A peer-reviewed version of this preprint was published in PeerJ on 5 September 2017.

View the peer-reviewed version (peerj.com/articles/3756), which is the preferred citable publication unless you specifically need to cite this preprint.

Effect of tire leachate on the invasive mosquito Aedes albopictus and the native congener Aedes triseriatus

Oswaldo Villena 1, Ivana Terry 1, Kayoko Iwata 2, Edward Landa 1, Shannon LaDeau 3, Paul Leisnham Corresp. 1

1 Department of Environmental Science and Technology, University of Maryland, College Park, Maryland, United States
2 Graduate School of Agriculture, Kyoto University, Kyoto, Japan
3 Cary Institute of Ecosystem Studies, Millbrook, New York, United States

Corresponding Author: Paul Leisnham
Email address: leisnham@umd.edu

Discarded vehicle tire casings are an important artificial habitat for the developmental stages of numerous vector mosquitoes. Discarded vehicle tires degrade under ultraviolet light and leach numerous soluble metals (e.g., barium, cadmium, zinc) and organic substances (e.g., benzothiazole and its derivatives [BTs], polyaromatic hydrocarbons [PAHs]) that could affect mosquito larvae that inhabit the tire casing. This study examined the relationship between soluble zinc, a common marker of tire leachate, on mosquito densities in tire habitats in the field, and tested the effects of tire leachate on the survival and development of newly hatched Aedes albopictus and Aedes triseriatus larvae in a controlled laboratory dose-response experiment. In the field, zinc concentrations were as high as 7.26 mg/L in a single tire and averaged as high as 2.39 (SE±1.17) mg/L among tires at a single site. A. albopictus (37/42 tires, 81.1%) and A. triseriatus (23/42, 54.8%) were the most widespread mosquito species, co-occurred in over half (22/42, 52.4%) of all tires, and A. triseriatus was only collected without A. albopictus in one tire. A. triseriatus was more strongly negatively associated with zinc concentration than A. albopictus, and another common mosquito, C. pipiens, which was found in 17 tires. In the laboratory experiment, A. albopictus per capita rate of population change (l′) was over 1.0, indicating positive population growth, from 0 to 10,000 mg/L tire leachate, corresponding to 8.9 mg/L zinc concentration, but steeply declined to zero from 50,000 to 100,000 mg/L tire leachate (44.5-89.0 mg/L zinc). In contrast, A. triseriatus l′ declined at the lower concentration of 100 mg/L tire leachate (0.05 mg/L zinc), and was zero at 500, 10,000, 50,000 and 100,000 mg/L tire leachate. These results indicate that tire leachate can have severe negative effects on populations of container utilizing mosquitoes at concentrations commonly found in the field. Superior tolerance to tire leachate of A. albopictus compared to A. triseriatus, and possibly other native mosquito species, may have facilitated the replacement of these native species as A. albopictus has invaded North America and other regions around the
world.
Effect of tire leachate on the invasive mosquito *Aedes albopictus* and the native congener *Aedes triseriatus*

Oswaldo C. Villena¹, Ivana Terry¹, Kayoko Iwata², Edward R. Landa¹,³, Shannon L. LaDeau⁴ and Paul T. Leisnham¹

1. Department of Environment Science and Technology, University of Maryland, College Park, MD, 20742
2. Graduate School of Agriculture, Kyoto University. Kitashirakawa Oiwake-Cho, Sakyō-ku, Kyoto 606-8502, Japan
4. Cary Institute of Ecosystem Studies, Millbrook, NY 12545

