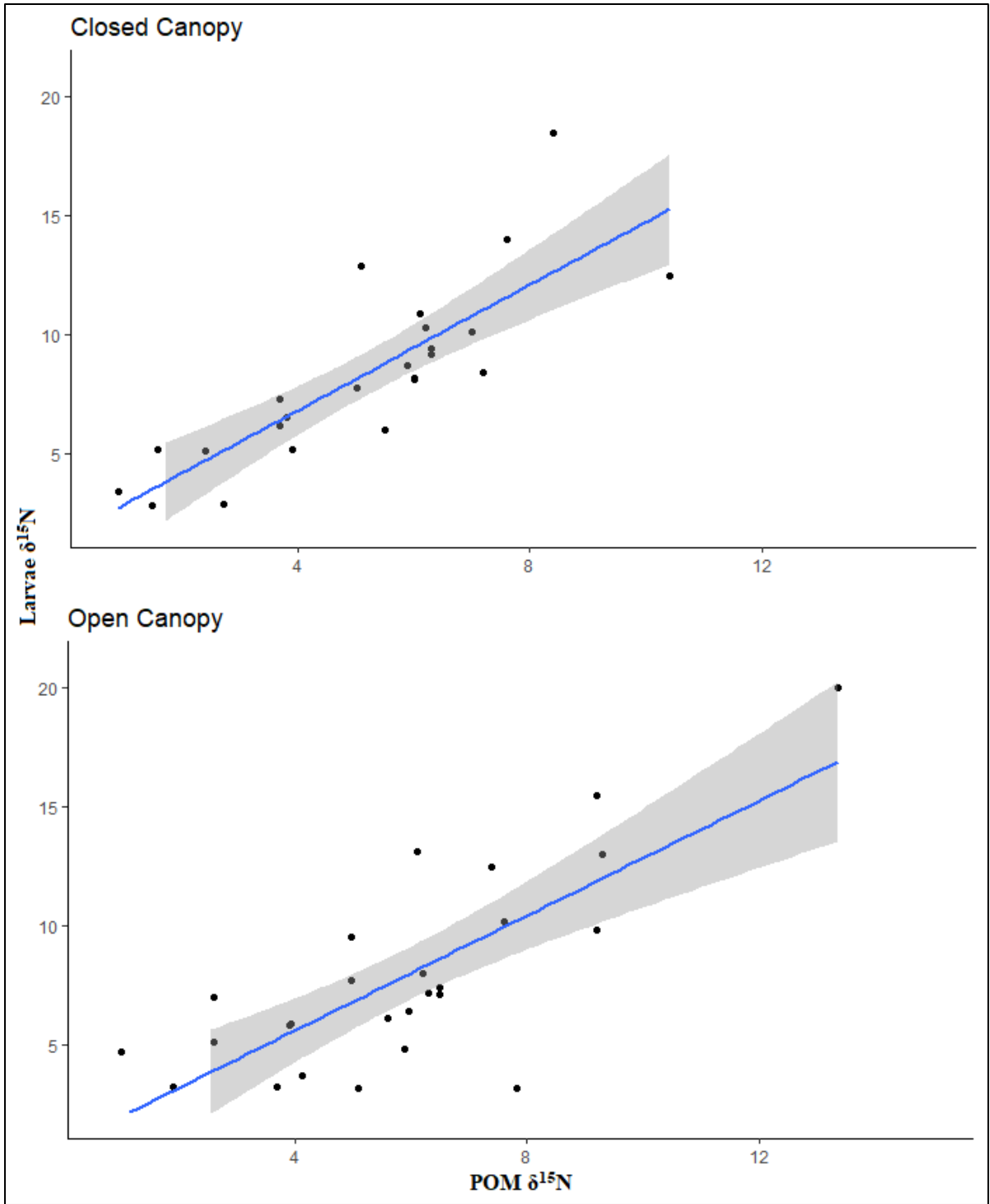
Figure 9. Regressions from closed and open canopy for the relationship between larvae and filter δ^{15}N.

Regressions relating leaf and larvae/POM samples had no need to be split between open and closed canopies because leaves were only collected from closed canopies. Regressions between leaf and larvae was not significant for $\delta^{13}C$ or $\delta^{15}N$ ($R^2 = 0.008$, $F_{1,16} = 0.008$; $p = 0.724$ and $R^2 = 0.067$, $F_{1,16} = 1.157$; $p = 0.298$, respectively). Likewise, regressions between leaf and POM were not significant for $\delta^{13}C$ or $\delta^{15}N$ ($R^2 = 0.003$, $F_{1,27} = 0.075$; $p = 0.786$ and $R^2 = 0.089$, $F_{1,27} = 2.648$; $p = 0.115$, respectively). Larvae and POM stable isotopes were not correlated to leaf stable isotopes.

The paired t-tests comparing larvae to the POM of the vase water they were collected from showed a significant relationship for both $\delta^{13}C$ and $\delta^{15}N$. The mean difference between the larvae and POM for $\delta^{13}C$ was -1.86 (t-value = -7.0557, df = 49, p < 0.0001). The mean difference between the larvae and POM for $\delta^{15}N$ was 4.64 (t-value = 13.943, df = 49, p < 0.0001).

