

ABSTRACT

Title of dissertation: THE EFFECTS OF UV-B RADIATION ON TIRE DETERIORATION AND THE ECOLOGY OF *Aedes albopictus* and *Culex pipiens* MOSQUITOES

Oswaldo Villena Carpio, Doctor of Philosophy, 2017

Dissertation directed by: Professor Paul Leisnham
Department of Environmental Science & Technology

Although recycling programs have reduced stockpiles of scrap tires in the U.S., vast numbers of tires are still deposited throughout landscapes nationwide. Among the most important environmental impacts of tires is their degradation when exposed to ultraviolet (UV) radiation and, in the presence of water, the resulting leaching of numerous soluble inorganic (e.g., zinc) and organic contaminants (e.g., benzothiazoles). Studies have shown clear toxicological effects of tire leachate on a few focal aquatic taxa, but there is a lack of knowledge on the effects of tire leachate on most aquatic communities. This project investigates the relationship of scrap tire contamination on the ecologies of the two most broadly distributed mosquitoes in the Eastern U.S., the invasive *Aedes albopictus* and resident *Culex pipiens*, which engage in strong competition for microbial food in tire habitats. The main objectives of this project were to: (1) Assess the impacts of UV-B radiation conditions that mimicked full-sun, shade, and no-UV settings in the field on the metabolic rates and fitness of *Ae. albopictus* and *Cx. pipiens* larvae and associated

microbial fauna; (2) Compare the effects of full-sun, shade, and no-UV radiation on the degradation of tires; (3) Test the hypothesis that tire leachate from tire degradation promotes condition-specific competition between *Ae. albopictus* and *Cx. pipiens*; and (4) Test the effects of tire leachate on *Ae. albopictus* and *Cx. pipiens* oviposition. Overall, the results of my dissertation indicate that UV-B can have strong effects on the ecologies of both *Ae. albopictus* and *Cx. pipiens*, both through direct negative effects on metabolic processes and fitness, and indirectly through the degradation of tires and the leaching of contaminants. The most ecologically interesting impacts of UV-B exposure may be via the promotion of condition-specific competition whereby *Cx. pipiens* appears to have greater competitive effects on *Ae. albopictus* under conditions that promote greater tire degradation. Tire leachate does not appear to alter the oviposition behavior of both *Ae. albopictus* and *Cx. pipiens*. Therefore, the effects of tire leachate on larval ecology is likely to be important in dictating the distribution and abundance of both species in tire habitats.

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ECOLOGY OF *Aedes albopictus* and *Culex pipiens* MOSQUITOES

by

Oswaldo Villena Carpio

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Advisory Committee:

Professor Paul T. Leisnham
Professor Daniel Fisher
Professor Sujay S. Kaushal
Professor Bahram Momen
Professor Joseph H. Sullivan

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Chapter 1 – General Introduction

Study organisms: *Aedes albopictus* and *Culex pipiens*

General biology and ecology of mosquitoes

Mosquitoes such as *Ae. albopictus* and *Cx. pipiens* are blood-feeding insects of the order Diptera and family Culicidae. Mosquitoes undergo complete metamorphosis, meaning they have four distinct life stages: egg, larva, pupa, and adult. The egg, larva, and pupal stages develop in aquatic environments, and the adult stage in the terrestrial environment (Lounibos, 2002a; Becker et al., 2010).

Mosquitoes oviposit eggs on or near still water in natural containers (e.g., water puddles, rock pools, tree holes) or in artificial containers (e.g., discarded tires, bottles, buckets, plastic pools). Mosquito eggs may be oviposited singly on the water surface (e.g., *Anopheles* mosquitoes), attached to container surfaces (e.g., *Ae. albopictus*, *Ae. aegypti*), or in clusters forming rafts of 100 to 300 eggs on top of the water surface (e.g., *Cx. pipiens*) (Becker et al., 2010). Hatching occurs when development is completed, or, for some species, when the water level rises sufficiently to immerse the eggs (Clements, 2000) or when environmental factors are adequate for the hatching of the offspring (i.e., photoperiod) (Lounibos, 2002a; Lounibos et al., 2011).

