Mosquito Larvae in Tires from Mississippi, United States: The Efficacy of Abiotic and Biotic Parameters in Predicting Spatial and Temporal Patterns of Mosquito Populations and Communities

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ABSTRACT Container systems, including discarded vehicle tires, which support populations of mosquitoes, have been of interest for understanding the variables that produce biting adults that serve as both nuisances and as public health threats. We sampled tires in six sites at three times in 2012 across the state of Mississippi to understand the biotic and abiotic variables responsible for explaining patterns of larvae of common species, species richness, and total abundance of mosquitoes. From 498 tires sampled, we collected >58,000 immatures representing 16 species, with the most common species including *Aedes albopictus* (Skuse), *Culex quinquefasciatus* (L.), *Orthopodomyia signifera* (Coquillett), *Aedes triseriatus* (Say), *Toxorhynchites rutilus septentrionalis* (Coquillett), and *Culex territans* (Walker) accounting for ~97% of all larvae. We also documented 32 new county records for resident species and recent arrivals in the state, including *Aedes japonicus japonicus* (Theobald) and *Culex coronator* (Dyar & Knab). Cluster analysis, which was used to associate sites and time periods based on similar mosquito composition, did reveal patterns across the state; however, there also were more general patterns between species and genera and environmental factors. Broadly, *Aedes* was often associated with factors related to detritus, whereas *Culex* was frequently associated with habitat variables (e.g., tire size and water volume) and microorganisms. Some *Culex* did lack factors connecting variation in early and late instars, suggesting differences between environmental determinants of oviposition and survival. General patterns between the tire environment and mosquito larvae do appear to exist, especially at the generic level, and point to inherent differences between genera that may aid in predicting vector locations and populations.

KEY WORDS arbovirus, *Aedes*, container, *Culex*, invasive

The ability to effectively predict the location and emergence of mosquito populations of public health concern has long been a goal of medical entomologists. Among aquatic habitats that produce mosquitoes, container systems, such as tree holes, cemetery vases, and tires are particularly tractable for observational and experimental studies (e.g., Juliano 1998, Kitching 2000, Vezzani 2007). Discarded vehicle tires are common and an important habitat for the larvae of >30 species of disease-carrying and nuisance mosquitoes in the United States (reviewed in Yee 2008). Furthermore, tires also serve an important conduit for the transportation and spread of both native (Berry

and Craig 1990) and invasive species (Hawley et al. 1987, Peyton et al. 1999). Because tires exist in a variety of human habitats, from urban to agricultural lands, they may support mosquito communities that have different habitat affinities, which may influence the disease threats experienced by the surrounding human population (e.g., LaDeau et al. 2013). A key feature lacking in many prior studies is how environmental conditions in and around tires affect mosquito populations and communities. This may be especially important for understanding species that are container specialists versus those that use containers as one of many aquatic habitats. As a consequence, our current conceptual understanding of how environmental factors affect mosquito populations in tires is lacking and in need of further examination.

Of the studies that have focused on how environmental factors affect mosquitoes that use tires, most have focused on a relatively small suite of factors (e.g., shading and volume; e.g., Beier et al. 1983b, Lampman

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et al. 1987) or focused on small geographic areas, where associations between mosquitoes and the tire environment have been limited to a single tire site within a single county (e.g., Beier et al. 1983a,b; Lampman et al. 1997; Costanzo et al. 2005; Kling et al. 2007). The limitation of many tire-related mosquito studies is perhaps the narrow geographic and temporal aspects that do not include larger more general patterns of emergence of different species. Recently, Yee et al. (2010) surveyed tires multiple times across Illinois to understand the potential mechanisms that explain the presence of mosquito larvae in tires. They found broad differences for some species between factors that explained early and late instars in tires, which they suggest points to a disconnect between oviposition choices by adult females and larval survivorship within a tire, especially of two common genera (*Aedes* and *Culex*). Such examinations of tire fauna are rare in the United States, which limits a more comprehensive understanding of general mosquito patterns at larger geographic scales. At the state level within the United States, relatively few investigations have examined the relationship between multiple environmental factors and mosquito community composition (e.g., Joy 2004, Kaufman et al. 2005), despite data suggesting that variation in species distribution and composition are influenced by environmental factors.

Here, we enumerated early- and late-instar larvae and the concomitant environmental conditions in tire container habitats within six counties across the state of Mississippi. This study represents the only multicounty tire survey in Mississippi, and one of the few for the southern coastal states (including Alabama, Louisiana, Florida, and Georgia; Yee 2008). The state of Mississippi has a population size of ~2,991,000 (31st) and is the 32nd largest among all 50 U.S. states with a land area of 75,515 km² (U.S. Census Bureau 2012). There are at least 61 species of mosquito found in Mississippi (Varnado et al. 2012) and mild winters and generally high spring and summer precipitation make it ideal for mosquito production. Historically, vector-borne diseases in Mississippi included La Crosse encephalitis, St. Louis encephalitis, and yellow fever; however, the potential for the spread of new infectious diseases (e.g., dengue and chikungunya) within the southern United States makes it essential to have a general understanding of the spatial and temporal pattern of species across this state (Varnado et al. 2012).

This study had three goals: 1) to understand the spatial and temporal variation in mosquito populations and communities across a wide geographic area, 2) to test the veracity of previous results of the relationship between specific environmental parameters and populations of mosquitoes in tires, established especially from northern United States surveys (e.g., Yee et al. 2010), and 3) to gather data from tires in Mississippi, a state that has historically received little scientific attention on mosquitoes in general, and container systems specifically (Varnado et al. 2012). Besides environmental factors in and around each tire, we enumerated early- and late-instar larvae. These data were used to test the hypotheses that environmental factors are

important for different 1) stages and 2) species of mosquitoes. As reported in previous work (Yee et al. 2010), it was assumed that because early instars (first and second) were only 1–2 d old their presence in a tire was reflective of oviposition responses of females to certain environmental factors, and less likely the result of factors related to survival. Conversely, we assumed that older instars (third, fourth, and pupae), having been in the aquatic tire environment for many days, would be most closely related to factors affecting larvae growth and survival. At present, little data exist linking oviposition or survival to aspects of the tire environment, and we affirm that these data could provide valuable predictive information regarding patterns of larval abundance.

Methods

Tire locations were distributed across the northern (Prentiss, Union, and Webster counties), central (Hinds), and southern (Adams, Harrison, and Lamar) portions of the state of Mississippi and comprised a geographic range of ~525 km north to south and 225 km east to west (Table 1; Figs. 1 and 2). In all cases, sites were located in different nonadjacent counties during all sampling times. Sites varied in distance from one another with the closest sites (Lamar and Harrison counties) ~110 km apart. Sites had been identified prior to sampling and were chosen based on the availability of tires. All sites were sampled three times at ~30-d intervals starting in mid-May 2012. Exceptions to this were the site in Prentiss, which was sampled only during the first period followed by a different site in Union (<1 km away) during the last two periods, and the tire repair facility in Hinds was replaced with a vehicle salvage business (<1 km away) during the last two sampling periods. These changes from the initial site to an alternative site nearby were made based on a lack of acceptable tires that met our criteria for subsequent sampling. Sites also varied in their location based on human habitation or active businesses (Table 1). Tire dealers in Hinds, Prentiss, and Adams counties sold a variety of new and used tires (i.e., automobile and truck), with each business having ~100 (Hinds, first sampling only, Adams) or 1,000 (Prentiss, first sampling only) tires located outdoors. The sites that contained abandoned tires (Harrison, Webster, and Union) each contained several hundred tires all located outdoors among vegetation. The salvage yards in Lamar and Hinds (second and third sampling only) contained ~100 tires distributed among vehicle salvage (Fig. 1). We recorded the Global Positioning System coordinates of each site, and the latitude was converted to decimals for analysis purposes.