Corresponding author: leisnham@umd.edu
Abstract

Discarded vehicle tire casings are an important artificial habitat for the developmental stages of numerous vector mosquitoes. Discarded vehicle tires degrade under ultraviolet light and leach numerous soluble metals (e.g., barium, cadmium, zinc) and organic substances (e.g., benzothiazole and its derivatives [BTs], polyaromatic hydrocarbons [PAHs]) that could affect mosquito larvae that inhabit the tire casing. This study examined the relationship between soluble zinc, a common marker of tire leachate, on mosquito densities in tire habitats in the field, and tested the effects of tire leachate on the survival and development of newly hatched *Aedes albopictus* and *Aedes triseriatus* larvae in a controlled laboratory dose-response experiment. In the field, zinc concentrations were as high as 7.26 mg/L in a single tire and averaged as high as 2.39 (SE±1.17) mg/L among tires at a single site. *A. albopictus* (37/42 tires, 81.1%) and *A. triseriatus* (23/42, 54.8%) were the most widespread mosquito species, co-occurred in over half (22/42, 52.4%) of all tires, and *A. triseriatus* was only collected without *A. albopictus* in one tire. *A. triseriatus* was more strongly negatively associated with zinc concentration than *A. albopictus*, and another common mosquito, *C. pipiens*, which was found in 17 tires. In the laboratory experiment, *A. albopictus* per capita rate of population change (\( \lambda' \)) was over 1.0, indicating positive population growth, from 0 to 10,000 mg/L tire leachate, corresponding to 8.9 mg/L zinc concentration, but steeply declined to zero from 50,000 to 100,000 mg/L tire leachate (44.5-89.0 mg/L zinc). In contrast, *A. triseriatus* \( \lambda' \) declined at the lower concentration of 100 mg/L tire leachate (0.05 mg/L zinc), and was zero at 500, 10,000, 50,000 and 100,000 mg/L tire leachate. These results indicate that tire leachate can have severe negative effects on populations of container utilizing mosquitoes at concentrations commonly found in the field. Superior tolerance to tire leachate of *A. albopictus* compared to *A. triseriatus*, and possibly other native
mosquito species, may have facilitated the replacement of these native species as *A. albopictus* has invaded North America and other regions around the world.

**Key Words:** Asian tiger mosquito, competition, invasion biology, pest control, tire leachate, urbanization, West Nile virus, zinc
Introduction

The distributions and abundances of adult mosquitoes are strongly affected by processes occurring at the developmental (eggs, larval) life stages (Washburn 1995, Juliano 2009). Larval densities in developmental habitats, including water-holding containers, are regulated by many biotic and physical factors, including resource competition, predation, temperature, and environmental toxins (Clements 1992, 1999). Not surprisingly, almost all the literature examining the responses of mosquitoes to environmental toxins is focused on commercial or easily sourced chemicals that may be used as mosquitocides to control vector and nuisance species (Floore 2006, Tisgratog 2016). However, many soluble chemicals in mosquito developmental habitats may occur at concentrations that are toxic to larvae. For example, in container habitats that receive high amounts of allochthonous leaf litter, foliar tannins can suppress mosquito survival and development, and are likely to alter local community composition (Sota et al. 1993, Mercer and Anderson 1994, Smith et al. 2013).

Discarded vehicles are an important artificial habitat for the developmental stages of numerous vector mosquitoes (Yee 2008). The Rubber Manufacturers Association (2014) estimates that in the United States, there are approximately 75 million tires in stockpiles. Tires are important because they are often in close proximity to human habitation and support higher abundances of larvae than other, usually smaller, developmental habitats (e.g., containers, plastic trash, bird baths) (Carrieri et al. 2003, Dowling et al. 2013). Furthermore, tires are a common means of accidentally transporting native and invasive species around the United States and the world (Lounibos 2002). Tires are a conglomerate of chemical compounds that degrade under ultraviolet (UV) light and leach numerous soluble metals (e.g., barium, cadmium, zinc) and organic (e.g.,
benzothiazole and its derivatives [BTs], polyaromatic hydrocarbons [PAHs]) compounds (Wik and Dave 2006, Wik et al. 2009). A growing body of literature has demonstrated acute toxicity of tire particles, leachate, or specific compounds on living organisms, and in particular aquatic taxa (reviewed by Wik and Dave 2009). Fewer studies have researched sub-lethal impacts of tire materials (e.g., Draper and Robinson 2001, Suwanchaichinda and Brattsten 2002, Gualtieri et al. 2005), and to our knowledge, no studies have tested the effects of tire leachate on the survival and overall population performances of mosquito species.