Mosquito larvae live in water from 5 to 15 days, depending on water temperature, photoperiod, and presence of food resources. Larvae of most mosquito species must come to the surface at frequent intervals to obtain oxygen through a siphon. During growth, the larva molts four times and the stages between molts are called instars. When the 4th instar

larva molts, it becomes a pupa (Becker et al., 2010). Mosquito larvae feed on aquatic microbial communities (e.g., bacteria, fungi, protists) that colonize organic detritus (e.g., leaf litter, bark, invertebrate carcasses) (Becker et al., 2010). Mosquitoes feed on microbial communities using their brush-like mouthparts to either filter-feed through the mouth and separating particulate material (e.g., *Cx. pipiens*) or browse by removing particles from surfaces (e.g., *Ae. albopictus*) (Merritt et al., 1992).

Mosquito pupae live in water from 1 to 4 days, depending on temperature and species. Pupae take in oxygen through two breathing tubes called trumpets. In the pupal stage, there is no food ingestion. Once pupae reach the maturation stage they go into a metamorphosis into adult mosquitoes, which emerge to the surface of the water where they rest until their bodies dry and harden (Becker et al., 2010). In the adult stage, only female mosquitoes require blood meals as a source of protein, which is essential for egg production. Only female mosquitoes bite. Male mosquitoes do not bite; they feed on the nectar of flowers (Becker et al., 2010).

***Aedes albopictus* invasion**

Aedes albopictus, also known as the Asian tiger mosquito, is a mosquito native to the tropical and sub-tropical areas of Southeast Asia. *Aedes albopictus* is about 2 to 10 mm in length with a striking white and black pattern and a characteristic white stripe down the center beginning at the dorsal surface of the head and continuing along the thorax. *Aedes albopictus* males are approximately 20% smaller than females. The female lays her eggs near water, not directly into it like other mosquitoes. *Aedes albopictus* are diurnal biters; they blood feed on a range of mammals besides humans and also on birds (Estrada-Franco and Craig, 1995).

In the past 40 years, *Ae. albopictus* has spread to 28 countries in four continents (North and South America, Europe and Africa), mainly through the international used tire trade (Benedict et al., 2007; Ngoagouni et al., 2015). *Aedes albopictus* is the most invasive mosquito in the world utilizing a range of artificial containers as developmental habitats, including trash receptacles, buckets, tires, birdbaths (Juliano and Lounibos, 2005). *Aedes albopictus* was introduced from Asia into the continental United States in the mid-1980s through tire shipments. It was first discovered in a shipment of used tires from Japan in Houston, Texas in 1985 (Sprenger and Wuithranyagool, 1986). Since then *Ae. albopictus* has become the most abundant container-dwelling mosquito in the eastern United States (Moore and Mitchell, 1997). Mosquitoes are ecologically and medically important (Juliano and Lounibos, 2005). Non-native mosquitoes, including *Ae. albopictus*, have had important ecological and human health impacts, such as declines in abundance and/or local extinction of resident mosquitoes (Lounibos et al., 2002b) and the spread of disease (Fonseca et al., 2004; Kim et al., 2005).

***Culex pipiens* mosquitoes**

Culex pipiens is known as the northern house mosquito or the common house mosquito. The northern house mosquito is about 3 to 12 mm in length and its color is brownish. It is present in 44 countries on 4 continents (North and South America, Europe, and Africa). *Culex pipiens* lays eggs on still water and at night. One *Cx. pipiens* female may lay up to 300 eggs. The eggs are laid side by side standing erect, and stuck together by the legs to form boat-shaped rafts which float on water. The eggs hatch in 1 to 3 days and a larva emerges from the lower end of each egg (Markowski, 2015).

Culex pipiens was introduced to North America between the 16th and 17th centuries, presumably from Europe, and has since become a resident member of the mosquito community in its introduced range (Vinogradova, 2000; Lounibos, 2002a). The establishment and spread of *Cx. pipiens* was probably facilitated by the absence of competitors at the time of the invasion (Carriere et al., 2003; Costanzo et al., 2005a). Currently, *Cx. pipiens* is distributed in the northern part of the United States and the southern part of Canada (Costanzo et al., 2005a). Along the eastern seaboard, it overlaps with *Ae. albopictus*, from northern Virginia to southern Connecticut (Darsie and Ward, 2005). *Culex pipiens* is associated with human development and urbanization (Vinogradova, 2000; Costanzo et al., 2005a). It is one of the most important vectors of West Nile virus (Fonseca et al., 2004). West Nile virus was introduced into the United States in 1999 with a genotype known as NY99, which mutated into a novel genotype known as WN2, a genotype that is better transmitted and disseminated by *Cx. pipiens* mosquitoes (Kilpatrick et al., 2008).