Chapter 4. Discussion

4.1. Patterns of Mosquito Larvae Assemblages in Cemeteries of New Orleans

A greater number of *Ae. aegypti* collected compared to *Ae. albopictus* (total of 559 and 304, respectively) could be linked to the urbanized environment of most New Orleans cemeteries. Rey *et al.* (2006) found that areas with more urban markers, like buildings and pavement, were more likely to have a higher relative abundance of *Ae. aegypti* to *Ae. albopictus*, whereas the opposite was true for areas with more rural markers, such as canopy. Although we did not quantify the urban/rural variable such as in that study, we would categorize New Orleans cemeteries as being more urban because they were all surrounded on most sides with buildings, walls, and pavement. This discrepancy between relative abundance of *Ae. aegypti* to *Ae. albopictus* in our study could be explained due to urbanization, and we would hypothesize that the larvae which were sampled further away from the city (e.g., in cemeteries in more rural areas) we would find fewer and fewer *Ae. aegypti*. In Florida cemeteries, more climate-driven egg mortality in *Ae. albopictus* than in *Ae. aegypti* has been observed which may also explain the greater abundance of *Ae. aegypti* than *Ae. albopictus* (Lounibos *et al.*, 2010). *Ae. albopictus* and *Ae. aegypti* only co-occurred in 7 samples. Most samples with both species occurred in the summer and autumn, and usually 1 species had a disproportionate number of individuals compared to the other, suggesting competition.

Our hypothesis that we would see a greater number of *Ae. albopictus* under closed canopy, and a greater number of *Ae. aegypti* under open canopy was only partially supported in the summer. When looking at the differences in season, canopy, and the interaction between season and canopy, *Ae. albopictus* abundance seemed to be the most stable of the other species.

This is to be expected, as *Ae. albopictus* tends to be a better competitor than the other collected species (Yee *et al.*, 2015). In summer, the majority of *Ae. albopictus* occurred under a closed canopy, while the majority of *Ae. aegypti* occurred under an open canopy. In the autumn, this was the reversed. *Ae. albopictus* tend to negatively affect *Ae. aegypti* distribution during periods of more rain (Leisnham, 2014). In the rainy but warm summer months, we assumed that *Ae. albopictus* excluded *Ae. aegypti*, whose larvae have more desiccation-resistant eggs, from the closed canopy. However, the open canopy in autumn, where more *Ae. albopictus* were found, had a lower POM C:N than in both canopy types in the summer. Since nitrogen is the most limiting nutrient in a container environment, this could represent a shift in nutrients that favored *Ae. albopictus* dominance in the open canopy in the autumn despite the weather being drier (Kaufman *et al.*, 2002).

Cx. quinquefasciatus were common from January to May, but by June disappeared from our samples in the cemeteries. Adult trapping of *Cx. quinquefasciatus* in New Orleans from 2008-2010 reported highest abundances generally from May to June (Moise *et al.*, 2018). This suggests that the *Cx. quinquefasciatus* exploit the cemeteries as larval habitat in the winter and spring. In the summer, *Cx. quinquefasciatus* probably prefers larger habitats, such as drains, that exclude *Aedes*. A study from Florida observed a seasonal shift in which *Ae. albopictus* began to exclude *Cx. quinquefasciatus* from cemeteries during the rainy season (Leisnham *et al.*, 2014). Similarly, we observed that as rainfall increased, *Cx. quinquefasciatus* abundance decreased, and *Ae. albopictus* abundance increased.

Many of the larvae we collected were 2nd and 3rd instars, so this does not necessarily equate to the number of mosquitoes surviving to adulthood. Larval survival to adulthood,

especially in vases with more leaves, may favor *Ae. albopictus* over *Ae. aegypti* (Yee *et al.*, 2015).

We believe this study should reflect an average year for mosquito abundances. COVID-19 closures should have only affected mosquito control during the initial shutdown which was not peak season for *Aedes* mosquitoes. By summer, mosquito spraying began operating as normal.

This study did not focus on abundance among the individual cemeteries. However, we do have data for each cemetery (Appendix A). Future studies could be done by looking at correlation between income and abundance of mosquito larvae since lower income areas tend to have more mosquitoes than higher income areas (LaDeau *et al.*, 2013, de Jesús Crespo *et al.*, 2021). For example, the 3 cemeteries in lower income areas (St. Roch, St. Vincent de Paul, and Lafayette No. 2) contained *Ae. aegypti* consistently year-round. Whereas, Metairie, the cemetery in the highest income area, contributed 54% of the *Cx. quinquefasciatus* collected, but only 8% of the *Ae. aegypti*.

4.2. The Role of Vegetation on Elemental Concentration and Stable Isotopes

The elemental concentration values of the POM currently reflect the percentages of %C, %N, and C:N from the amount of material that was analyzed. However, it is important to consider that not only do these percentages matter, the actual amount of POM to the volume of water in a container is perhaps even more important. Murrell *et al.* notes that fine particulate organic matter from cemetery vases appears to be nutrient poor, thus a larger amount of it in a vase may support *Ae. albopictus* dominance (2011). More important differences for season and

canopy may be revealed if we took this new concentration by weighing the rest of the POM that was filtered from the water such as they did in the Yee *et al.* paper (2019).

Canopy seemed to be the most important factor for POM δ^{13}C. For POM, the δ^{13}C range was much smaller under the closed canopy than under the open canopy. Reflecting that, larval δ^{13}C varied by an interaction in season × canopy, which suggests that larvae undergo a switch in food source as the seasons change. δ^{15}N was not significantly different for any variables tested. The source of δ^{15}N seems to be consistent and similar between canopy types. Regardless of canopy or season, δ^{15}N in larvae remained similar among species, suggesting that larvae species were at the same trophic level.

Larval δ^{13}C and δ^{15}N were correlated to the POM in open and closed containers. Similarly, Yee *et al.* also found that larval stable isotopes correlated to the POM in container water samples (2019). On the other hand, neither of the δ^{13}C and δ^{15}N from either the larvae or POM were correlated with leaf values. This suggests that the nutrient content of the cemetery vases was influenced by a variety of detritus sources, not only leaves. In this study, leaf samples were treated the same, undivided among species, because most trees in the cemeteries were southern live oak (*Quercus virgiana*). In the future, separating leaves by stage of decomposition or by species could provide a better insight into the role of leaf litter in mosquito nutrition. Many of the vases contained algae, grasses, tree nuts, flowers, biofilms, or organisms, such as decaying invertebrates, which could contribute to nutrient source of the larvae. It is also important to note that the container that the larvae were collected from was not the same as the container they encountered one or two weeks ago when they first hatched.