*Aedes albopictus*, the Asian tiger mosquito, was introduced to the continental United States in the mid-1980s through shipments of eggs and larvae in used tires (Tatem et al. 2006). It has since spread throughout the eastern part of the country where its range overlaps with the native treehole mosquito, *Aedes triseriatus*. Both *A. albopictus* and *A. triseriatus* commonly inhabit tire habitats (Yee 2008). Laboratory and field experiments have shown *A. albopictus* to be competitively superior to *A. triseriatus* under most conditions (e.g., Livdahl & Willey 1991, Teng & Apperson 2000, Aliabadi & Juliano 2002). Ecological theory and empirical work suggests that with one limiting factor in a constant environment, interspecific competition should result in competitive exclusion (Tilman 1982, Chase and Leibold 2003). Yet *A. triseriatus* has persisted in many areas despite the spread of *A. albopictus* (Lounibos et al. 2001). One hypothesis for the coexistence of *A. triseriatus* with *A. albopictus* may be superior tolerance of the native to environmental toxins, including those in important developmental habitats such as tires. Inferior competitors can escape local extinction by a number of mechanisms, including superior tolerance to environmental conditions, and trade-offs between competitive ability and tolerances to environmental conditions have been well documented in the ecological literature (e.g., Chesson 1986, 2000, Dunson and Travis 1991, Chesson and Huntly 1997).
In this study, we will examine the relationship between tire leachate and densities of mosquitoes in discarded tires in the field, and test the effects of tire leachate on the survival and development of *A. albopictus* and *A. triseriatus* larvae in a controlled laboratory dose-response study. Zinc has been implicated as an important tire toxicant to aquatic organisms and is one of the most common compounds in tire material (Wik et al. 2009), but a range of potentially organic compounds are present in tire leachate that may also induce acute toxicity or sub-lethal impacts (Wik et al. 2009). Therefore, in this study we will use zinc concentration as a marker of tire leachate consistent with past studies (Wik et al. 2009). In our laboratory study, we will examine the effects of tire leachate on per capita rate of change \([r, (Goldberg and Fleedwood 1987)]\). In mosquito experiments, population performance can be estimated by calculating an estimate of the finite rate of population increase \(\left(\lambda^{'},\right)\), which is a composite index based on individual fitness parameters: survivorship, female development time, and female wing length (as a fecundity surrogate).

**Materials and Methods**

**Field Study.** Mosquitoes were sampled from 42 tires among five sites (6-14 tires per site) in College Park and Baltimore, Maryland, USA, from July to August 2011, which is the period of peak mosquito activity in the area (Dowling et al. 2013). Three sites were where tires had been illegally dumped and two sites were a part of stacked tire piles at auto-repair shops (Table 1). All immature (larvae and pupae) were identified to species level and counted. Entire contents of each tire (i.e., water, detritus, and all biota) were collected. Detritus was dried at 35°C for >48 hours and weighed as a broad measure of resource quantity. A 75 mL water subsample was acidified at
pH 2.0 and refrigerated for later analysis of total nitrogen (TN) and total phosphorus (TP) using Hach test kits (TNT826 and TNT844, respectively) and a Hach 3800 spectrophotometer (Hach Company, Colorado, USA). A second water subsample was filtered immediately after field collection through a syringe-mounted, 0.45 um pore-size nylon filter. The filtrate was acidified with double distilled nitric acid prior to analysis for zinc using a PerkinElmer Optima 4300 DV Inductively Coupled Plasma-Optical Emissions Spectrophotometer (ICP-OES) (PerkinElmer Inc.; Waltham, Massachusetts, USA).