Coexistence of Ae. albopictus and Cx. pipiens: Potential ecological mechanisms

Competition among larval mosquitoes is common in container systems where allochthonous leaf litter and associated microbial communities (bacteria, fungi, and protists) provide the main food resource (Walker et al., 1991; Murrell and Juliano, 2008). Understanding how larval competition and species richness affects mosquito survival, growth, fecundity and overall population performance under a range of environmental conditions is important in better understanding mosquito invasions and vector-borne disease risk (Juliano and Lounibos, 2005; Juliano, 2009).

Numerous studies have shown that in almost all conditions *Ae. albopictus* is a superior competitor over other mosquito species, including *Ae. aegypti* (e.g., Murrell and Juliano, 2008; Alto et al., 2013), *Ae. japonicus* (Freed and Leisnham, 2014), *Ae. triseriatus* (Smith et al., 2013), *Ae. sirrensis* (Kesavaraju et al., 2014), and *Cx. pipiens* (Carrieri et al., 2003; Costanzo et al. 2005a; Costanzo et al., 2011). However, although resident mosquito species are usually competitively inferior to *Ae. albopictus*, they appear to coexist with the invasive in some areas. Mechanisms governing the coexistence of resident mosquito species after *Ae. albopictus* invasion are still not well understood. Some mechanisms have been relatively well documented including interspecific competition, keystone predation, aggregation of species, apparent competition, and intraguild predation (Blaustein and Chase, 2007), among the most ecologically interesting variation in competition across habitat gradients (Juliano, 2009).

Some studies have shown how changes in environmental conditions by abiotic factors have altered or reversed competitive superiority. This ecological phenomenon is called condition-specific competition (Blaustein and Chase, 2007). Probably the best studied case of condition-specific competition is the competition of *Ae. albopictus* and *Ae. aegypti* in the Florida region, where Costanzo et al. (2005b) showed that competitive superiority of *Ae. albopictus* over *Ae. aegypti* under wetter environmental conditions could be reversed under dryer environmental conditions. This mechanism probably explains the co-existence of *Ae. albopictus* and *Ae. aegypti* in Florida (Juliano and Lounibos, 2005; Costanzo et al., 2005b; Costanzo et al. 2011). Another example of condition-specific competition was reported by Alto et al. (2013), who assessed the effect of Malathion, an organophosphate insecticide, widely used in agriculture, on the coexistence of *Ae.*

albopictus and *Ae. aegypti*. In the absence of Malathion, *Ae. aegypti* survivorship was negatively affected by increasing densities of *Ae. albopictus*, while *Ae. albopictus* survivorship was not affected by increasing densities of *Ae. aegypti*. But in presence of Malathion, the negative effect of *Ae. albopictus* competition on *Ae. aegypti* survivorship was eliminated. Apart from these two examples, there has been no research on the role of condition-specific competition upon mosquito invasions.

Study system: Discarded tires

Discarded tires and mosquitoes

In the last 40 years, discarded tires have been implicated in the spread and introduction of mosquitoes in many parts of the world, many of them bringing with them novel pathogens or vectoring resident pathogenic fauna (Hedberg et al., 1985; Lounibos, 2002a; Yee 2008a). Invasions of vector mosquitoes like *Ae. aegypti*, *Cx. pipiens*, and most recently, *Ae. albopictus* have been facilitated by worldwide ship and aircraft transport, especially in water-holding objects like used tires (Lounibos, 2002a). One of the most recent examples is the introduction of *Ae. albopictus*. An established population was discovered for the first time at a roadside tire dump in Houston, Texas in 1985 (Moore and Mitchell, 1997; Mitchell et al., 1998; McMahon, 2008).