No differences were found among species for elemental concentration or stable isotopes. This is unexpected since *Aedes* and *Culex* have different feeding behaviors that may lead to

differences in enrichment of ^{15}N (Yee *et al.*, 2015). Many collected samples did not have enough larvae to contribute the amount of material needed for elemental concentration and stable isotope analysis. Perhaps with more statistical power, we could observe some differences among species.

4.3. Conclusions

This study provides new insights into the ecology of container mosquito species in urban cemeteries. Although more research is needed to parse out differences among species and the specific nutrient sources of the larvae, this study found some differences in nutrient sources between open and closed canopies. New Orleans cemeteries provide an important habitat for invasive, vector *Aedes* species. *Ae. albopictus* did not seem to be affected by canopy cover or season, but *Ae. aegypti* was overall more abundant especially in the summer months. Although differences among species existed for abundance, no difference among species existed for elemental concentration or stable isotopes. Canopy cover seems to be the most important factor for $\delta^{13}C$. Values from a closed canopy seems to be much less variable when compared to open canopy. Therefore, the closed canopy could represent a less diverse, but more stable, nutrient source for larvae. Larvae were more similar to the POM than they were to leaves. More studies of this kind are needed in order to explore the importance of cemeteries in mosquito production and the influence of container habitats in driving mosquito segregation patterns within a city.

Appendix A. Larvae and Cemetery Raw Data

#	Cemetery	Temp	Total Canopy	Date	*Ae. albopictus*	*Ae. aegypti*	*Cx. quinquefasciatus*	*Cx. restuans*
1	Lafayette 2	10.9	0	1/24/2020	4			
2	Lafayette 2	13.2	83.36	1/24/2020		2		
3	St. Roch	10.6	0	1/24/2020				
4	Holt	12.9	100	1/24/2020			77	
5	Metairie	21.4	0	1/24/2020			57	
6	Metairie	14.3	0	1/24/2020			65	
7	Metairie	16.9	98.7	1/24/2020			9	
8	Metairie	20.7	96.1	1/24/2020			31	
9	St. Roch	7.8	0	2/28/2020	2			
10	St. Vincent	17.8	0	2/28/2020		4		
11	Holt	13.3	39.42	2/28/2020	1			
12	Metairie	14.8	0	2/28/2020			31	
13	Metairie	15.6	0	2/28/2020			1	
14	Metairie	22	0	2/28/2020			29	
15	Metairie	20.3	0	2/28/2020			32	
16	Carrollton	17.9	83.88	4/28/2020			70	
17	Carrollton	21.1	0	4/28/2020			97	
18	St. Vincent	30.2	0	4/28/2020		6		
19	Metairie	23.6	40.46	4/28/2020			13	
20	Metairie	25.6	91.16	4/28/2020			8	
21	Metairie	30	0	4/28/2020	5			
22	Holt	29.1	77.9	4/28/2020	6			

table cont'd.

#	Cemetery	Temp	Total Canopy	Date	Ae. albopictus	Ae. aegypti	Cx. quinquefasciatus	Cx. restuans
23	Lafayette 1	24.3	0	5/30/2020			80	
24	Lafayette 1	26.3	99.22	5/30/2020			3	
25	Lafayette 2	24.6	64.9	5/30/2020		59		
26	Lafayette 2	28.3	0	5/30/2020		2		
27	St. Vincent	27.5	0	5/30/2020	2	21		
28	Carrollton	24.3	98.96	5/30/2020		10		
29	Carrollton	27.8	0	5/30/2020		7		
30	Holt	25.9	100	5/30/2020	1			
31	Holt	25.8	87	5/30/2020	5			
32	Metairie	28.2	0	5/30/2020			44	
33	Metairie	26.9	100	5/30/2020			2	
34	Metairie	26.4	17.32	5/30/2020			86	
35	Metairie	29.9	100	5/30/2020	11		2	
36	Lafayette 1	26.7	0	6/27/2020		8		
37	Lafayette 1	26.2	100	6/27/2020			11	
38	Lafayette 2	26.8	75.82	6/27/2020		7		
39	Lafayette 2	27.6	0	6/27/2020		32		
40	St. Vincent	27.8	0	6/27/2020		13		
41	Carrollton	29	0	6/27/2020		31		
42	Carrollton	28.6	30.84	6/27/2020		17		
43	Holt	27.4	89.08	6/27/2020	15	1		
44	Metairie	28.1	100	6/27/2020		1		
45	Metairie	29.3	8.48	6/27/2020		2		
46	Metairie	29.7	0	6/27/2020		13		
47	Metairie	29.8	97.4	6/27/2020	4	1		

table cont'd.

#	Cemetery	Temp	Total Canopy	Date	*Ae. albopictus*	*Ae. aegypti*	*Cx. quinquefasciatus*	*Cx. restuans*
48	Metairie	31.4	0	6/27/2020	1			
49	St. Roch	31.7	0	8/1/2020		5		
50	St. Vincent	28.7	0	8/1/2020	3	4		
51	Lafayette 2	26.6	86.48	8/1/2020		6		
52	Lafayette 2	29.3	0	8/1/2020		6		
53	Carrollton	27	86.22	8/1/2020		26		
54	Holt	27.8	100	8/1/2020	44			
55	Metairie	28.2	100	8/1/2020		10		
56	Metairie	29.5	86.22	8/1/2020	22			
57	Metairie	31.1	52.42	8/1/2020	8			
58	Lafayette 2	27.1	0	8/29/2020		2		
59	Lafayette 2	28	71.4	8/29/2020		16		
60	St. Vincent	34.2	0	8/29/2020	1	2		
61	Carrollton	27.8	88.82	8/29/2020	9	7		
62	Metairie	28.4	100	8/29/2020		18		
63	Metairie	28.6	0	8/29/2020	1			
64	Lafayette 2	22.4	94.28	9/26/2020		46		
65	Lafayette 2	21.8	0	9/26/2020		1		
66	St. Vincent	22.7	0	9/26/2020		5		
67	Carrollton	24.6	61.26	9/26/2020		36		
68	Holt	24.1	100	9/26/2020	1			
69	Metairie	24.8	14.2	9/26/2020	47			
70	Metairie	27.5	0	9/26/2020	6			
71	Metairie	25.4	98.96	9/26/2020	25			
72	Metairie	28.9	0	9/26/2020	4			

table cont'd.