We designated three categories of tires to test our hypothesis and predictions. The minimum criteria for the first tire category (hereafter 1° tires) was the presence of water, plant and animal detritus, and mosquito larvae. The second tire category (2° tires) was defined as containing water, plant, and animal detritus, and may or may not have contained larvae. The third tire category (3° tires) was defined as tires with the

Table 1. Information for tires sites sampled in Mississippi during 2012

County	Lamar	Harrison	Hinds	Adams	Prentiss/Union	Webster
City	Oloh	Gulfport	Jackson	Natchez	Booneville/Blue Springs	Eupora
Site description	Rural vehicle salvage yard	Urban roadside	Urban Tire repair facility/ Urban vehicle salvage yard	Urban tire repair facility	Urban tire repair facility/Rural roadside	Rural property
Sampling dates (2012)	15 May, 25 June, 30 July	21 May, 25 June, 30 July	15 May, 29 June, 2 Aug.	24 May, 5 July, 9 Aug.	28 May, 12 July, 14 Aug.	29 May, 12 July, 14 Aug.
Number of samples by date	31, 20, 30	30, 21 30	31, 20, 30	34, 20, 30	42, 20, 30	29, 20, 30
Human population density (km ²) ⁻¹	26.53	487.54	892.56	177.79	276.48/21.48	5.75

The number of tires sampled are indicated, with 1°, 2°, and 3° tires sampled during the first and last sampling dates and only 1° and 2° sampled during the second sampling date.

Fig. 1. Tire sites from across the state of Mississippi sampled during three sampling periods (May–August) 2012. Sites were located in (A) Lamar, (B) Harrison, (C) Hinds (May only), (D) Hinds, (E) Adams, (F) Prentiss (May only), (G) Webster, and (H) Union counties. Details and locations for individual sites can be found in Table 1 and Figure 2.

presence of larvae only, though any type of detritus may be present. These tire categories were determined based on visual inspection of tires in the field prior to sampling. Tire categories were used to test the relationship between larvae and environmental patterns (1° and 2° tires) and to identify more general spatial and temporal patterns in abundance and richness of mosquitoes (1°, 2°, and 3° tires). During the first and last sampling periods, we sampled all categories, but omitted 3° tires in the second sampling period. Sample size for each category was generally 15–16 for 1°, 4–5 for 2°, and 10–22 for 3° tires (total of 498 tires). Once

categorized, all tires were then sampled for invertebrates, and 1° and 2° tires were also sampled for a suite environmental factors that have been shown to affect container mosquito populations (Yee 2008, Yee et al. 2010): canopy cover, tire diameter, water depth and volume, water temperature, pH, protozoan abundance and richness, bacterial biomass production, algal biomass, and detritus. These environmental factors found elsewhere to be important for explaining larval abundance patterns (summarized in Yee 2008) were placed into three broad categories : microorganisms (protozoan richness, protozoan total abundance, bacterial

Fig. 2. Maps of Mississippi showing cluster analysis relationships among sites based on mosquito composition. Maps for each of the three clusters show the sites within counties and their time period (1 = early [15–29 May], 2 = middle [25 June–12 July], 3 = late [30 July–14 August, 2012]). County names are listed along the right side of Cluster 1. Time periods and sites that were associated with one another based on mosquito species composition are listed on the same cluster map.

biomass production, and algal biomass), nutrients (leaves, twigs, seeds, animal detritus, fine detritus, and pH), and those related to habitat attributes or the terrestrial matrix (hereafter, habitat; human density, canopy cover, tire diameter, water volume, water depth, and water temperature).

Canopy cover above each tire was measured using a spherical densimeter (range from 0 = no cover to 37 = total cover). The tire's rim diameter (tire size) was indicated on the sidewall of each tire. A hand ruler was used to measure the water depth and was placed in the middle of the fluid within the tire. Water temperature was measured using a handheld digital thermometer (YSI, Yellow Springs, OH), whereas pH was measured using test strips (Whatman, Florham Park, NJ). Before sampling the tire contents, we removed 125 ml of the fluid for microorganism measurements that was then kept in the dark and on ice for transport to the laboratory. The remaining contents of the tire were then removed with kitchen basters, hand pumps, or by cutting a hole in the side wall of the tire and pouring out the contents out. The volume of water from each tire also was measured at this time. After removal of the contents, the tire was rinsed with 125 ml of tap water and this was added to the original sample. Tire contents and the rinse were taken to the laboratory for quantification of mosquitoes and detritus. We divided detritus into six categories: wood (including twigs and bark), deciduous leaves, coniferous leaves (mostly pine needles), plant reproductive material (including seeds and fruit), fine particulates (including soil), and animal detritus (dead invertebrates); inorganic material (e.g., trash and rocks) was discarded. All detritus was

dried at 50°C for ≥ 48 h and weighed to the nearest 0.001 mg using a Ohaus Explorer Pro EP214 (Parsippany, NJ).

The 125 ml sample of water was used to measure algal biomass, protozoans, and bacterial productivity. Algal biomass ($\mu\text{g/liter}$) associated with collected water samples was estimated from chlorophyll *a* concentrations. One milliliter from each replicate sample was placed into sterile 15-ml polypropylene conical tubes containing 9 ml of high-performance liquid chromatography-grade ethanol. Samples were mixed thoroughly. After steeping overnight in the refrigerator (in darkness at 4°C), chlorophyll concentrations were determined by high-performance liquid chromatography using fluorescence detection (Murray et al. 1986). Production of new bacterial biomass was quantified by estimating protein synthesis (hereafter PS) using a tritiated L-leucine ($4,5\text{-}^3\text{H}$, 50 Ci mmol $^{-1}$) incorporation assay. The L-leucine incorporation assay technique is specific to bacteria in aquatic systems (Riemann and Azam 1992) and has been used to quantify bacterial productivity in container mosquito experiments (Kaufman et al. 2001; Yee et al. 2007a,b, 2010, 2014; Murrell and Juliano 2008). Specifically, we measured the water column PS, as in Yee et al. 2012. Protozoans (richness and abundance) in each tire were measured by first gently mixing samples, extracting 1.0 ml into a Sedgewick rafter cell, and enumerating at 100 $\times$ magnification in a compound light microscope (Kneitel and Chase 2004; Yee et al. 2010, 2012). If protozoans were too numerous to count, they were diluted and the final values adjusted based on that dilution. Protozoan identifications were made

based on the methods used by Pennak (1989) and Foissner and Berger (1996).

We counted and identified all mosquito larvae and separated them into two size classes based on instar: early (first and second instars) and late (third, fourth, and pupae). Because of the large numbers of early instars and potential mortality incurred during development, we used a subsampling protocol to assign individuals to species (e.g., Yee et al. 2012). Briefly, this procedure involved counting all early instars within a sample and then rearing them to the third or fourth stage. Of these, we identified 100 randomly chosen larvae to species (Darsie and Ward 2004) and all larvae were identified if <100 remained. We used the proportion of identified larvae (if >100) to assign species affiliations to the initial number of early instars. Larvae collected as late instars all were identified to species. We allowed pupae to eclose and then identified the adults to species, except for *Culex*, which, because of difficulties in identification among species, were identified only to genus. We hypothesized that *Aedes* and *Culex* mosquitoes would be found in association with different environmental factors. This hypothesis is based on past work in tire communities for these two common genera, which suggests that *Aedes* are often affected by factors related to food resources (e.g., detritus), whereas some *Culex* are affected by habitat factors (e.g., tire size) or microorganisms (e.g., protozoans; Yee et al. 2010, 2012). We assumed that some factors we measured (microorganism and detritus factors) could directly affect larvae success (late instars) or oviposition patterns (early instars). Although factors related to the habitat also could directly influence larvae (e.g., via effects on temperature), we assumed that it was much more likely to influence larvae indirectly by affecting oviposition patterns (early instars).

For each location, we generated an estimate of human population density based on data from the 2012 U.S. Census Bureau (<http://www.census.gov/>). Specifically, we identified the census tract that contained each site, and then determined the area and population size of that tract to generate a population density (km^2) coincident with each tire site (Table 1).

Statistical Analyses. We tested for differences in total mosquito richness and abundance ($\log + 1$ in both cases to meet assumptions of statistical tests) and the abundance of each species, regardless of stage across sampling periods (early, middle, and late summer) and sites using two-way multivariate analysis of variance with sampling period and site as the independent variables (PROC GLM; SAS Institute Inc. 2004, Cary, NC). Contributions to significant multivariate effects were interpreted using standardized canonical coefficients (Scheiner 2001). For these analysis all tires (1°, 2°, and 3°) were used. Although we did sample one of the sites only during the first sampling period (Prentiss County), it was geographically close enough to the one we used to replace it (Union County) that we considered it the same site for analyses purposes. The two sites we sampled in Hinds County were less than a km apart and were similar in terms of habitat parameters were also considered the same site.