**Tire leachate preparation.** Tire leachate was prepared by extracting ground tire material, minus 30 mesh (less than 0.59 mm) 99.51% dry content, with deionized water (DI) in either Teflon or Teflon coated polypropylene bottles at a solid to liquid ratio of 1:10 (100 g of ground tire material to a liter of DI water). A blank, containing only DI water was also prepared. The samples were shaken for a one-week period on an orbital table shaker at approximately 100 rpm at room temperature (19–22ºC). At the conclusion of the one-week period, the samples were filtered in a class 1000 laminar flow clean bench through a sterile 0.2µm filter, into a sterile 1000 ml polycarbonate container. The filter membrane was surfactant-free Cellulose Acetate (CA); this membrane has no wetting agents that might affect sensitive cell culture lines and is cleaner than ordinary CA membranes for tissue culture use. The sample in the sterile container was bagged and refrigerated prior to usage. For conformity and comparison with earlier studies in the literature, this stock solution was designated as 100% tire leachate at a concentration of 100,000 mg/L. Analysis on this 100% solution using an ICP-OES indicated that it had a zinc concentration of 89 mg/L.
Laboratory Experiment. *Aedes albopictus* and *A. triseriatus* eggs sourced from F_{1-2} colonies at University of Maryland were synchronously hatched in nutrient broth solutions in plastic trays. Within 24 hours, 25 newly hatched larvae of each species were rinsed and added to 400 ml cups containing one of six concentrations of tire leachate using dilutions of the 100% stock solution and DI water. The six dilutions and their zinc concentrations (mg/L) were: 100.0% (89.0 mg/L), 50.0% (44.5 mg/L), 10.0% (8.9 mg/L), 1% (0.89 mg/L), 0.5% (0.445 mg/L), and 0.1% (0.089 mg/L). Each concentration x species combination had 5 replicates to yield 70 total experimental cups (7 concentrations x 2 species x 5 replicates). Cups were housed in an incubator at 25°C and 16:8h light-dark cycle. Each cup received 0.1 mg of bovine liver powder:lactalbumin (diluted 1:10). Pupae were removed from containers daily and placed into individual vials until adult emergence. Adults were sexed, identified, dried (>24 h, 50°C) and weighed, and females’ wings were measured. For each cup, proportion survivorship, median female development time, and median female mass were calculated. These fitness parameters were used to estimate the finite rate of population growth for each species $\lambda'$, (Juliano 1998):

$$\lambda' = \exp \left[ \ln \left( \frac{1}{N_0} \sum_x A_x f(w_x) \right) \right]$$

where $N_0$ is the initial number of females (assumed to be 50% per microcosm), $x$ is the mean time to eclosion (measured in days), $A_x$ is the mean number of females eclosing on day $x$, $w_x$ is the mean body size on day $x$, and $f(w_x)$ is a function describing size dependent fecundity for each
species, estimated from the mean wing length on day $x$, $wx$, of female mosquitoes (Livdahl and Sugihara 1984, Juliano 1998). The function for *A. albopictus* was $f(wx) = -121.240 + 78.02wx$, where $wx$ is wing length (millimeters) (Lounibos et al. 2002). The function for *A. triseriatus* was $f(wx) = (1/2) \exp[4.5801 + 0.8926(\ln wx)] - 1$ (Nannini and Juliano 1998). $D$ is the mean days it takes for an adult mosquito to mate, bloodfeed, and oviposit, and is estimated at 14 days for *A. albopictus* (Lounibos et al. 2002) and 12 days for *A. triseriatus* (Nannini and Juliano 1998).

**Statistical Analyses.** Relationships between zinc concentrations and field abundances of each of the three collected mosquito species: *A. albopictus*, *A. triseriatus*, and *C. pipiens* (see Results) were analyzed using negative binomial regressions (PROC GENMOD, SAS Institute). Because a species’ density in a tire may be predicted by various physiochemical and biological container characteristics as well as surrounding landscape variables, we also included site, detritus amount, TN, TP, and the abundances of the other two mosquito species in an initial multi-factor model for each species. Final multi-factor models were selected using backward selection. If there was no significant loss of fit, as evaluated by AIC, we continued to eliminate the next least significant variable until all non-significant variables were removed or until there was significant loss of model fit (e.g., $-2 \Delta AIC$). Correlations among predictor variables were tested using Pearson or Spearman rank correlations (SAS PROC CORR) and multicolinearity was checked by means of Variance Inflation Characteristics (VIF) (SAS PROC REG), with a VIF above 5 for a variable indicating a problem (Kutner et al. 2004); however, none were evident. We tested for effects of tire leachate concentration and mosquito species from the laboratory experiment using randomization ANOVAs (Randomization wrapper for SAS PROCs; Cassell 2011) because data (survival, mean development time, mean body size, $\lambda$) failed to meet parametric assumptions despite transformations, and generalized models using variable distribution models (e.g.,
Poisson, negative binomial) routinely failed to converge. Tire leachate concentration was treated as a categorical variable because preliminary data plots clearly showed that its relationship with each response variable was not linear. For all analyses, randomization ANOVA yielded conclusions identical to those of parametric ANOVA, probably because trends among treatment groups were clear (see Results). For brevity and accuracy, we report only the results from randomization ANOVA. Experimentwise $\alpha = 0.05$ for all statistical analyses.