#	Cemetery	Temp	Total Canopy	Date	Ae. albopictus	Ae. aegypti	Cx. quinquefasciatus	Cx. restuans
73	Metairie	31.4	0	9/26/2020	57			
74	Lafayette 2	22.8	76.08	10/24/2020		23		
75	St. Roch	23.2	0	10/24/2020		1		
76	St. Vincent	23.5	0	10/24/2020	11	2		
77	Metairie	23.9	94.28	10/24/2020	4			
78	Lafayette 1	17.4	0	11/21/2020		1		
79	Lafayette 2	17.3	74	11/21/2020		7		
80	St. Roch	19	0	11/21/2020		1		
81	St. Vincent	19.8	0	11/21/2020		27		
82	Lafayette 2	11.8	85.7	12/19/2020		43		
83	Lafayette 2	10.6	0	12/19/2020		19		
84	St. Roch	12.6	0	12/19/2020		8		
85	St. Vincent	17.2	0	12/19/2020	1			
86	Metairie	21.9	0	12/19/2020	3			
87	Metairie	17.2	0	12/19/2020				4

Appendix B. Elemental Concentration and Stable Isotope Raw Data

#	Material	Specific Material	Date	Canopy	Carbon Content (%)	Nitrogen Content (%)	C:N	δ¹³C (‰)	δ¹⁵N (‰)
1	filter	filter	1/24/20	open	28.9	2.13	13.5	-28.6	3.2
2	filter	filter	1/24/20	closed	20.21	2.19	9.22	-27.3	4.8
2	leaf	leaf	1/24/20	closed	45.38	1.53	29.69	-29.8	3.9
3	filter	filter	1/24/20	open	12.76	1.06	12.01	-24.3	2.2
4	larvae	Cx. quinquefasciatus	1/24/20	closed	39.76	9.53	4.18	-23.5	12.9
4	filter	filter	1/24/20	closed	9.73	0.94	10.36	-26.7	5.1
4	leaf	leaf	1/24/20	closed	48.21	1.1	43.71	-29.6	7
5	larvae	Cx. quinquefasciatus	1/24/20	open	40.75	8.86	4.61	-24.1	7.4
5	filter	filter	1/24/20	open	16.28	1.58	10.27	-25.6	6.5
6	larvae	Cx. quinquefasciatus	1/24/20	open	44.84	10.38	4.32	-26.2	8
6	filter	filter	1/24/20	open	14.76	1.81	8.14	-26.6	6.2
7	larvae	Cx. quinquefasciatus	1/24/20	closed	42	10.49	4	-25.7	12.5
7	filter	filter	1/24/20	closed	25.15	2.47	10.15	-27.3	10.4
7	leaf	leaf	1/24/20	closed	48.85	0.88	55.73	-30.4	2.8
8	larvae	Cx. quinquefasciatus	1/24/20	closed	42.61	10.7	3.98	-25.6	18.5
8	filter	filter	1/24/20	closed	19.1	2.08	9.19	-28.5	8.4
8	leaf	leaf	1/24/20	closed	50.07	1.12	44.86	-29.7	3.8
9	filter	filter	2/28/20	open	21.55	1.06	20.29	-26	2.33
10	filter	filter	2/28/20	open	34.49	2.33	14.81	-22.67	3.7
11	filter	filter	2/28/20	open	10.74	0.61	17.65	-26.3	5.57
11	leaf	leaf	2/28/20	open	44.46	1.17	37.91	-27.5	7.1
12	larvae	Cx. quinquefasciatus	2/28/20	open	38.35	9.5	4.03	-29.05	5.9
12	filter	filter	2/28/20	open	29.45	2.44	12.08	-35.7	3.93

table cont'd.