Discarded tires can be used by mosquitoes for breeding, representing a potential human health threat (Evans, 1997). There are millions of used tires discarded annually around the globe. The Rubber Manufacturers Association (2017) estimates that each year 280 million scrap tires are generated in the U.S. and around 67 million tires are in stockpiles in the U.S. Of the 32 mosquito species found in tires, *Ae. albopictus* and *Cx. pipiens* are two of the most abundant species (Yee, 2008a). *Aedes albopictus* and *Cx. pipiens* species

co-occur in high abundance in tires in residential areas, sharing limited space and resources (Costanzo, 2005a; Macia, 2006; Juliano, 2009). Similar to other container systems, tire dwelling mosquitoes often undergo strong inter- and intra-specific interactions that can generate phenotypical variations, in addition to genetic variants, which can have an important effect on population dynamics (Fish, 1985; Macia, 2006). Inter- and intra-specific competition during the larval stage can alter adult fitness and vectorial capacity (Bradshaw and Holzapfel 1986; Himeidan et al., 2013). A shortage of resources in earlier instars can result in lower percentages of larval survival and longer larval development as well as lower success and smaller size at metamorphosis, which could affect fecundity, mating success, and flight capacity as adults (Fisher et al., 1990; Bradshaw and Holzapfel, 1992; Bradshaw et al., 1993; Hard and Bradshaw, 1993).

Tire composition

Tires are composed of a complex mixture of chemical substances. Evans and Evans (2006) stated that a common sized all-season passenger tire fabricated by Goodyear contains 30 kinds of synthetic rubber, 8 kinds of natural rubber, 8 kinds of carbon black, steel cord for belts, polyester and nylon fiber, steel bead wire, waxes, oils, pigments, silicas and clays. Tires components vary according to the brand and use of the tire (Wik and Dave, 2005; Wik and Dave, 2009); for this reason, different authors have cited different kinds and amounts of metals and organic compounds in tires. Horner (1996) analyzed 10 different tire makes in the UK for cadmium, lead and zinc. To do this, he cut 5 small pieces of 10 g each from discarded tires, and then samples were ashed in a muffle furnace, digested in concentrated nitric acid, and analyzed in an atomic absorption spectrophotometer. He found that zinc concentrations ranged from 2,524 to 6,012 mg/kg, cadmium concentrations

ranged from 0 to 2 mg/kg, and lead concentrations ranged from 8.1 to 22.3 mg/kg. Kreider et al. (2010) found concentrations of 9,000 mg/kg of zinc, 12,000 mg/kg of sulfur, 470 mg/kg of aluminum, 76.5 mg/kg of antimony, and 21.5 mg/kg of copper in tire particles that were obtained from unused tires, which were cryogenically ground and sieved to 180 μm to obtain the ground material for analysis. Tires used by Kreider et al. (2010) were Goodyear and Michelin brands, which were analyzed using microwave assisted digestion and ICP-AES. Kreider et al. (2010) also analyzed these tires for polycyclic aromatic hydrocarbons (PAHs) using gas chromatography coupled with 2D mass spectrometry, finding concentrations of 2.95 mg/L of chrysene, 2.87 mg/L of benzo(a)Anthracene, 1.77 mg/L of benzo(ghi)perylene, 1.62 mg/L of fluoranthene, 1.24 mg/L of acenaphthalene, 1.21 mg/L of phenanthrene, and 1.18 mg/L of naphthalene.

Toxicity of tire leachate

Tires have historically been considered to be relatively benign in the environment unless subjected to high temperatures (Andrady et al. 2003). However, different studies suggest that tires contain water soluble compounds that leach into water and have lethal or chronic detrimental effects on some aquatic biota (Gualtieri et al., 2005a; Gualtieri et al. 2005b; Wik and Dave 2006). Most of these studies assessed toxicity of tire leachate using the leachate as a combination of pollutants which have a combined effect on biological organisms, instead of trying to identify specific contaminants. Wik and Dave (2009) highlight that this method is very common in assessing the toxicity of complex chemical mixtures like tire leachate, where most of toxic contaminants are unknown. Summarized below is an overview of the existing literature on the toxicity of tire leachate on aquatic organisms.