Different mosquito stages (early or late) were related to the suite of environmental factors using canonical correspondence analysis (CCA) using 1° and 2° tires (we did not measure environmental factors for 3° tires). CCA is an ordination technique that considers species and environmental variables simultaneously (ter Braak 1986) and produces canonical axes that are constrained to optimize their relationship with a set of environmental variables represented, as vectors with species are represented by points on the diagram that characterize approximate weighted means of the species with respect to environmental variables. The distance between species points and vectors identifies the relative importance of environmental variables in explaining species occurrence; points closer to vectors are more influenced by that environmental variable than vectors farther away. The statistical significance of each environmental axis was evaluated by permutation tests, after first reducing the overall suite of environmental variables using a step-wise method based on Akaike's Information Criterion (AIC) (Akaike 1973). AIC is analogous to a goodness-of-fit test, and allows for the determination of which of various models provide a better fit (i.e., have a lower AIC score, Motulsky and Christopoulos 2004). We restricted CCA to taxa that had an average of ≥ 1 individual per tire regardless of stage in all tires during each sampling period (hereafter, common species). Tires lacking these common species were excluded from analyses. CCA was performed using the statistical package R (<http://www.r-project.org>). To avoid potential spurious relationships between taxa and environmental factors at sites that lacked those taxa, we used cluster analysis to group sites and time periods based on mosquito composition. Cluster analysis is a data mining technique that uses a dissimilarity matrix of values based on the original species present at each site and has been used to categorize habitats that produce mosquitoes (Stein et al. 2011, Amarasinghe and Rathnayake 2014). Hierarchical cluster analysis (Ward 1963) was run for all sites and sampling periods for a presence/absence matrix of all mosquito species. Individual groupings (i.e., clusters) of sites and time periods were then subjected to CCA. Cluster analysis and CCA were conducted in R (<http://www.r-project.org>).

Results

In total, 498 tires were sampled (1° + 2° $n = 361$, 3° $n = 137$) from which we encountered 16 species, with the most common, *Aedes albopictus* (Skuse), found in all sites in all sampling periods, and the rarest, *Anopheles quadrimaculatus* (Say), with two individuals found only in Adams County in the early sampling period (Table 2). Some species were geographically isolated [e.g., *Culex coronator* (Dyar & Knab) and *Aedes japonicus japonicus* (Theobald)], whereas others were found across all sites. The most common genera encountered from highest to lowest abundance were *Aedes* (three species), *Culex* (six species), and *Anopheles* (four species).

Mosquito richness and abundance per tire varied among sites (Pillai's Trace_{10, 960} = 0.201; $P < 0.001$),

Table 2. Mean abundance per tire across sites within counties across sampling periods (E, early; M, middle; L, late)

County →	Harrison			Lamar			Adams			Hinds			Webster			Prentiss/Union			Total
Sampling Period → Species ↓	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	
<i>Ae. albopictus</i>	9.7	8.5	68.2	127.0	54.8	117.9	86.8	73.1	193.9	36.1	55.1	40.1	127.3	157.9	173.2	38.7	117.7	55.1	43398
<i>Ae. japonicus</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.7	0.1	4.4	204
<i>Ae. triseriatus</i>	5.5	0.8	0.2	7.9	0.1	0.0	3.7	0.1	3.6	6.4	0.0	0.0	2.0	4.7	2.8	0.7	0.4	2.3	1221
<i>An. barberi</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33
<i>An. crucians</i>	0.0	0.1	0.0	0.2	0.2	0.0	0.0	0.0	0.1	0.8	0.1	0.0	0.1	0.0	0.8	0.1	0.0	0.0	69
<i>An. punctipennis</i>	0.0	1.3	0.0	0.0	0.2	0.0	<u>0.0</u>	<u>0.0</u>	<u>0.0</u>	<u>0.0</u>	<u>0.0</u>	<u>0.0</u>	0.1	0.0	0.0	0.0	0.0	<u>0.4</u>	53
<i>An. quadrimaculatus</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	<u>0.0</u>	<u>0.0</u>	<u>0.0</u>	<u>0.0</u>	0.0	0.0	0.0	0.0	0.0	0.0	2
<i>Cx. coronator</i>	0.0	0.0	0.0	0.9	19.9	4.4	0.0	0.4	1.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	655
<i>Cx. nigripalpus</i>	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	66
<i>Cx. quinquefasciatus</i>	8.5	23.2	0.0	4.0	10.1	2.8	59.4	11.9	52.1	69.1	9.4	1.4	0.7	0.2	<u>0.0</u>	0.4	4.5	0.2	7526
<i>Cx. restuans</i>	0.0	0.0	0.0	9.9	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	1.3	0.0	0.3	0.0	0.0	360
<i>Cx. salinarius</i>	0.3	0.2	0.0	0.0	0.0	2.0	0.0	0.0	0.0	1.9	0.0	0.4	0.0	0.0	0.0	0.0	0.0	<u>0.0</u>	132
<i>Cx. territans</i>	0.2	0.2	0.0	0.4	0.2	6.9	0.0	0.1	0.0	0.1	0.7	0.3	6.8	0.9	1.8	1.3	0.0	2.6	676
<i>Or. signifera</i>	0.0	0.0	0.0	0.0	0.7	0.0	0.1	23.7	33.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1479
<i>Ps. howardii</i>	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	10
<i>Tx. r. septentrionalis</i>	8.1	1.7	0.0	0.6	0.5	0.0	1.6	5.3	13.8	0.8	0.1	0.3	0.9	0.0	0.1	0.0	0.0	0.2	957
Total per site	969	765	2053	4665	1797	4051	5158	2292	5958	3595	1307	1295	3998	3309	5365	2740	843	3680	56840

Values with an underline are >0 but <0.1. The grand total from all sites and times is in bold.

sampling periods (Pillai's $\text{Trace}_{4, 960} = 0.063$; $P < 0.001$) and the interaction (Pillai's $\text{Trace}_{20, 960} = 0.223$; $P < 0.001$). Standardized canonical coefficients were large but opposite for N and S for the effects of site (SCC N = 1.253, S = -0.692), period (N = 1.137, S = -1.039), and site by period (N = -0.887, S = 1.221), and thus both generally contributed to the multivariate effects.

For the site by period interaction for richness, the southernmost county, Harrison, had more species per tire in the early sample compared with the other sampling times, whereas Lamar County had higher richness during the middle sampling time compared with the early period, with the late period intermediate to both (Fig. 3a). All other sites showed no variation in mean richness per time over time. In general, northern sites had fewer species compared with the south. Specifically, Adams had significantly more mean species per tire compared with Prentiss/Union, Webster, Hinds, and Harrison, with Lamar intermediate (Fig. 3a). For cumulative species richness, Lamar (12 species) had the highest, followed by Adams (11), Prentiss/Union, Webster, and Hinds (all with 10), with Harrison (9 species) the lowest.

For mosquito abundance, only Prentiss/Union showed variation in the number of mosquito larvae per tire, with the middle sampling period having fewer individuals than the late sampling periods and the early sampling period showing intermediate values (Fig. 3b); all other sites showed no variation in abundance per tire across sampling periods. There was variation in abundance across sites irrespective of sampling period, but unlike richness, the pattern did not follow a latitudinal gradient. Specifically, abundance was highest at Adams and Webster compared with Harrison, Hinds, and Prentiss/Union, and intermediate at Lamar (Fig. 3b).

Similar to the community patterns, the abundance of individual mosquito species also varied with site (Pillai's

$\text{Trace}_{80, 2345} = 0.609$; $P < 0.001$), by sampling period (Pillai's $\text{Trace}_{32, 932} = 0.192$; $P < 0.001$), and with their interaction (Pillai's $\text{Trace}_{60, 4740} = 0.714$; $P < 0.001$). The SCCs for the interaction of site and sampling period were largest for *Cx. coronator* (SCC = 0.921), *Culex quinquefasciatus* (L.) (0.287), *Culex nigripalpus* (Theobald) (-0.429), *Culex restuans* (Theobald) (-0.379), *Psorophora howardii* (Coquillett) (-0.160), *Ae. albopictus* (-0.154), and *Orthopodomyia signifera* (Coquillett) (-0.112). All other species had absolute values of SCCs < 0.010. Thus, these seven species likely contributed most to variation among sites and sampling times, however patterns for all species are detailed below.