#	Material	Specific Material	Date	Canopy	Carbon Content (%)	Nitrogen Content (%)	C:N	δ¹³C (‰)	δ¹⁵N (‰)
13	filter	filter	2/28/20	open	13.87	1.57	8.85	-27.47	9
14	filter	filter	2/28/20	open	23.32	2.43	9.6	-25.8	3.9
15	larvae	*Cx. quinquefasciatus*	2/28/20	open	46.5	10.28	4.52	-23.75	9.5
15	filter	filter	2/28/20	open	19.8	2.54	7.8	-25.27	4.97
16	larvae	*Cx. quinquefasciatus*	4/28/20	closed	31.06	6.36	4.88	-24.3	8.4
16	filter	filter	4/28/20	closed	4.88	0.53	9.17	-26.33	7.2
16	leaf	leaf	4/28/20	closed	41.12	1.33	30.84	-30.8	8.3
17	larvae	*Cx. quinquefasciatus*	4/28/20	open	41.76	8.91	4.69	-21.1	20
17	filter	filter	4/28/20	open	20.34	1.93	10.54	-22.93	13.37
18	larvae	*Ae. aegypti*	4/28/20	open	31.64	6.61	4.79	-23.8	4.8
18	filter	filter	4/28/20	open	25.38	0.96	26.45	-27.53	5.9
19	larvae	*Cx. quinquefasciatus*	4/28/20	open	47.07	9.15	5.15	-24.17	12.5
19	filter	filter	4/28/20	open	28.64	3.34	8.57	-27.8	7.4
19	leaf	leaf	4/28/20	open	47.03	1.21	38.9	-30.9	1.3
20	larvae	*Cx. quinquefasciatus*	4/28/20	closed	42.87	10.02	4.28	-28.45	2.85
20	filter	filter	4/28/20	closed	15.47	1.93	8.03	-30.17	2.73
20	leaf	leaf	4/28/20	closed	46	1.57	29.38	-32.3	2.1
21	larvae	*Ae. albopictus*	4/28/20	open	41.8	9.24	4.53	-26	3.17
21	filter	filter	4/28/20	open	31.4	2.86	10.98	-28.17	7.83
22	filter	filter	4/28/20	closed	15.61	1.25	12.5	-27.9	6.6
22	leaf	leaf	5/30/20	closed	45.62	1.07	42.75	-31.1	6.7
23	larvae	Cx. quinquefasciatus	5/30/20	open	40.51	3.93	10.31	-27.77	3.17
23	filter	filter	5/30/20	open	26.88	1.87	14.37	-26.33	5.1
24	filter	filter	5/30/20	closed	38.34	2.83	13.53	-27.27	6.33
24	leaf	leaf	5/30/20	closed	38.4	1.89	20.35	-31.6	4.2
25	larvae	*Ae. aegypti*	5/30/20	closed	44.13	7.68	5.75	-28.7	7.75

table cont'd.

#	Material	Specific Material	Date	Canopy	Carbon Content (%)	Nitrogen Content (%)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
25	filter	filter	5/30/20	closed	27.56	2.62	10.52	-27.73	5.03
25	leaf	leaf	5/30/20	closed	39.16	1.8	21.78	-31.7	4.5
26	filter	filter	5/30/20	open	24.31	2.26	10.77	-23	5.6
27	larvae	*Ae. aegypti*	5/30/20	open	28.98	6.41	4.52	-22	6.4
27	filter	filter	5/30/20	open	21.47	1.54	13.92	-22.43	5.97
28	filter	filter	5/30/20	closed	2.11	0.24	8.97	-26.1	6.17
28	leaf	leaf	5/30/20	closed	44.17	1.14	38.87	-29.8	3.8
29	filter	filter	5/30/20	open	9.34	0.91	10.23	-24.5	8.4
30	filter	filter	5/30/20	closed	13.79	1.16	11.93	-26.77	6.73
30	leaf	leaf	5/30/20	closed	45.15	1.22	36.89	-30.4	7.1
31	filter	filter	5/30/20	closed	13.98	1.45	9.63	-26.73	5.3
31	leaf	leaf	5/30/20	closed	33.2	1.09	30.52	-29.9	6.1
32	larvae	*Cx. quinquefasciatus*	5/30/20	open	34.76	4.61	7.53	-28.1	7.7
32	filter	filter	5/30/20	open	27.94	2.37	11.81	-27.3	4.97
33	filter	filter	5/30/20	closed	19.76	2	9.89	-27.53	7.43
33	leaf	leaf	5/30/20	closed	43.49	1.17	37.24	-29.8	3.5
34	larvae	*Cx. quinquefasciatus*	5/30/20	open	43.39	5.59	7.76	-25.2	3.67
34	filter	filter	5/30/20	open	32.51	2.43	13.38	-26.73	4.13
34	leaf	leaf	5/30/20	open	44.04	1.01	43.77	-29.9	3.3
35	larvae	*Ae. albopictus*	5/30/20	closed	37.62	8.88	4.24	-23.5	5.1
35	filter	filter	5/30/20	closed	10.78	0.73	14.82	-27.7	2.4
35	leaf	leaf	5/30/20	closed	47.06	1.79	26.32	-28.9	1.8
36	larvae	*Ae. aegypti*	6/27/20	open	37.44	9.72	3.85	-21.8	3.2
36	filter	filter	6/27/20	open	15.44	1.88	8.22	-24.2	1.9
37	larvae	*Cx. quinquefasciatus*	6/27/20	closed	41.79	10.03	4.17	-25.7	7.3
37	filter	filter	6/27/20	closed	24.05	1.82	13.12	-28.1	3.7

table cont'd.

#	Material	Specific Material	Date	Canopy	Carbon Content (%)	Nitrogen Content (%)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
37	leaf	leaf	6/27/20	closed	42.63	1.81	23.68	-30.2	6.2
38	larvae	*Ae. aegypti*	6/27/20	closed	38.26	9.64	3.97	-22.9	6.5
38	filter	filter	6/27/20	closed	23.49	2.17	10.83	-28.4	3.8
38	leaf	leaf	6/27/20	closed	39.86	2.09	19.08	-30.5	3.9
39	larvae	*Ae. aegypti*	6/27/20	open	42.53	8.34	5.09	-19.6	13
39	filter	filter	6/27/20	open	33.21	4.94	6.74	-19.8	9.3
40	filter	filter	6/27/20	open	27.71	1.85	15.24	-23.6	5.2
41	larvae	*Ae. aegypti*	6/27/20	open	39.13	7.64	5.12	-21.5	6.1
41	filter	filter	6/27/20	open	11.17	1	11.04	-23.4	5.6
42	larvae	*Ae. aegypti*	6/27/20	open	45.64	7.82	5.93	-23.2	3.2
42	filter	filter	6/27/20	open	15.01	1.2	12.43	-27	3.7
42	leaf	leaf	6/27/20	open	43.68	1.01	43.19	-30.3	3.6
43	larvae	*Ae. albopictus*	6/27/20	closed	41.47	8.67	4.79	-25	8.7
43	filter	filter	6/27/20	closed	13.8	1.23	11.35	-27.6	5.9
43	leaf	leaf	6/27/20	closed	38.9	1.86	21.01	-30	4.8
44	filter	filter	6/27/20	closed	20.73	1.74	11.87	-29	9.5
44	leaf	leaf	6/27/20	closed	46.04	1.19	38.98	-31.8	7.7
45	filter	filter	6/27/20	open	19.67	1.63	12.34	-27.5	7.8
45	leaf	leaf	6/27/20	open	42.29	0.82	51.91	-29.1	0.5
46	larvae	*Ae. aegypti*	6/27/20	open	44.84	7.17	6.26	-27.6	13.1
46	filter	filter	6/27/20	open	33.85	2.24	15.41	-29.3	6.1
47	filter	filter	6/27/20	closed	38.32	2.69	14.28	-28.5	3.4
47	leaf	leaf	6/27/20	closed	38.57	1.84	21.02	-31.9	4.2
48	filter	filter	6/27/20	open	30.2	2.68	11.3	-26.1	7.8
49	larvae	*Ae. aegypti*	8/1/20	open	41.69	9.45	4.41	-18.9	4.7
49	filter	filter	8/1/20	open	20.84	1.59	13.08	-21	1