Results

Field Survey. *Aedes albopictus*, *A. triseriatus* and *C. pipiens* were collected from 88% (37/42), 55% (23/42), and 41% (17/42) of tires, and constituted 55% (2941/5383), 8% (415/5383), and 38% (2027/5383) of total immatures, respectively. *Aedes albopictus* and *A. triseriatus* co-occurred in individual tires the most frequently among all pairwise species combinations, being collected together in 22 tires. *Aedes triseriatus* was only collected without *A. albopictus* in one tire. *Aedes albopictus* and *A. triseriatus* co-occurred with *C. pipiens* in 17 and 15 tires, respectively. There was considerable variation among individual tires and sites in mosquito abundances and physiochemical characteristics of tires (Table 1). Zinc concentrations were as high as 7.26 mg/L, recorded in a single tire at Site 5, and as low as 0.05 mg/L, recorded at Sites 1, 2, and 4 (Table 1). The three dump sites had higher average zinc concentrations compared to the two sites at auto-repair shops (Table 1). In final multi-factor models, *A. triseriatus* (Estimate: -0.8019; $\chi^2_1 = 5.37, P = 0.0204$) was most strongly negatively associated with zinc concentration, followed by *A. albopictus* (Estimate: -0.6340; $\chi^2_1 = 6.51, P = 0.0107$), and *C. pipiens* (Estimate: -0.2190; $\chi^2_1 = 5.02, P = 0.0251$). Additionally, *A. albopictus* was associated positively with detritus amount (Estimate: 0.6340; $\chi^2_1 = 5.37, P = 0.0204$); *A. triseriatus* was positively
associated with *A. albopictus* (Estimate: 0.0112; $\chi^2 = 6.34$, $P = 0.0118$) and *C. pipiens*

(Estimate: 0.0092; $\chi^2 = 5.74$, $P = 0.0166$); and *C. pipiens* varied with site ($\chi^2 = 13.79$, $P = 0.0080$) and was positively associated to *A. triseriatus* (Estimate: 0.1624; $\chi^2 = 16.31$, $P < 0.0001$) and TN (Estimate: 0.2627; $\chi^2 = 4.44$, $P = 0.0351$). TP was excluded from final models because it was always non-significant (data not shown).

**Laboratory Experiment.** Estimated finite rate of population increase ($\lambda'$, $P < 0.0001$) and survival ($P < 0.0001$) both showed a significant interaction between species and tire leachate concentration. *Aedes albopictus* had similar $\lambda'$ than *A. triseriatus* in the control treatment (0 mg/L) but clearly divergent responses to increasing tire leachate concentration. *Aedes albopictus* $\lambda'$ was consistently high and similar from 0-10,000 mg/L tire leachate but steeply declined to zero from 50,000 to 100,000 mg/L. In contrast, *A. triseriatus* $\lambda'$ declined at the lower concentration of 100 mg/L tire leachate, and was zero at 500, 10,000, and 100,000 mg/L (Fig. 1A). Survivorship results were broadly similar to $\lambda'$ for both species. *Aedes albopictus* survivorship declined steeply at 10,000 mg/L tire leachate whereas *A. triseriatus* survivorship declined steeply at 100 mg/L tire leachate (Fig. 1B). There was no *A. albopictus* survivorship at 100,000 mg/L tire leachate, whereas there was either very low or no *A. triseriatus* survivorship from 100 to 100,000 mg/L. Tire leachate concentration had no effect on development time (male: $P = 0.6040$; female = 0.3080) and mass (male: $P = 0.9870$; female = 0.2210) of both sexes, nor were any significant interactions of tire leachate with species (P-values > 0.1000) (Fig 2). *Aedes triseriatus* were on average larger than *A. albopictus* for both sexes (male: $P < 0.0100$; female: $P < 0.0001$), and *A. albopictus* had faster female development than *A. triseriatus* ($P < 0.0001$) (Fig 2). Development time for males did not vary between species ($P = 0.3000$).
This study showed that although tire leachate had a negative effect on $\lambda'$ and survival of both the invasive mosquito *A. albopictus* and the native congener *A. triseriatus*, but the invasive *A. albopictus* was clearly more tolerant to tire compounds in the water. In field tires, *A. triseriatus* was more strongly negatively associated with zinc concentration compared to *A. albopictus* and another common co-occurring mosquito, *C. pipiens*. In our laboratory experiment, *A. albopictus* $\lambda'$ was over 1.0, indicating positive population growth, and survival was high from 0-10,000 mg/L tire leachate, but both $\lambda'$ and survival steeply declined to zero from 10,000 to 100,000 mg/L. In contrast, *A. triseriatus* $\lambda'$ and survival declined at the lower concentration of 100 mg/L, and was zero at 500, 10,000, and 100,000 mg/L.