Ae. albopictus was the most common species collected at all sites in almost all times and represented 76.4% of all larvae collected (Table 2). In general, *Ae. albopictus* was more abundant during the late compared with the early sapling periods, and appeared to be least abundant at the more urban sites (e.g., Hinds and Harrison). *Ae. albopictus* was more common as early instars (mean $\pm$ SE, 71.58 ± 4.01 per tire) compared with early instars (15.57 ± 0.90). *Cx. quinquefasciatus*, the second most abundant mosquito encountered (13.2%), was present at all sites at most times, but showed different abundance patterns at different sites and was generally more common at urban sites (e.g., Hinds and Adams; Table 2). It too was most common as early (14.01 ± 2.12) compared with late instars (1.04 ± 0.28). The third most common species, *Or. signifera* (2.6%), was restricted to two sites (Lamar and Adams) and became more common at later sampling times (Table 2). Another *Aedes*, *Aedes triseriatus* (Say), representing 2.2% of all larvae collected, was also found at all sites in most time periods and appeared to peak generally during the first sampling period (Table 2). The predatory species, *Toxorhynchites rutilus septentrionalis* (Coquillett), was found at most sites but did not show any consistent patterns, being

Fig. 3. Data for richness (a) and abundance (b) per tire from across six sites (arranged in a south to north gradient) and three summer sampling periods (early = 15–29 May, middle = 25 June–12 July, late = 30 July–14 August) in Mississippi during 2012. Sites or groups of sites that share the same letter are not significantly different (lowercase letters compare sampling periods within each county, whereas capital letters compare counties regardless of sampling period).

higher in abundance early at some sites (Harrison) and higher later at others (Adams); this species accounted for 1.7% of all larvae. Other *Culex*, including *Culex territans* (Walker) (1.2%), *Cx. restuans* (0.6%), *Culex salinarius* (Coquillett) (0.2%), *Cx. nigripalpus* (0.12%), and *Cx. coronator* (1.2%) had variable patterns of abundance. *Anopheles* in general were uncommon, with the four species collected, *Anopheles barberi* (Coquillett), *Anopheles crucians* (Wiedmann), *Anopheles punctipennis* (Ludlow), and *An. quadrimaculatus* representing <0.12% of all larvae. A new invader to the state, *Ae. j. japonicus*, was found in the two northern counties and increased in abundance toward the end of the sampling periods (Table 2). Finally, the predator *Ps. howardii* was only found at one time in Harrison and Webster counties (Table 2). This latter species has not been recorded from tires previously (Yee 2008).

Besides general patterns in abundance and occurrence, some collections represented new county records (based on Varnado et al. 2012). These include *An. barberi* (Adams), *An. crucians* (Adams, Webster, and Union), *An. punctipennis* (Lamar, Adams, Webster,

and Union), *An. quadrimaculatus* (Adams), *Ae. j. japonicus* (Webster, Prentiss, and Union), *Ae. triseriatus* (Adams, Webster, and Union), *Cx. coronator* (Lamar and Hinds), *Cx. nigripalpus* (Lamar), *Cx. quinquefasciatus* (Union), *Cx. restuans* (Prentiss), *Cx. salinarius* (Lamar and Union), *Cx. territans* (Harrison, Adams, Hinds, Prentiss, and Union), *Ps. howardii* (Webster), and *Tx. r. septentrionalis* (Harrison, Webster, and Union).

Cluster analysis reduced the 18 combinations of site by sampling periods down to three clusters (Fig. 2). Cluster 1 included sites in the north sampled early as well as the southernmost sites and Harrison during the early and middle sampling periods. This cluster had high richness (mean = 8.4 species) and all sites shared six species, including *Ae. albopictus*, *Ae. triseriatus*, *An. punctipennis*, *Cx. quinquefasciatus*, *Cx. territans*, and *Tx. r. septentrionalis*. Cluster 2 was similar to Cluster 1 except that it included those sites sampled during the middle and late sampling times. This cluster had the lowest overall mean richness (4.8 species), and all sites shared only two species, *Ae. albopictus* and *Ae. triseriatus*. Finally, Cluster 3 represented the remaining sites

Fig. 4. CCA biplot for mosquito species and stages and the environmental factors measured in tires for sites and sampling periods in Cluster 1 (Union, Webster, Hinds period 1, and Harrison periods 1 and 2) during 2012 in Mississippi ($n = 96$). The amount of variation explained by each axis is shown. Arrows indicate the direction and relative importance of the environmental factors microorganism category (PS, bacterial productivity via protein synthesis; ProtoN, protozoan abundance), detritus category (Fine, Leaf, Repro - Reproductive tissue, Animal, Pine, pH), Habitat category (Dia, tire diameter; Vol, water volume; Temp, water temperature; depth, water depth; human, human population density); species (a, *Ae. albopictus*; t, *Ae. triseriatus*; q, *Culex quinquefasciatus*; r, *Cx. territans*; x, *Tx. r. septentrionalis*) and stages (E, early instars; L, late instars) are indicated within symbols.

and times, including all sampling periods for Lamar and Adams counties, as well as the middle and late sampling periods for Hinds and late for Webster. This cluster had moderate richness (mean = 7.00 species), and sites shared only two species, *Ae. albopictus* and *Cx. quinquefasciatus*, although at least six of the sites also included *Ae. triseriatus*, *Cx. coronator*, *Cx. territans*, and *Tx. r. septentrionalis*.

Using step-wise selection, CCA for Cluster 1 reduced the 17 environmental variables down to 13, including two in the microorganism category (protozoan total abundance and bacterial biomass production), six nutrients (leaves, animal, pine, reproductive, fine detritus, and pH), and five habitat attributes (human density, tire diameter, water volume, water depth, and water temperature). These variables accounted for 46.9% of the variation among species ($F_{13, 76} = 4.670$; $P = 0.005$; Fig. 4). For Cluster 2, there were four variables selected, including one microorganism (protozoan richness), two nutrients (wood and reproductive detritus), and one habitat (water volume). This model explained 15.8% of the variation among species ($F_{4, 69} = 3.227$; $P = 0.010$; Fig. 5). Finally, the CCA for Cluster 3 resulted in selection of six variables, including one microorganism (bacterial biomass production), one nutrient (pH), and four habitat (water depth, water volume, canopy cover, and tire diameter). This model explained 14.9% of the variation among species ($F_{6, 157} = 6.158$; $P = 0.005$; Fig. 6).

Fig. 5. CCA biplot for mosquito species and stages and the environmental factors measured in tires for sites and sampling periods in Cluster 2 (Union periods 2 and 3, Webster 2, and Harrison 3) during 2012 in Mississippi ($n = 78$). The amount of variation explained by each axis is shown. Arrows indicate the direction and relative importance of the environmental factors Microorganism category (ProtoS, protozoan richness), Detritus category (Wood and Repro, Reproductive tissue), Habitat category (Vol, water volume); Species (a, *Ae. albopictus*; t, *Ae. triseriatus*; q, *Cx. quinquefasciatus*; r, *Cx. territans*) and stages (E, early instars; L, late instars) are indicated within symbols.

CCA for Cluster 1 generally resulted in two environmental gradients: habitat (human density, tire diameter, water volume, and temperature) and leaf detritus (pine needles and deciduous leaves) versus other detritus (reproductive, animal, fine, and pH) and microorganism (protozoan abundance and bacterial biomass). The first axis delineated *Ae. albopictus* (more common in large diameter tires with high pine detritus at lower temperatures with low human density) from *Cx. quinquefasciatus* (more common in higher temperature tires near high human densities with higher volume; Fig. 4). Other species were generally found along the second axis. Higher densities of *Tx. r. septentrionalis* were associated with high amount of animal and fine detritus, whereas *Cx. territans* densities were higher in tires with higher (more basic) pH and higher protozoan abundance. A greater mix of factors appeared to influence *Ae. triseriatus*, including animal and fine detritus and protozoan abundance. Although early and late instars for each species were generally associated with the same environmental factors (e.g., *Ae. albopictus* and *Tx. r. septentrionalis*), *Ae. triseriatus* early instars were more influenced by detritus types compared with late instars who were explained more by microorganisms (Fig. 4).