table cont'd.

#	Material	Specific Material	Date	Canopy	Carbon Content (%)	Nitrogen Content (%)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
50	larvae	*Ae. aegypti*	8/1/20	open	39.25	7.28	5.39	-19.3	15.5
50	filter	filter	8/1/20	open	16.34	1.37	11.91	-20.6	9.2
51	larvae	*Ae. aegypti*	8/1/2020	closed	43.59	7.28	6.07	-25.4	6
51	filter	filter	8/1/20	closed	26.09	2.45	10.65	-27.4	5.5
51	leaf	leaf	8/1/2020	closed	42.52	2.28	18.62	-30.1	4.4
52	larvae	*Ae. aegypti*	8/1/20	open	45.23	7.56	5.98	-23	7
52	filter	filter	8/1/20	open	41.52	1.78	23.33	-27.4	2.6
53	larvae	*Ae. aegypti*	8/1/20	closed	39.46	9.1	4.34	-27.8	9.4
53	filter	filter	8/1/20	closed	26.34	2.2	11.95	-28.4	6.3
54	larvae	*Ae. albopictus*	8/1/20	closed	40.87	8.37	4.89	-24	10.9
54	filter	filter	8/1/20	closed	17.3	1.41	12.35	-27.4	6.1
55	larvae	*Ae. aegypti*	8/1/20	closed	42.46	8.56	4.96	-25	14
55	filter	filter	8/1/20	closed	37.43	2.91	12.72	-27.4	7.6
56	larvae	*Ae. albopictus*	8/1/20	closed	33.57	8.06	4.17	-24.5	5.2
56	filter	filter	8/1/20	closed	12.09	1.1	10.99	-28	3.9
57	larvae	*Ae. albopictus*	8/1/20	open	41.3	7.7	5.36	-25.6	5.1
57	filter	filter	8/1/20	open	17.73	1.21	14.71	-27.5	2.6
58	filter	filter	8/29/2020	open	11.37	0.91	12.54	-24.9	2.1
59	larvae	*Ae. aegypti*	8/29/2020	closed	46.11	8.17	5.66	-26.1	5.2
59	filter	filter	8/29/2020	closed	37.17	3.74	9.95	-26.5	1.6
60	filter	filter	8/29/2020	open	19.11	2.18	8.76	-21.2	10.7
61	larvae	*Ae. aegypti*	8/29/2020	closed	42.6	9.3	4.58	-28.7	8.1
61	larvae	*Ae. albopictus*	8/29/2020	closed	43.11	9.53	4.52	-29.8	8.2
61	filter	filter	8/29/2020	closed	35.45	2.66	13.31	-29.6	6
61	leaf	leaf	8/29/2020	closed	45.24	0.83	54.45	-28.1	8.4
61	leaf	leaf	8/29/2020	closed	47.5	1.43	33.28	-28.9	6

table cont'd.

#	Material	Specific Material	Date	Canopy	Carbon Content (%)	Nitrogen Content (%)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
62	larvae	*Ae. aegypti*	8/29/2020	closed	40.41	8.62	4.69	-26.6	9.2
62	filter	filter	8/29/2020	closed	23.76	2.29	10.34	-28.4	6.3
62	leaf	leaf	8/29/2020	closed	45.93	1.4	32.92	-30.9	1.1
63	filter	filter	8/29/2020	open	18.62	1.62	11.47	-28.8	2.9
64	larvae	*Ae. aegypti*	9/26/2020	closed	37.94	6.55	5.82	-25.5	10.3
64	filter	filter	9/26/2020	closed	25.89	2.77	9.34	-27.3	6.2
64	leaf	leaf	9/26/2020	closed	45.73	1.15	39.64	-31.5	1.9
65	filter	filter	9/26/2020	open	29.59	4.05	7.3	-19.1	5.2
66	filter	filter	9/26/2020	open	28.72	4.23	6.79	-21.3	11.2
67	larvae	*Ae. aegypti*	9/26/2020	closed	51.61	8.24	6.27	-28.9	3.4
67	filter	filter	9/26/2020	closed	19.23	1.72	11.22	-28.3	0.9
67	leaf	leaf	9/26/2020	closed	47.28	0.89	52.97	-28.9	3.1
68	filter	filter	9/26/2020	closed	4	0.42	9.47	-27.6	4.4
68	leaf	leaf	9/26/2020	closed	35.55	1.2	29.53	-28.8	4.1
69	larvae	*Ae. aegypti*	9/26/2020	open	51.91	8.46	6.14	-25.6	5.8
69	filter	filter	9/26/2020	open	11.88	1.63	7.29	-28.1	3.9
69	leaf	leaf	9/26/2020	open	46.24	0.91	50.71	-29.1	2.7
70	larvae	*Ae. albopictus*	9/26/2020	open	46.38	8.35	5.56	-19.7	7.1
70	filter	filter	9/26/2020	open	21.33	2.67	8.01	-22.7	6.5
71	larvae	*Ae. albopictus*	9/26/2020	closed	45.43	8.53	5.34	-29	6.2
71	filter	filter	9/26/2020	closed	19.91	1.59	12.58	-28	3.7
71	leaf	leaf	9/26/2020	closed	47.81	1.39	34.45	-29.5	2.7
72	filter	filter	9/26/2020	open	14.46	1.76	8.23	-23.5	3.5
73	larvae	*Ae. albopictus*	9/26/2020	open	52.38	7.15	7.55	-20.7	7.2
73	filter	filter	9/26/2020	open	13.65	1.71	7.99	-23.4	6.3
74	larvae	*Ae. aegypti*	10/24/2020	closed	45.15	8.32	5.43	-27.2	10.1