The results of our study are inconsistent with the idea that tire leachate may facilitate the persistence of *A. triseriatus* after *A. albopictus* invasion by releasing compounds that are more toxic to *A. albopictus*. Instead, our data suggest that tire habitats may in fact support the spread of *A. albopictus* and the concomitant decline in *A. triseriatus*. Three of the five field sites that we sampled had tire water with mean zinc concentrations (1.35-2.39 mg/L) in excess of concentrations that caused a decline in *A. triseriatus* survival and $\lambda'$ (0-0.45 mg/L) in the laboratory experiment. This result, combined with the strong negative association between *A. triseriatus* and zinc concentrations in the field, suggests that *A. triseriatus* is likely to be negatively affected by tire leachate under many field conditions. Mean *A. triseriatus* $\lambda'$ was < 1.0 across all tire leachate concentrations in our experiment, including in the control concentration that consisted only of DI water. This result suggests that our baseline experimental conditions were generally unfavorable to *A. triseriatus*, possibly because of food limitations. Nevertheless,
the decline in _A. triseriatus_ performance with even relatively small increases in leachate concentration was clear and not apparent in _A. albopictus_, which only showed declines in population performance in conditions higher than 8.9 mg zinc/L. Since its establishment in North America, _A. albopictus_ has rapidly become the most common urban mosquito in many areas in the eastern United States. There are several explanations for this dominance; the most well documented being that _A. albopictus_ is a superior competitor at the larval stage (Juliano 2009, Smith et al. 2015) and asymmetric reproductive competition (Lounibos, 2007). Our study suggests that tire habitats may also facilitate the dominance of _A. albopictus_ through this species’ superior tolerance to compounds that leach from tire rubber.

No tires that we sampled in the field had zinc concentrations in excess of 8.9 mg/L suggesting that _A. albopictus_ may be minimally affected by tire leachate in the field. In our field study, we visited three dump sites, where tires had been illegally discarded, and two sites at auto-repair shops, where used tires had been stacked. Interestingly, the three dump sites had tires with zinc concentrations at least 3 times higher than the sites at auto-repair shops. Tires leach compounds as their rubber deteriorates, and the rate of deterioration is the result of the time and intensity of exposure to combined effects of temperature, UV radiation, and humidity. These environmental variables cause discoloration, cracking, and splitting of tire sidewall tire rubber and oxidation of the steal belt (Andrady et al., 1998, Andrady et al., 2003, Yu et al., 2015). It is possible that the discarded tires at the illegal dumping sites were exposed to the environment longer than tires at auto-repair shops, which would likely be eventually sold or recycled. Future research needs to examine the rate of tire degradation and resultant leaching of tire materials (and associated compounds) under different environmental conditions, especially UV exposure, and the effects
Variation in $\lambda'$ of both species as a result of tire leachate appears to be primarily driven by survival. Interestingly however, despite an approximately four-fold decline of mean $A. albopictus$ survival from 1,000 to 10,000 mg/L tire leachate, $A. albopictus$ $\lambda'$ remained unchanged (Fig. 1B). This result was probably due a small but important concomitant increase in female mass from 1,000 to 10,000 mg/L tire leachate concentration (Fig 2). An important limitation of past studies that have tested the effects of tire leachate on aquatic organisms, and of many toxicological studies, is that inferences on toxicological impacts are limited to individual parameters of fitness, and usually that of survival. Toxicological studies that focus solely on the effects on survival, yield limited inference of population impacts over multiple generations. Some prior experiments using $\lambda'$ have generated different conclusions for both $\lambda'$ and of survival, reiterating the importance of including an analysis of $\lambda'$ for a more thorough examination of environmental impacts on populations (Livdahl and Sugihara 1984, Juliano 1998). Further, $\lambda'$ is a more biologically meaningful measure of population performance than considering individual fitness parameters, such as survival, development time, and body size, separately, as it accounts for nonlinear interactions among these parameters (Livdahl and Sugihara 1984). Thus, although the increase in $A. albopictus$ female mass from 1,000 to 10,000 mg/L tire leachate was non-significant, it may still have an important effect in preventing a decline in $A. albopictus$ $\lambda'$. 