The CCA for the Cluster 2 resulted in fewer environmental factors and generally explained a lower amount of variation in mosquito abundance patterns compared with Cluster 1 (Fig. 5). This CCA had two environmental gradients: protozoan abundance and tire volume versus detritus (wood and reproductive tissue). These axes pointed to two mosquito assemblages based on genus, *Culex* associated with the first gradient and

Fig. 6. CCA biplot for mosquito species and stages and the environmental factors measured in tires for sites and sampling periods in Cluster 3 (Webster period 3, Hinds 2 and 3, Adams 1, 2, 3, and Lamar 1, 2, 3) during 2012 in Mississippi ($n = 173$). The amount of variation explained by each axis is shown. Arrows indicate the direction and relative importance of the environmental factors Microorganism category (PS - Bacterial productivity via protein synthesis), Detritus category (pH), Habitat category (Dia, tire diameter; Depth, water depth; Vol; water volume; Canopy, canopy cover); Species (a, *Ae. albopictus*; t, *Ae. triseriatus*; q, *Cx. quinquefasciatus*; r, *Cx. territans*; c, *Cx. coronator*; o, *O. signifera*; x, *Tx. r. septentrionalis*) and stages (E, early instars; L, late instars) are indicated within symbols.

Aedes associated with the second. Specifically, *Cx. quinquefasciatus* and *Cx. territans* were most likely to be found in high volume tires with high protozoan richness. Alternatively, *Ae. triseriatus* were found in higher abundance in tires with lots of wood, whereas *Ae. albopictus* were in high abundance in tires with higher reproductive detritus. Generally, the environmental factors for all species were similar between early and late instars (Fig. 5).

The final cluster produced a gradient that contained mostly habitat variables (canopy cover and water volume) and pH, whereas the second gradient was associated with water depth and bacterial productivity; tire diameter was intermediate to these axes (Fig. 6). Unlike Clusters 1 and 2, there were fewer obvious associations between species and the environmental gradients. In this case, *Culex* was found to be associated with various factors. For instance, high densities of *Cx. quinquefasciatus* were associated with high bacterial abundance and high (basic) pH, *Cx. territans* was related with high water depth, and late-instar *Cx. coronator* were found with high pH (Fig. 6). Patterns for *Aedes* were just as variable, with late-instar *Ae. triseriatus* were associated with tires with high pH and high bacterial productivity, whereas *Ae. albopictus* generally found in low volume and small diameter tires. Two other species were also apparent in this cluster, including *Or. signifera* (high abundance in large diameter tires) and *Tx. r. septentrionalis* (high abundance in high volume tires). There were greater differences in the environmental factors between mosquito stages

compared with Cluster 2 (e.g., *Cx. coronator*, *Cx. territans*, and *Ae. triseriatus*; Fig. 6).

Patterns among clusters for individual species or genera were variable, and no single factor(s) important for the same species or genera across clusters. However, there were some general patterns regardless of time periods and locations. Tires with high densities of *Aedes* (i.e., *Ae. albopictus* and *Ae. triseriatus*) generally contained high amounts of various types of detritus (i.e., pine, animal, wood, and reproductive). *Culex*, especially *Cx. quinquefasciatus* and *Cx. territans*, in contrast, were found in high densities in tires with high water volumes at higher water temperatures containing high protozoan abundances or species richness. *Tx. r. septentrionalis*, the only other species found across clusters, was mostly found in high abundance in larger water volumes (Figs. 4 and 6). In most cases, different stages of the same species were likely to be explained by the same environmental factor(s), with the exceptions of *Ae. triseriatus* (Figs. 4 and 6), *Cx. territans* (Fig. 6), and *Cx. coronator* (Fig. 6).

Discussion

We collected mosquitoes from discarded vehicle tires from across the state of Mississippi to document spatial and temporal variation in mosquito populations and communities, to test the robustness of previous results for the relationships between specific environmental parameters and stages and species of mosquitoes documented elsewhere, and to gather mosquito occurrence data from tires in Mississippi. Although no tire surveys exist for the state of Mississippi, our data identified mosquito species known to occur in the state (Varnado et al. 2012, Yee et al. 2012) and that occur in tires in adjacent states (e.g., Alabama; Qualls and Mullens 2006). However, the fact that we were able to establish almost three dozen new country records speaks to the general paucity of distributional information for species in Mississippi.

Analyses of communities and populations of mosquitoes for the state suggest some broad patterns. Richness of mosquito communities generally followed a latitudinal gradient, with southern sites having more species on average than northern sites; however, abundance of mosquitoes did not follow this pattern. The data for latitude as a predictive factor explaining species richness are uncommon for mosquito communities (e.g., Yee et al. 2007c), although such examinations could be important for understanding larger geographic trends. Individual sites, at least based on the presence and absence of species, did follow a strong pattern across the state. Cluster analysis produced three groupings of sites based on species composition, with the first two containing basically the same set of sites that were sampled at different times, whereas the last cluster grouped sites mainly in the south-central region at all times. Cluster 1 was generally the strongest (most shared species) and contained high overall richness per site but generally only included early sampling. In contrast, Cluster 2, which had essentially the same sites as Cluster 1, was weaker (fewer shared species) and also

had much lower richness. Cluster 2 contained sites sampled during the second sampling period when we did not sample 3° tires. Thus, lower sample size may have affected the data by reducing the chances of sampling all possible species. However, a lower number of tires seem an unlikely explanation for the lower similarities among sites given that some sites (e.g., Hinds and Harrison) sampled during the middle period grouped in other clusters. It is more likely that differences between clusters were because of temporal changes in mosquito populations among sites. The final cluster contained sites in the southeastern part of the state, and were usually located at active tire businesses (Adams) or salvage faculties (Lamar and Hinds). Whether or not this specific characteristic of a site helps to explain why these sites clustered together is untested, as we did not include the site category in any of our examinations. Based on cluster analysis, it does appear that simple geographic ranges cannot easily explain the presence of a species at a site.

By far the dominant species we encountered was *Ae. albopictus*, which now occurs across much of the eastern United States after invading during the mid-1980s (Hawley et al. 1987). Similar to the neighboring state of Alabama (Qualls and Mullen 2006), this species represented >70% of all mosquitoes recovered from tires in our study. *Aedes albopictus* showed peak abundance generally during the middle or late sampling periods (e.g., Varnado et al. 2012), although it was found at all sites at all times (Table 2). Considered to be an urban and suburban species (Juliano and Lounibos 2005, Bartlett-Healy et al. 2012), *Ae. albopictus* was still the dominant species at the most rural sites (i.e., with the lowest human population densities; Webster and Union counties). This species is of relatively high medical importance as a vector of several arboviruses (Mitchell et al. 1992; Gerhardt et al. 2001; Turell et al. 2001, 2005). *Cx. quinquefasciatus*, the second most common species in tires, was especially common in more urban areas (e.g., Hinds and Adams counties). Work in tires by others in the adjacent state of Alabama showed that *Cx. quinquefasciatus* occurred at much lower densities and was found to exhibit a much smaller geographic range (Qualls and Mullen 2006). Differences between the distributions for these states may point to differences in sampling methods, selection of sites, or sampling times. *Cx. quinquefasciatus* is considered an important vector of West Nile virus and St. Louis encephalitis in bird populations, and a bridge vector of these same diseases to humans (Sardelis et al. 2001, Foster and Walker 2002, Reisen et al. 2005). The two most dominant species (*Ae. albopictus* and *Cx. quinquefasciatus*) appear to shown signs of density dependence, given that far fewer late instars were found in tires compared with early instars. A variety of mechanism may explain the overall low production of late instars in tires (e.g., predation); however, density dependence regulation due to limited resources is often hypothesized for containers (Juliano 2009). Another *Culex*, *Cx. territans*, was found at all sites, albeit at low densities. Past studies elsewhere have led to the conclusion that this species is not an important tire inhabitant (Jamieson et al.