table cont'd.

#	Material	Specific Material	Date	Canopy	Carbon Content (%)	Nitrogen Content (%)	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
74	filter	filter	10/24/2020	closed	31.83	2.92	10.91	-28.2	7
74	leaf	leaf	10/24/2020	closed	46.94	1.38	34.12	-30.6	2.3
75	larvae	*Ae. aegypti*	10/24/2020	open	46.53	7.94	5.86	-23.6	9.8
75	filter	filter	10/24/2020	open	20.88	2.39	8.73	-22.4	9.2
76	larvae	*Ae. aegypti*	10/24/2020	open	39.95	9.19	4.35	-21.9	10.2
76	filter	filter	10/24/2020	open	32.11	3.64	8.83	-22.2	7.6
77	larvae	*Ae. albopictus*	10/24/2020	closed	45.96	8.59	5.35	-25.8	2.8
77	filter	filter	10/24/2020	closed	18.75	1.74	10.82	-29.6	1.5
77	leaf	leaf	10/24/2020	closed	47.1	0.92	51.27	-28.5	2.6

References

Allgood, D.W., and D.A. Yee. 2014. Asian tiger mosquito *Aedes albopictus* (*Stegomyia albopicta*) and the southern house mosquito *Culex quinquefasciatus*. *Medical and Veterinary Entomology* 28: 273-286.

Alto, B.W., L.P. Lounibos, S. Higgs, and S.A. Juliano. 2005. Larval competition differentially affects arbovirus infection in *Aedes* mosquitoes. *Ecology* 86: 3279-3288.

American Community Survey (ACS). 2017. 2013-2017 ACS 5 Year Data Profile. Available online: https://www.census.gov/acs/www/data/data-tables-and-tools/data-profiles/2017/.

Costanzo, K.S., B. Kesavaraju, and S.A. Juliano. 2005. Condition-specific competition in container mosquitoes: the role of noncompeting life-history stages. *Ecology* 86: 3289 3295.

Clements, A.N. 2000. *The Biology of Mosquitoes: Development, Nutrition, and Reproduction*. Cambridge, MA: CABI Publishing.

Darsie, R.F., and R.A. Ward. 2005. *Identification and Geographical Distribution of the Mosquitoes of North American, North of Mexico*. Gainesville, FL: University Press of Florida.

Daugherty, M.P, B.W. Alto, and S.A. Juliano. 2008. Invertebrate carcasses as a resource for competing *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology* 37: 364-372.

Deerman, J. 2018. Ecological Stoichiometry: What role does it play in the competition and spatial distribution patterns of *Aedes albopictus* and *Aedes aegypti*? [Master's thesis, The University of Southern Mississippi]. The Aquila Digital Community.

de Jesús Crespo, R., M. Harrison, R. Rogers, and R. Vaeth. 2021. Mosquito vector production across socio-economic divides in Baton Rouge, Louisiana. *International Journal of Environmental Research and Public Health* 18: 1420.

Dieng, H., G.M.S. Rahman, A.A. Hassan, M.R.C. Salmah, T. Satho, F. Miake, M. Boots, and A. Sazaly. 2012. The effects of simulated rainfall on immature population dynamics of *Aedes albopictus* and female oviposition. *International Journal of Biometeorology* 56: 113-120.

Harrington, L.C., T.W. Scott, K. Lerdthusnee, R.C. Coleman, A. Costero, G.G. Clark, J.J. Jones, S. Kitthawee, P. Kittayapong, R. Sithiprasasna, and J.D. Edman. 2005. Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. *The American Journal of Tropical Medicine and Hygiene* 72: 209-220.

Hawley, W.A., P. Reiter, R.S. Copeland, C.B. Pumpuni, and G.B. Craig. 1987. *Aedes albopictus* in North America: probable introduction in used tires from Northern Asia. *Science,* 236: 1114—1116.

Juliano, S.A. 2010. Coexistence, exclusion, or neutrality? A meta-analysis of competition between *Aedes albopictus* and resident mosquitoes. *Israel Journal of Ecology and*

Evolution 56: 325-351.

Juliano, S.A., G.F. O'Meara, J.R. Morrill, and M.M. Cutwa. 2002. Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. *Oecologia* 130: 458-469.

Kaufman, M.G., W. Goodfriend, A. Kohler-Garrigan, E.D. Walker, and M.J. Klug. 2002. Soluble nutrient effects on microbial communities and mosquito production in *Ochlerotatus triseriatus* habitats. *Aquatic Microbial Ecology* 29: 73-88.