The study of Day et al. (1993) showed a 96-h median lethal concentration (LC₅₀) for *Oncorhynchus mykiss* (rainbow trout) that ranged from 11.8% to 19.3% (v/v) for leachate from used tires and 52.1% to 80.4% (v/v) for leachate from new tires, showing that leachate from discarded tires is more toxic than leachate from new tires. This is probably because antioxidants and antiozonants have been removed from the surface of the tire through wear of tire tread. This in turn could cause cracking of tires which could expose fillers and metals inside the tire to humidity and heat, producing higher leachate of toxicants. Gualtieri et al. (2005a) found a 72-h half maximal effective concentration (EC₅₀) of 0.47 to 1.64 g/L for *Pseudokirchneriella subcapitata* (a green algae) exposed to tire leachate. Wik and Dave (2005) found a 24-h EC₅₀ that ranged from 0.29 to 32 g/l and a 48-h EC₅₀ that ranged from 0.0625 to 2.41 g/l for *Daphnia Magna* exposed to leachate from tire particles from seven different tire brands. Wik and Dave (2005) also showed that tire leachate could increase its toxicity by more than 10-fold after exposure to UV-A radiation at 340 nm with a light intensity of 0.5 mW/cm².

Tire leachate also exhibits chronic toxicity effects on species such as the marine bacterium *Photobacterium phosphoreum* where bioluminescence is inhibited, *Escherichia coli* where the enzyme β -galactosidase is inhibited, and *Spirillum volutans* where some motility inhibition occurs (Day et al., 1993). Gualtiere et al. (2005b) reported 20% mortality of embryos and 80% of malformed larvae in *Xenopus laevis* (African clawed frog) when they are exposed for 120 hours to tire leachate with a concentration of 44.73 mg/l of zinc, obtained from tire particles. *Oncorhynchus mykiss* shows sub-lethal effects when exposed to high aromatic oils from tire leachates. After one day of exposure, cytochrome P4501A1 (CYP1A1) was induced; and, after 2 weeks of exposure, antioxidant

activity decreased, and the liver showed high concentration of glutathione groups and glucose-6-phosphate dehydrogenase (Stephensen et al., 2003). Camponelli et al. (2009) reported that zinc from tire wear particles increased the time of metamorphosis by 6.5 days of *Rana sylvatica* (wood frog).

The most common constituents of tires that are used as markers to assess tire contamination in the environment are styrene butadiene, benzothiazoles, and zinc (Fauser et al., 1999; Wik and Dave, 2009). The study of Nelson et al. (1994) is the only study that identified zinc from tire leachate as the toxicant that caused acute toxicity to *Ceriodaphnia dubia* with a 24-h LC₅₀ of 147.1 ug/L. Nelson et al. (1994) used ethylenediaminetetraaceticacid (EDTA) and sodium thiosulfate to remove the toxicity of copper, cadmium, and lead from the solution and found zinc as the toxic component for *C. dubia*. To reconfirm his results, he added zinc chloride to deionized water, and he found it was toxic to *C. dubia*. Nelson et al. (1994) found concentrations of 751 ug/L of zinc, 6.7 ug/L of copper, 6.7 ug/L of lead, and 0.6 ug/L of cadmium from leachate of 29 tire plugs of 10.25 cm of diameter and 100 g of weight. All the tire leachate samples tested for organic compounds were under the detection limit, which was 1.0 ug/L (Nelson et al. 1994). In our study, I assessed the effect of tire leachate on the overall population performance of competing *Ae. albopictus* and *Cx. pipiens* mosquitoes, using zinc as a marker of the presence of contaminants, metals and organic compounds, in tire leachate.

Ultraviolet radiation and its impact on mosquito populations and associated microbial communities