of tire leachate across a larger suite of mosquito species so that we might be able to predict field sites with favorable or deleterious leachate conditions. Numerous studies have related larval habitat variables to larval mosquito communities in discarded vehicle tires (e.g., Beier et al. 1983, Kling et al. 2007, Yee et al. 2010). Our study suggest that tire leachate should be another variable to consider to better predict vector distributions and disease risks.
Addressing parameters of adult fitness, such as female body size, is also be important when considering disease vector mosquitoes since the adult life-stage is of public health importance and adult traits that affect disease transmission can be affected by the conditions of larval habitat. For example, recent modeling research has shown that smaller adult female *Aedes* have decreased probability of dengue infection, primarily because of they have reduced nutritional reserves and shorter lifespans, and hence live out the extrinsic incubation period and become infectious (Juliano et al. 2016).

To our understanding this is amount the first studies to rigorously examine the effects of tire leachate on the population performance of mosquitoes. Our study did not, however, address the mechanism by which sub-lethal concentrations of tire leachate might negatively affect individual mosquito larvae and differentially affect *A. albopictus* and *A. triseriatus*. One likely mechanism that tire leachate may affect mosquito larvae is by changing their microbial food resource.

Numerous studies have demonstrated decreased overall bacterial abundances and altered bacterial communities under increasing tire leachate concentrations (e.g., Leff et al. 2007, Vukanti et al. 2009). *A. albopictus* is more efficient at harvesting microbial food resources primarily because it spends more time feeding and more efficient converts these resources into biomass (Kesavaraju et al. 2008, 2011). Overall decreases in microbial food or changes in the composition of microbial communities as a result of tire leachate toxicity might therefore be expected to more severely negatively affect *A. triseriatus*. Another mechanism that tire leachate could affect mosquitoes is by being directly toxic to the larvae, and individuals from different species may vary in their tolerance. Insects detoxify natural (e.g., tannins) and synthetic (commercial insecticides) toxins via a number of biochemical mechanisms, and among the most
well documented are P450 monoxygenases (P450s) (Scott, 1999). It possible that selection for increased upregulation of P450s in *A. albopictus* than *A. triseriatus* allows *A. albopictus* to better tolerate tire leachate. Suwanchaichinda and Brattsten (2002) demonstrated increased induction of P450s in *A. albopictus* larvae exposed to the common tire compound, benzothiazole and its derivatives (BZTs). The focus of Suwanchaichinda and Brattsten (2002) was to test if the exposure to tire compounds, particularly BZT, made *A. albopictus* mosquito larvae more tolerant to commercial insecticides through P450 induction, and the study found evidence that it did (Suwanchaichinda and Brattsten 2002).

The effects of tire leachate on mosquitoes are likely to complex and act at both immature (egg and larval) and adult life-stages. The main goal of this study was to compare effects of tire leachate on the population performance of two co-occurring *Aedes* mosquito species, *A. albopictus* and *A. triseriatus*, and it showed clear interspecific differences. Additional research needs to examine the suite of density dependent and trait-mediated impacts related to vector mosquitoes, including the effects on population performances of a larger number of tire-inhabiting species, on community processes, such as competition, predation, parasitism and mutualism, and on vector competence across a range of disease systems.
We thank R. Pozzatti, N. Kirchoff, and D. Bodner for assistance in data collection, Kathryn M. Conko (USGS) for preparing the tire leachate and help planning the laboratory experiment, and S.A. Juliano for statistical advice.
References


Chesson, P., and Huntly, N. 1997. The roles of harsh and fluctuating conditions in the


sequential leachates of tire powder using a battery of toxicity tests and toxicity identification evaluations. Chemosphere 77:922-927.