Kaufman, M.G., K.S. Pelz-Stelinski, D.A. Yee, S.A. Juliano, P.H. Ostrom, and E.D. Walker. 2010. Stable isotope analysis reveals detrital resource base sources of the tree hole mosquito, *Aedes triseriatus*. *Ecological Entomology* 35: 586-593.

LaDeau, S.L., P.T. Leisnham, D. Biehler, and D. Bodner. 2013. Higher mosquito production in low-income neighborhoods of Baltimore and Washington, D.C.: Understanding ecological drivers and mosquito-borne disease risk in temperate cities. *International Journal of Environmental Research and Public Health* 10: 1505-1526.

Leisnham, P.T., S.L. LaDeau, and S.A. Juliano. 2014. Spatial and temporal habitat segregation of mosquitoes in urban Florida. *PLOS One,* 9(3).

Lounibos, L.P., G.F. O'Meara, S.A. Juliano, N. Nishimura, R.L. Escher, M.H. Reiskind, M. Cutwa, and K. Greene. 2010. Differential survivorship of invasive mosquito species in South Florida cemeteries: Do site-specific microclimates explain patterns of coexistence and exclusion? *Annals of the Entomological Society of America* 103: 757-770.

Lounibos, L.P., and S.A. Juliano. 2018. Where vectors collide: the importance of mechanisms shaping the realized niche for modeling ranges of invasive *Aedes* mosquitoes. *Biological Invasions* 20: 1913-1929.

Marini, F., B. Caputo, M. Pombi, G. Tarsitani, and A.D. Torre. 2010. Study of *Aedes albopictus* dispersal in Rome, Italy, using sticky traps in mark-release-recapture experiments. *Medical and Veterinary Entomology* 24: 361-368.

Moise, I.K., C. Riegel, and E.J. Muturi. 2018. Environmental and social demographic predictors of the southern house mosquito *Culex quinquefasciatus* in New Orleans, Louisiana. *Parasites and Vectors* 11: 249.

Murrell, E.G., and S.A. Juliano. 2008. Detritus type alters the outcome of interspecific competition between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology* 45: 375-383.

Murrell, E.G., K. Damal, L.P. Lounibos, and S.A. Juliano. 2011. Distributions of competing container mosquitoes depend on detritus types, nutrient ratios, and food availability. *Annals of the Entomological Society of America* 104: 688-698.

National Oceanic and Atmospheric Administration (NOAA). 2010. Data Tools: 1981-2010 Normals. Available online: https://www.ncdc.noaa.gov/cdo-web/datatools/normals (accessed on 28 February 2021).

National Oceanic and Atmospheric Administration (NOAA). 2020. Climate Data Online. Available online: https://www.ncdc.noaa.gov/cdo-web/search (accessed on 15 February 2021).

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703-718.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reinhold, J.M., C.R. Lazzari, and C. Lahondère. 2018. Effects of the environmental temperature on *Aedes aegypti* and *Aedes albopictus* mosquitoes: A review. *Insects* 9: 158.

Rey, J.R., N. Nishimura, B. Wagner, M.A.H. Braks, S.M. O'Connell, and L.P. Lounibos. 2006. Habitat segregation of mosquito arbovirus vectors in south Florida. *Journal of Medical Entomology* 43: 1134-1141.

Robert, M.A., R.C. Christofferson, N.J.B. Silva, C. Vasquez, C.N. Mores, H.J. Wearing. 2016. Modeling mosquito-borne disease spread in U.S. urbanized areas: the case of dengue in Miami. *PLOS One*, 11 (8).

Sota, T. and M. Mogi. 1992. Interspecific variation in desiccation survival time of *Aedes* (*Stegomyia*) mosquito eggs is correlated with habitat and egg size. *Oecologia*, 90(3): 353—358.

Sterner, R.W., and J.J. Elser (2002). *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton: Princeton UP.

Tabachnick, W.J. 1991. Evolutionary genetics and arthropod-borne disease: the yellow fever mosquito. *American Entomologist.* 37:14–26.

United States Census Bureau. 2010. QuickFacts. Available online: https://www.census.gov/quickfacts/fact/table/neworleanscitylouisiana,US/POP010210

Vezzani, D. 2007. Review: Artificial container-breeding mosquitoes and cemeteries: a perfect match. *Tropical Medicine and International Health*, 12(2): 299–313.

Winters, A.E., and D.A. Yee. 2012. Variation in performance of two co-occurring mosquito species across diverse resource environments: insights from nutrient and stable isotope analyses. *Ecological Entomology* 37:54-64.

Walker, E.D., R.W. Merritt, M.G. Kaufman, M.P. Ayres, and M.H. Riedel. 1997. Effects of variation in quality of leaf detritus on growth of the eastern tree-hole mosquito, *Aedes triseriatus* (Diptera: Culicidae). *Canadian Journal of Zoology* 75: 1529–1546.

Yee, D.A., M.G. Kaufman, and N.F. Ezeakacha. 2015. How diverse detrital environments influence nutrient stoichiometry between males and females of the cooccurring container mosquitoes *Aedes albopictus*, *Ae. aegypti*, and *Culex quinquefasciatus*. *PLOS One,* 10(8).

Yee, S.H., D.A. Yee, R. de Jesus Crespo, A. Oczkowski, F. Bai, and Stephanie Friedman. 2019. Linking water quality to *Aedes aegypti* and Zika in flood-prone neighborhoods. *Ecohealth* 16: 199-209.

Vita

Rachel Rogers is from Vicksburg, Mississippi. She earned her Bachelor of Science in Biology from the University of Southern Mississippi in 2018 where she wrote an honors thesis. Her undergraduate honors thesis has since been published in the Journal of Medical Entomology titled "Response of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) survival, life history, and population growth to oak leaf and acorn detritus." Rachel is passionate about the natural world and hopes to make a difference through her future career and volunteer work. She plans to receive her master's degree from Louisiana State University in August 2021.