The ozone layer, whose formation in the stratosphere started 3.5 billion years ago, protects organisms and its environment from the harmful ultraviolet radiation (UVR)

emitted by the sun (McMichael et al., 2003). Ultraviolet radiation is electromagnetic radiation with a wavelength shorter than that of visible light, but longer than X-rays. The wavelength range of UVR is between 400 and 100 nm (Andrady et al., 1998). Ultraviolet radiation is subdivided into three subtypes, UV-A (400-315 nm), UV-B (315-280 nm) and UV-C (280-100 nm). The amount of UVR reaching the earth's surface is about five percent of all the energy received by the earth. Of the UVR that reaches the surface about five percent corresponds to UV-B radiation and ninety five percent to UV-A radiation. Most of UV-B radiation is absorbed by the ozone layer in the stratosphere (Mazza et al., 2010). The amount of UVR reaching the earth's surface depends on many factors: thickness of the ozone layer, the cosine law that states that solar radiation is highest at the equator and decreases towards the poles (Scotto et al., 1988), the incident intensity which is inversely related to the altitude, cloudiness and the presence of particulate matter in the atmosphere (Caldwell et al., 2003). Between the 1970's and 1990's, due to the increase of the emissions of Chlorofluorocarbons, a depletion of the ozone layer was observed (Newman, 2009). As a consequence of ozone depletion, UVR reaching the earth's surface increased by an average of 5% in the northern hemisphere mid-latitudes (40° N-50° N) and by 8 % in the southern hemisphere mid-latitudes (40° S-50° S), and by 2 to 3% in the tropics (20° N-20° S) (McKenzie et al., 2003).

Research on the direct impact of UVR on mosquitoes dates back to the 1930's, with the study of MacGregor (1932), which assessed which levels of UVR cause negative effects on larvae and pupae of *Ae. aegypti* and *Cx. pipiens* mosquitoes. MacGragor (1932) found that the pupal state is more resistant to UV-C and UV-B radiation levels than the larval state. He also found that *Cx. pipiens* larvae are more resistant to UVR than *Ae.*

aegypti larvae. Larvae irradiated with UV-C showed damage at the cuticle level after 45 seconds of exposure and paralysis, followed by death after 24 hours of exposure. Larvae exposed to UV-B radiation were affected when exposed for longer periods of time (more than 48 hours). Larvae lost movement coordination and increased swimming rates after 24 hours of exposure but still 60 % of larvae were able to pupate. However, none were able to become adult.

There may also be some indirect effects of UVR on mosquito communities. Ultraviolet radiation plays a major role in tire deterioration and the generation of leachate of pollutants in the aquatic environment where mosquitoes breed. Tire leachate affects mosquitoes in different ways. For example, the study of Suwanchaichinda and Brattsten (2002), showed that mosquito larvae from *Ae. albopictus* that were pre-exposed to tire leachate mainly containing benzothiazole were more tolerant to some pesticides such as carbaryl, rotenone, and temephos. On the other hand, there was no change in toxicity for aldrin treatment. The mechanism involved in this tolerance is that benzothiazole induces the cytochrome P450s, which detoxify insecticides and thus cause insecticide tolerance in the mosquito larvae (Scott, 1999). Burke et al. (1983) described the negative effect of UVR on the spore viability and larvicidal effect of *Bacillus sphaericus* strain 1593, used in the biological control for mosquitoes. Spore viability of *B. sphaericus* was drastically affected diminishing from 3.7×10^7 CFU/ml to 3.7×10^4 CFU/ml after 7.5 minutes of exposure to UV levels of 675 W/m^2 . The diminished spore viability decreased the mortality rate of *Cx. quinquifasciatus* by 25% after 24 hours of application. Also, spore viability of the indigenous ISPC-8 *Bacillus sphaericus* strain Neide was reduced to 2.5% and larvicidal activity was reduced by 50% against *Cx. quinquifasciatus* after 8 hours of exposure to UV-

B radiation that was provided by a 20W Philips lamp with a maximum wavelength of 315 nm, placed a 12.5 cm of distance (Hadapad et al., 2008).