Table 1 Mean ± SE (range in parentheses) *A. albopictus* density, *A. triseriatus* density, *C. pipiens* density, soluble zinc concentration, detritus amount, total nitrogen concentration, and total phosphorus concentration among tires in six sample sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Site Type</th>
<th>Number of Tires</th>
<th><em>A. albopictus</em> per tire</th>
<th><em>A. triseriatus</em> per tire</th>
<th><em>C. pipiens</em> per tire</th>
<th>Soluble zinc (mg/L)</th>
<th>Detritus (g)</th>
<th>Total Nitrogen (mg/L)</th>
<th>Total Phosphorus (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dump</td>
<td>6</td>
<td>161.7 ± 94.6 (3-489)</td>
<td>20.3 ± 10.4 (1-67)</td>
<td>192.8 ± 142.4 (0-887)</td>
<td>1.37 ± 0.81 (0.05-4.85)</td>
<td>10.56 ± 3.44 (3.35-25.17)</td>
<td>4.08 ± 4.05 (0.00-12.18)</td>
<td>1.76 ± 0.81 (0.38-3.89)</td>
</tr>
<tr>
<td>2</td>
<td>Auto repair shop</td>
<td>10</td>
<td>37.5 ± 12.3 (1-135)</td>
<td>10.0 ± 4.5 (0-43)</td>
<td>23.5 ± 22.9 (0-230)</td>
<td>0.09 ± 0.04 (0.05-0.45)</td>
<td>6.26 ± 1.38 (1.62-14.06)</td>
<td>7.76 ± 1.72 (3.12-18.10)</td>
<td>6.29 ± 3.59 (0.45-34.60)</td>
</tr>
<tr>
<td>3</td>
<td>Dump</td>
<td>14</td>
<td>27.0 ± 8.1 (0-91)</td>
<td>2.7 ± 1.8 (0-24)</td>
<td>8.8 ± 8.7 (0-122)</td>
<td>1.35 ± 0.26 (0.19-3.14)</td>
<td>2.43 ± 0.42 (0.00-5.34)</td>
<td>4.88 ± 0.48 (1.35-9.42)</td>
<td>0.38 ± 0.04 (0.08-0.65)</td>
</tr>
<tr>
<td>4</td>
<td>Auto repair shop</td>
<td>6</td>
<td>191.0 ± 56.3 (28-367)</td>
<td>23.5 ± 12.5 (0-81)</td>
<td>85.3 ± 61.3 (1-385)</td>
<td>0.43 ± 0.13 (0.05-0.97)</td>
<td>5.4 ± 1.9 (1.03-14.21)</td>
<td>2.82 ± 1.15 (0.38-7.16)</td>
<td>0.85 ± 0.12 (0.65-1.32)</td>
</tr>
<tr>
<td>5</td>
<td>Dump</td>
<td>6</td>
<td>12.5 ± 11.3 (0-69)</td>
<td>2.3 ± 2.0 (0-12)</td>
<td>0.0 ± 0.0 (0)</td>
<td>2.39 ± 1.17 (0.09-7.26)</td>
<td>0.68 ± 0.4 (0.00-2.39)</td>
<td>4.32 ± 1.31 (1.97-9.73)</td>
<td>0.26 ± 0.07 (0.12-0.56)</td>
</tr>
</tbody>
</table>
Fig 1 Mean ± SE $A.\ albopictus$ and $A.\ triseriatus$ $\lambda'$ (A) and survivorship (B) exposed to varying concentrations of tire leachate in dose-response laboratory experiment. The symbol for $A.\ albopictus$ $\lambda'$ and survival at 100,000 mg/L tire leachate is hidden behind the corresponding symbol for $A.\ triseriatus$. 
Fig 2 Mean ± SE *A. albopictus* and *A. triseriatus* female development time (days to eclosion) (A), male development time (days to eclosion) (B), female mass (g) (C), and male adult body size (g) (D) exposed to varying concentrations of tire leachate in dose-response laboratory experiment.