1994); however, our data and past work (Yee et al. 2012) suggest that this species does represent a common tire species across the state. Other species were either geographically widespread but occurred at low abundance (e.g., *Ae. triseriatus*, *An. punctipennis*, *Cx. restuans*, and *Tx. r. septentrionalis*) or were more abundant at only a few sites (e.g., *Or. signifera*, *Ae. j. japonicus*, and *Cx. coronator*). Of these, the presence of two relatively new species to the state, *Ae. j. japonicus* (Thorn et al. 2012) and *Culex coronator* (Varnado et al. 2005) were of note. Previous work has shown each species being restricted to 1 (*Ae. j. japonicus*, Varnado et al. 2012) or 14 counties (*Cx. coronator*, Varnado et al. 2012), and thus our additional county records indicate a wider distribution across the state. However, laboratory studies suggest that neither species is likely to be competitively dominant as larvae compared with other common container species (e.g., Freed and Leisnham 2014, Kaufman and Fonseca 2014, Yee and Skiff 2014); therefore, although their range may expand over time, it is unlikely it will be at the expense of other container species.

One of our main goals was to test the efficacy of patterns between environmental factors in tires and the abundance of container species, especially with reference to different stages of larvae. Our hypothesis that *Aedes* and *Culex* would be found in association with different environmental factors was supported. As with previous work regarding the relationship between environmental factors and the presence of early and late instars (Yee et al. 2010, 2012), we did find congruence with factors that were related to the two major mosquito genera in tires. Specifically, the most common *Aedes* (*Ae. albopictus* and *Ae. triseriatus*) were found associated with various forms of detritus, whereas the most common *Culex* (*Cx. quinquefasciatus*, *Cx. territans*, and *Cx. coronator*) were associated with factors related to the tire habitat, including tire size and water volume, as well as to the presence of microorganisms. This study also identified environmental factors important for two other species not found in other studies, *Tx. r. septentrionalis* (animal and fine detritus and water volume) and *Or. signifera* (tire diameter); however, these species were found at low abundance or were more geographically isolated (Table 2), making broad conclusions about observed patterns difficult. In the case of *Tx. r. septentrionalis*, given their predatory behavior, it is possible that they respond to factors like prey densities that were not measured here, although one could speculate that detrital concentrations and water volume may both indirectly indicate prey presence as well.

Environmental factors that affect patterns of abundance for *Culex* and *Aedes* could be related to a number of generic-level attributes, including oviposition behavior of adults and feeding behavior and resource requirements of larvae (Yee et al. 2010). *Culex* populations were often related to microorganisms, and as a group, their larvae tend to filter feed on microorganisms and dissolved nutrients in the water column (Dahl et al. 1988, Merritt et al. 1992), although feeding style can be flexible (Yee et al. 2004, Skiff and Yee 2014).

Thus, the water-column microorganisms we measured, including bacteria and protozoans, may be more predictive for *Culex* compared with *Aedes*, the latter group primarily feeding on microorganisms attached to container or detrital surfaces (Merritt et al. 1992). Conversely, *Aedes* were affected more by types of detritus, with *Ae. albopictus* responding to pine needles and plant reproductive tissue, whereas *Ae. triseriatus* were affected by animal, wood, and fine detritus. As mentioned, *Aedes* often graze on container surfaces, and this feeding behavior may give them an advantage in some low resources environments, especially when resources such as nitrogen are limiting (Winters and Yee 2012). In two of our clusters (1 and 2), protozoans were important for *Cx. quinquefasciatus* and *Cx. territans*. Protozoans are member of the aquatic prey food web that larval mosquitoes consume (Cochran-Stafira and von Ende 1998, Eisenberg et al. 2000, Kaufman et al. 2002, Yee et al. 2007c). However, no evidence of a unique nutritional role for protozoans on performance of larval *Cx. quinquefasciatus* and *Cx. coronator* has been found (Skiff 2013), and oviposition responses to these same microorganisms have not been documented. Thus, it remains unknown the nature of the specific role of protozoans in explaining *Culex* populations in tires. Noticeably absent from CCA was algal biomass, suggesting a limited or inconsequential role for algae in affecting mosquitoes, a result found in other tire studies (Beier et al. 1983b, Kling et al. 2007) and specifically for oviposition of others (i.e., *A. j. japonicus*, Lorenz et al. 2013).

Besides responding to general factors associated with tires, the segregation of genera indicated by CCA could be explained by larval interactions or avoidance by gravid females to conspecific or congeneric larvae (Yee et al. 2010). *Ae. albopictus* has been shown to competitively dominate some container inhabiting *Culex*, including *Cx. coronator* (Yee and Skiff 2014), *Cx. quinquefasciatus* (Allgood and Yee 2014), and *Culex pipiens* L. (Carrieri et al. 2003, Costanzo et al. 2005) and thus avoidance may explain differences in occurrence patterns for these species. Some container inhabiting *Culex* have been shown to increase their egg laying to conspecific eggs (Bruno and Laurence 1979) and larvae (Resikind and Wilson 2004, Allan et al. 2005), perhaps because of egg aggregation pheromones (Laurence and Pickett 1982). Studies that may reveal oviposition responses of *Culex* to *Aedes* and vice versa do not exist, although such studies may prove useful in helping to explain patterns of larval occurrence.

Differences in environmental factors important for each stage of mosquito larvae were more evident for *Culex*, especially *Cx. quinquefasciatus*, *Cx. Territans*, and *Cx. coronator*. *Culex restuans*, a species collected in tires but with densities too low to evaluate here, has been shown to oviposit in high- and low-resource environments equally, even though larvae perform better with higher nutrients (Reiskind et al. 2004). This is one of the few examples to show a potential disconnect between oviposition and larval performance that was illustrated in this work and previous studies (Yee et al. 2010). Besides *Culex*, *Ae. triseriatus* did exhibit some

disconnect between factors that explain early- and late-instar larvae. For instance, in the first cluster, early instars were related to animal and fine detritus, whereas later instars were related to protozoan abundance and pH. Different stages of *Ae. triseriatus* were previously shown to generally respond to the same factors in tires (Yee et al. 2010), and it is unclear why these data sets differ; however, this species occurred at lower abundance in these collections. Lower abundance may have led to more noise in the data, and thus may have led to a greater heterogeneity of response to environmental factors among tires.

The differences between genera were indeed consistent with those identified from tires sampled in Illinois, a distance of many hundreds of kilometers away; therefore, the responses of these genera to certain nonshared environmental factors do seem to be robust to changes in geography and species composition. The reality that some species of *Aedes* and *Culex* do not overlap between these states (e.g., *Aedes atropalpus* (Coquillett) and *Cx. pipiens* only in Illinois) does speak to the importance of these broad environmental factors to these genera. Moreover, *Culex*, unlike most *Aedes*, are not container specialists and, therefore, the kinds of environmental factors that they respond to may be more reflective of their common open-water habitats (e.g., ponds and ditches). Although little work has been conducted to explore what may affect the differences between populations of *Culex* and *Aedes* within tires, differences between genera in oviposition behavior and performance in different habitats may also reflect past evolutionary divergences, as recent phylogenetic work suggests that the formation of *Culex* and *Aedes* was separated by many millions of years (Reidenbach et al. 2009). The fact that *Aedes* and *Culex* presence in tires are influenced by different suites of environmental variables may be useful when designing vector control strategies, especially given differences in their capability to vector disease. For instance, as some *Culex* are affected by aspects of the tire itself, perhaps steps could be taken to affect tire storage and placement in areas where tires cannot be quickly removed. Tire stacking methods do affect larval abundance (Novak et al. 1990) and this approach could prove cost-effective for controlling some groups of mosquitoes. Similarly, as *Aedes* are affected by types of detritus, much of which emanates from overhanging vegetation, moving tires away from trees or other plants would reduce detrital loads and thus decrease larval production for *Aedes* specifically, and mosquitoes in general. Strategies such as this would not eliminate all adult production from nuisance tire piles, but may reduce densities down to manageable levels to make local control efforts more effective.