Microbial organisms are the predominant food resources for mosquito larvae and influence the composition of container inhabitants via competitive interactions (Pelz-Stelinski et al., 2011). Microbial growth is influenced by various physical factors (e.g., temperature, pH, solar radiation) and chemical factors (e.g., oxygen, carbon, nitrogen, phosphorus). There are two different points of view about the effect of UV-B on microbial communities. Some authors indicate that UV-B radiation has negative effects on microorganisms and algae (Gao et al., 2008; Wu et al., 2009; Hader et al., 2007). Gao et al (2008) reported that UV-B radiation damages the DNA, proteins, membranes, and photochemical efficiency of photosynthetic prokaryote organisms like *Arthrospira platensis* (cyanobacteria), affecting photosynthesis and biomass production. The spiral structure of *Ar. platensis* is broken, and there is inhibition of photosynthetic activity after exposure to UV-B radiation in a temperature range of 18 to 20 °C. The damage to these cells is temperature and density dependent (Gao et al., 2008). Wu et al. (2005) also found that exposure to 4 to 6 hours of UV-B radiation breaks the spiral filaments of *Ar. platensis* into small pieces and it also affects photosynthesis activity. UV-B radiation specifically affects the photosynthetic electron transport and pigment-protein complexes of *Ar. platensis* (Wu et al., 2005). Hader et al. (2007) showed that UVR affects negatively algae and microbial communities in aquatic ecosystems on which mosquito larvae feed. Ultraviolet radiation penetrates to significant depths in aquatic systems, depending on water transparency, with effects ranging from effects on major biomass producers such as phytoplankton to effects on consumers in the food web such as mosquito larvae. Davidson

and Belbin (2002) found that marine phytoplankton and protozoan community assemblages exposed to UVR at less than 2 meters depth for more than a day suffer a reduction in biomass and concentration per cubic meter of water, which would represent less availability of food for mosquito larvae that feeds on these microbial assemblages. On the other hand, other authors have suggested that UVR could be beneficial to microbial communities because of increased availability of dissolved organic carbon, which leads to bacterial growth and bacterial abundance, leading to the increase of food resources for mosquito larvae (De Lange et al., 2003).

Dissertation goal and structure

The overarching goal of my dissertation is to test the role of UV-B radiation and tire contaminants in the coexistence of *Ae. albopictus* and *Cx. pipiens* mosquitoes in discarded tire habitats. By addressing this goal, my research is of importance both to the applied field of medical entomology and to basic ecological theory that may explain invasion success, impacts, and species coexistence. My thesis consists of four content chapters that address different aspects of the goal above, with each chapter describing a separate study and written as a stand alone paper.

In Chapter 2, I describe a laboratory experiment that tested the effects of UV-B radiation on the metabolic rate of larvae and fitness parameters (survival, development time, and body size) of *Ae. albopictus* and *Cx. pipiens*, and on the production of microbial resources on which mosquito larvae feed, in aquatic microcosms. I set up three UV-B radiation conditions that mimicked three common conditions in the field: (1) full-sun: 10.82 $\mu\text{mol}/\text{m}^2/\text{s}$ (FS); (2) shade: 6.1 $\mu\text{mol}/\text{m}^2/\text{s}$ (S); and (3) no UV radiation: 0.6 $\mu\text{mol}/\text{m}^2/\text{s}$

(NUV). The metabolic rates for larvae and microbial communities were measured at days 1, 8, and 15.

In Chapter 3, I used a controlled greenhouse experiment to test the effects of UV-B radiation on the degradation of discarded vehicular tires, using zinc as an indicator of tire leachate. To do this, whole discarded tires were exposed to one of my three UV-B conditions: FS, S, and NUV. Tires were filled with 4 L deionized water and were routinely topped up throughout the experiment. At days 1, 50, 100 and 150 water samples were collected to be analyzed for total and dissolved zinc. At day 210, each tire was destructively sampled for biofilm and biofilm was analyzed for total recoverable zinc.

In Chapter 4, in a controlled greenhouse experiment, I tested the hypothesis that tire leachate, released by tire degradation under three different UV-B radiation conditions (chapter 3), may promote condition-specific competition for limited food resources between *Ae. albopictus* and *Cx. pipiens*, thereby facilitating their coexistence. To do this, varying densities of newly hatched *Ae. albopictus* and *Cx. pipiens* larvae (*Ae. albopictus*: *Cx. pipiens*, 0:100, 0:50, 100:0, 50:0, 50:50) were added to tires that had been exposed to FS, S, or NUV conditions. From each tire, I measured three fitness parameters for both species: proportion ♀ survivorship, median ♀ development time, and median ♀ wing length. Using these fitness parameters, I calculated λ' , a composite index of population increases based on r' , the realized per capita rate of population change ($dN/N dt = r$, the exponential growth rate) (Juliano 1998). Furthermore, I measured total recoverable zinc in *Ae. albopictus* and *Cx. pipiens* mosquitoes.