The mosquito fauna of Mississippi is diverse, and contains at least 61 species (Varnado et al. 2012), a richness comparable with other southern Gulf coast states (Darsie and Ward 2005). We identified 16 species that use tires from just 6 of the 82 counties across the state, and thus it is likely more species would be identified with further sampling. This data set does add to a growing body of evidence that posits that patterns

of larvae of the two most common genera, *Culex* and *Aedes*, can be explained by different factors found in the tire environments. The veracity of these patterns needs to be explored across other locations where differences in underlying environmental patterns may help to reinforce or refute them. In addition, there are few mechanistic studies of the causes of the responses of either oviposition or larval performance to these factors most important for each genus. Such tests to understand the nature of response of either gravid females or larvae to certain features of these containers is the logical next step to better understand tire mosquito communities.

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References Cited

- Akaike, H. 1973.** Information theory and an extension of the maximum likelihood principle, pp. 267–281. In B. N. Petrov and F. Csaki (eds.), 2nd International Symposium on Information Theory (Tsahkadsor, 1971). Akademia Kiado, Budapest, Hungary.
- Allan, S. A., U. R. Bernier, and D. L. Kline. 2005.** Evaluation of oviposition substrates and organic infusions on collection of *Culex* in Florida. *J. Am. Mosq. Control Assoc.* 21: 268–273.
- Allgood, D. W., and D. A. Yee. 2014.** Influence of resource levels, organic compounds, and laboratory colonization on interspecific competition between *Aedes albopictus* and *Culex quinquefasciatus* (Diptera: Culicidae). *Med. Vet. Entomol.* 28: 273–286.
- Amarasinghe, L. D., and A.R.L.K. Rathnayake. 2014.** Prevalence of micro fauna associated with different mosquito breeding habitats in a selected area of Sri Lanka. *Int. J. Curr. Microbiol. Appl. Sci.* 3: 587–598.
- Bartlett-Healy K., I. Unlu, P. Obenauer, T. Hughes, S. Healy, T. Crepeau, A. Farajollahi, B. Kesavaraju, D. Fonseca, G. Schoeler, et al. 2012.** Larval mosquito habitat utilization and community dynamics of *Aedes albopictus* and *Aedes japonicus* (Diptera: Culicidae). *J. Med. Entomol.* 49: 813–824.
- Beier, J. C., C. Patricoski, M. Travis, and J. Krantzfelder. 1983a.** Influence of water chemical and environmental parameters on larval mosquito dynamics in tires. *Environ. Entomol.* 12: 434–438.
- Beier, J. C., C. Patricoski, and J. Krantzfelder. 1983b.** Habitat segregation among larval mosquitoes in tire yards in Indiana, USA. *J. Med. Entomol.* 20: 76–80.
- Berry, W. J., and G. B. Craig, Jr. 1984.** Bionomics of *Aedes atropalpus* breeding in scrap tires in northern Indiana. *Mosq. News* 44: 476–484.
- Bruno, D. W., and B. R. Laurence. 1979.** The influence of the apical droplet of *Culex* egg rafts on oviposition of *Culex pipiens fatigans* (Diptera: Culicidae). *J. Med. Entomol.* 16: 300–305.
- Carrieri, M., M. Bacchi, R. Bellini, and S. Maini. 2003.** On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. *Environ. Entomol.* 32: 1313–1321.
- Cochran-Stafira, D. L., and C. N. von Ende. 1998.** Integrating bacteria into food webs: Studies with *Sarracenia purpurea* ingulines. *Ecology* 79: 880–898.
- Costanzo, K. S., K. Mormann, and S. A. Juliano. 2005.** Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *J. Med. Entomol.* 42: 559–570.
- Dahl, C., L.-E. Widahl, and C. Nilsson. 1988.** Functional analysis of the suspension feeding system in mosquitoes (Diptera: Culicidae). *Ann. Entomol. Soc. Am.* 81: 105–127.
- Darsie, R. F., and R. A. Ward, Jr. 2005.** Identification and geographical distribution of the mosquitoes of North America, North of Mexico. Gainesville University Press, Gainesville, FL.
- Eisenberg, J.N.S., J. O. Washburn, and S. J. Schreiber. 2000.** Generalist feeding behaviors of *Aedes sierrensis* larvae and their effects on protozoan populations. *Ecology* 81: 921–935.
- Foissner, W., and H. Berger. 1996.** A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. *Freshw. Biol.* 35: 375–482.
- Foster, W. A., and E. D. Walker. 2002.** Mosquitoes (Culicidae), pp. 245–249. In G. Mullen and L. Durden (eds.), *Medical and veterinary entomology*. Academic Press, New York, NY.
- Freed, T. Z., and P. T. Leisnham. 2014.** Roles of spatial partitioning, competition, and predation in the North American invasion of an exotic mosquito. *Oecologia* 175: 601–611.
- Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001.** First isolation of LaCrosse virus from naturally infected *Aedes albopictus*. *Emerg. Infect. Dis.* 37: 807–811.
- Hawley, W. A., P. Reiter, R. S. Copeland, C. B. Pumpuni, and G. B. Craig, Jr. 1987.** *Aedes albopictus* in North America: Probable introduction in used tires from northern Asia. *Science* 236: 1114–1116.
- Jamieson, D. H., L. A. Olson, and J. D. Wilhide. 1994.** A larval mosquito survey in northeastern Arkansas including a new record for *Aedes albopictus*. *J. Am. Mosq. Control Assoc.* 10: 236–239.
- Joy, J. E. 2004.** Larval mosquitoes in abandoned tire pile sites from West Virginia. *J. Am. Mosq. Control Assoc.* 20: 12–17.
- Juliano, S. A. 1998.** Species introduction and replacement among mosquitoes: Interspecific resource competition or apparent competition? *Ecology* 79: 255–268.
- Juliano, S. A. 2009.** Species interactions among larval mosquitoes: Context dependence across habitat gradients. *Ann. Rev. Entomol.* 54: 37–56.
- Juliano, S. A., and L. P. Lounibos. 2005.** Ecology of invasive mosquitoes: Effects on resident species and on human health. *Ecol. Lett.* 8: 558–574.
- Kaufman, M. G., and D. M. Fonseca. 2014.** Invasion biology of *Aedes japonicus japonicus* (Diptera: Culicidae). *Ann. Rev. Entomol.* 59: 31–49.
- Kaufman, P. E., L. C. Harrington, J. K. Waldron, and D. A. Rutz. 2005.** The importance of agricultural tire habitats for mosquitoes of public health importance in New York state. *J. Am. Mosq. Control Assoc.* 21: 171–176.
- 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. *Aquat. Microb. Ecol.* 29: 73–88.
- Kaufman, M. G., S. N. Bland, M. E. Worthen, E. D. Walker, and M. J. Klug. 2001.** Bacterial and fungal biomass responses to feeding by larval *Aedes triseriatus* (Diptera: Culicidae). *J. Med. Entomol.* 38: 711–719.
- Kitching, R. L. 2000.** Food webs and container habitats. The natural history and ecology of phytotelmata. Cambridge University Press, Cambridge, United Kingdom.
- Kling, L. J., S. A. Juliano, and D. A. Yee. 2007.** Larval mosquito communities in discarded vehicle tires in a forested and unforested site: Detritus type, amount, and water nutrient differences. *J. Vector Ecol.* 32: 207–217.
- Kneitel, J. M., and J. M. Chase. 2004.** Disturbance, predator, and resource interactions alter container community composition. *Ecology* 85: 2088–2093.
- LaDeau, S. L., P. T. Leishnam, D. Biehler, and D. Bodner. 2013.** Higher mosquito production in low-income neighborhoods of Baltimore and Washington, DC: Understanding ecological drivers and mosquito-borne disease risk in temperate cities. *Int. J. Environ. Res. Public Health* 10: 1505–1526.
- Lampman, R., S. Hanson, and R. J. Novak. 1997.** Seasonal abundance and distribution of mosquitoes at a rural waste tire site in Illinois. *J. Am. Mosq. Control Assoc.* 13: 193–200.
- Laurence, B. R., and J. A. Pickett. 1985.** An oviposition attractant pheromone in *Culex quinquefasciatus* Say (Diptera: Culicidae). *Bull. Entomol. Res.* 75: 283–290.
- Lorenz, A. R., E. D. Walker, and M. G. Kaufman. 2013.** Does autochthonous primary production influence oviposition by *Aedes japonicus japonicus* (Diptera: Culicidae) in container habitats? *J. Med. Entomol.* 50: 67–78.
- Merritt, R. W., R. H. Dadd, and E. D. Walker. 1992.** Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annu. Rev. Entomol.* 37: 349–376.
- Mitchell, C. J., M. L. Niebylski, N. Karabatsos, D. Martin, and J. P. Mutebi. 1992.** Isolation of eastern equine encephalitis from *Aedes albopictus* in Florida. *Science* 257: 526–527.
- Motulsky, H., and A. Christopoulos. 2004.** Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting. Oxford University Press, Oxford, United Kingdom.
- Murray, A. P., C. F. Gibbs, and A. R. Longmore. 1986.** Determination of chlorophyll in marine waters: Intercomparison of a rapid HPLC method with full HPLC, spectrophotometric and fluorometric methods. *Mar. Chem.* 19: 211–227.
- Murrell, E. G., and S. A. Juliano. 2008.** The role of detritus type in interspecific competition and population distributions of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *J. Med. Entomol.* 45: 375–383.
- Novak, R. J., B. A. Steinly, D. W. Webb, L. Haramis, J. Clarke, Jr., B. Farmer, and R. Cieslik. 1990.** Penetration rate of two pesticide carriers at a large used-tire storage facility in Chicago, Illinois. *J. Am. Mosq. Control Assoc.* 6: 188–196.
- Pennak, R. W. 1989.** Fresh-water invertebrates of the United States: Protozoa to mollusca. Wiley-Interscience, New York, NY.
- Peyton, E. L., S. R. Campbell, T. M. Canaeletti, M. Romanowski, and W. J. Crans. 1999.** *Aedes* (Finlaya) *japonicus japonicus* (Theobald), a new introduction into the United States. *J. Am. Mosq. Control Assoc.* 15: 238–241.
- Qualls, W. A., and G. R. Mullen. 2006.** Larval survey of tire-breeding mosquitoes in Alabama. *J. Am. Mosq. Control Assoc.* 22: 601–608.
- Reidenbach, K. R., S. Cook, M. A. Bertone, R. E. Harbach, B. M. Wiegmann, and N. J. Besansky. 2009.** Phylogenetic analysis and temporal diversification of mosquitoes (Diptera: Culicidae) based on nuclear genes and morphology. *BMC Evol. Biol.* 9: 298.
- Riemann, B., and F. Azam. 1992.** Measurements of bacterial protein synthesis in eutrophic aquatic environments by means of leucine incorporation. *Mar. Microb. Food Webs* 6: 91–105.
- Reisen, W. K., Y. Fang, and V. M. Martinez. 2005.** Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis Encephalitis virus transmission. *J. Med. Entomol.* 42: 367–375.
- Resikind, M. H., and M. L. Wilson. 2004.** *Culex restuans* (Diptera: Culicidae) oviposition behavior determined by larval habitat quality and quantity in southeastern Michigan. *J. Med. Entomol.* 41: 179–186.
- Reiskind, M. H., E. T. Watson, and M. L. Wilson. 2004.** Nutrient-dependent reduced growth and survival of larval *Culex restuans* (Diptera: Culicidae): Laboratory and field experiments in Michigan. *J. Med. Entomol.* 41: 650–656.
- Sardelis, M. R., M. J. Turell, D. J. Dohm, and M. L. O'Guinn. 2001.** Vector competence of selected North American *Culex* and *Coquilletidia* mosquitoes for West Nile virus. *Emerg. Infect. Dis.* 7: 1018–1022.
- SAS Institute. 2004.** SAS/STAT 9.1 user's guide. SAS Institute, Cary, NC.
- Scheiner, S. M. 2001.** MANOVA. Multiple response variables and multi species interactions, pp. 99–133. In S. M. Scheiner and J. Gurevitch (eds.), *Design and analysis of ecological experiments*, 2nd ed. Oxford University Press, Oxford, United Kingdom.
- Skiff, J. J. 2013.** Interactions between protozoan prey (Phylum Protista) and their mosquito (Order Diptera; Family Culicidae) predators: Prey size and predator behavior effects. Master thesis, University of Southern Mississippi, Hattiesburg.
- Skiff, J. J., and D. A. Yee. 2014.** Behavioral differences among four co-occurring species of container mosquito larvae: Effects of depth and resource environments. *J. Med. Entomol.* 51: 375–381.
- Stein, M., F. Ludueña-Almeida, J. A. Willener, and W. R. Almirón. 2011.** Classification of immature mosquito species according to characteristics of the larval habitat in the subtropical province of Chaco, Argentina. *Mem. Inst. Oswaldo Cruz* 106: 400–407.
- ter Braak, C. J. F. 1986.** Canonical correspondence analysis: A new eigenvector technique for multivariate direct gradient analysis. *Ecology* 67: 1167–1179.
- Thorn, M., W. C. Varnado, and J. Goddard. 2012.** First record of *Aedes japonicus japonicus* in Mississippi. *J. Am. Mosq. Control Assoc.* 28: 43–44.
- Turell, M. J., M. L. J. O'Guinn, D. J. Dohm, and J. W. Jones. 2001.** Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *J. Med. Entomol.* 38: 130–134.
- Turell, M. J., D. J. Dohm, M. R. Sardelis, M. L. O'Guinn, T. G. Andreadis, and J. A. Blow. 2005.** An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. *J. Med. Entomol.* 42: 57–62.
- U.S. Census Bureau 2012** (131st Edition). Mississippi Quick Facts. Washington, DC (<http://quickfacts.census.gov/qfd/states/28000.html>)
- Varnado, W. C., J. Goddard, and B. Harrison. 2012.** Identification guide to adult mosquitoes in Mississippi. Mississippi State University Extension Service, Starkville, MS.
- Varnado, W. C., J. Goddard, and B. A. Harrison. 2005.** New state record of *Culex coronator* Dyar and Knab (Diptera: Culicidae) from Mississippi. *Proc. Entomol. Soc. Wash.* 107: 476–477.
- Vezzani, D. 2007.** Review: Artificial container-breeding mosquitoes and cemeteries: A perfect match. *Trop. Med. Intern. Health* 12: 299–313.

- Ward, J. H., Jr. 1963. Hierarchical grouping to optimize an objective function. *J. Am. Stat. Assoc.* 58: 236–244.
- 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. *Ecol. Entomol.* 37: 56–64.
- Yee, D. A. 2008. Tires as habitats for mosquitoes: A review of studies within the eastern United States. *J. Med. Entomol.* 45: 581–593.
- Yee, D. A., and J. J. Skiff. 2014. Interspecific competition of a new invasive mosquito, *Culex coronator*, and two container mosquitoes, *Aedes albopictus* and *Cx. quinquefasciatus*, across different detritus environments. *J. Med. Entomol.* 51: 89–96.
- Yee, D. A., D. Allgood, J. M. Kneitel, and K. A. Kuehn. 2012. Constitutive differences between natural and artificial container mosquito habitats: Microorganisms, resources, and habitat parameters. *J. Med. Entomol.* 49: 482–491.
- Yee, D. A., J. M. Kneitel, and S. A. Juliano. 2010. Environmental correlates of abundances of mosquito species and stages in discarded vehicle tires. *J. Med. Entomol.* 47: 53–62.
- Yee, D. A., M. G. Kaufman, and S. A. Juliano. 2007a. The significance of ratios of detritus types and microorganism productivity to competitive interactions between aquatic insect detritivores. *J. Anim. Ecol.* 76: 1105–1115.
- Yee, D. A., B. Kesavaraju, and S. A. Juliano. 2007b. Direct and indirect effects of animal detritus on growth, survival, and mass of the invasive container mosquito *Aedes albopictus* (Diptera: Culicidae). *J. Med. Entomol.* 44: 580–588.
- Yee, D. A., S. H. Yee, J. M. Kneitel, and S. A. Juliano. 2007c. Richness-productivity relationships between trophic levels in a detritus-based system: Significance of abundance and trophic linkage. *Oecologia* 154: 377–385.
- Yee, D. A., B. Kesavaraju, and S. A. Juliano. 2004. Larval feeding behavior of three co-occurring species of container mosquitoes. *J. Vector Ecol.* 29: 315–322.

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