In Chapter 5, I conducted an oviposition choice bioassay, where single species cohorts of *Ae. albopictus* and *Cx. pipiens* had access to cups containing tire leachate with

high vs. low zinc concentrations and high vs. low amounts of food resources (i.e., filtered and unfiltered). The variables that I measured were number of eggs and hatching percentage for *Ae. albopictus* and number of egg rafts and number of eggs per egg raft for *Cx. pipiens*.

From these 4 studies, I gather critical information on the ecologies of *Ae. albopictus* and *Cx. pipiens* in tire habitats, testing both basic ecological theory that can help explain species coexistence as well as explore how the significant environmental problem of discarded tires may affect human health by altering species interactions between an invasive and a resident mosquito.

All of the chapters have been written as manuscripts to be submitted to journals for publication. Purposely, the collective term “we” was used through the chapters to reflect the collaborative nature of our chapters for publication.

References

- Alto, B.W., Lampman, R.L., Kesavaraju, B., and Muturi, E.J. 2013. Pesticide-induced release from competition among competing *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, 50:1240-1249.
- Andrady, A.L., Hamid, H.S., Hu, X., and Torikai, A. 1998. Effects of increased solar ultraviolet radiation on materials. *Journal of Photochemistry and Photobiology*, 46:96-103.
- Andrady, A.L., Hamid, H.S., and Torikai, A. 2003. Effects of climate change and UV-B on materials. *Journal of Photochemistry and Photobiology*, 2:68-72.
- Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., and Kaiser, A. 2010. Mosquitoes and Their Control. Chapter 2: Biology of Mosquitoes. Springer, 2nd ed. Heidelberg, Germany.
- Benedict, M.Q., Levine, R.S., Hawley, W.A., and Lounibos, L.P. 2007. Spread of the Tiger: Global risk of invasion by the mosquito *Aedes albopictus*. *Vector Borne Zoonotic Diseases*, 7:76-85.
- Blaustein, L. and Chase, J.M. 2007. Interactions between mosquito larvae and species that share the same trophic level. *Annual Review of Entomology*, 52:489-507.
- Bradshaw, W.E., and Holzapfel, C.M. 1986. Habitat segregation among tree-hole mosquitoes. *National Geographic Research*, 2:167-178.
- Bradshaw, W.E., and Holzapfel, C.M. 1992. Reproductive consequences of density-dependent size variation in the Pitcherplant mosquito, *Wyeomyia smithii* (Diptera: Culicidae). *Annual Entomological Society of America*, 85:274-281.
- Bradshaw, W.E., Holzapfel, C.M., and O'Neill T. 1993. Egg size and reproductive allocation in the Pitcherplant mosquito, *Wyeomyia smithii* (Diptera: Culicidae). *Journal of Medical Entomology*, 30:384-390.
- Burke, W.F., McDonald, K.O. and Davidson, E.W. 1983. Effect of UV light on spore viability and mosquito larvicidal activity of *Bacillus sphaericus* 1593. *Applied and Environmental Microbiology*, 46:954-956.
- Caldwell, M.M., Ballare, C.L., Bornman, J.F., Flint, S.D., Bjorn, L.O., Teramura A.H., Teramura, A.H., Kulandaivelu, G., and Tevini, M. 2003. Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climatic change factors. *Photochemical and Photobiological Sciences*, 2:29-38.

- Camponelli, K.M., Casey, R.E., Snodgrass, J.W., Lev, S.M., and Landa, E.R. 2009. Impacts of weathered tire debris on the development of *Rana sylvatica* larvae. *Chemosphere*, 74:717-722.
- Carrieri, M., Bacchi, M., Bellini, R., and Maini, S. 2003. On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. *Environmental Entomology*, 32:1313-132.
- Clements, A.N. 2000. The biology of mosquitoes. Development, nutrition and reproduction. Volume 1. CABI publishing, New York, NY, USA.
- Costanzo, K.S., Mormann, K., and Juliano, S.A. 2005a. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology*, 42:559-570.
- Costanzo, K.S., Kesavaraju, B., and Juliano, S.A., 2005b. Condition-specific competition in container mosquitoes: the role of noncompeting life-history stages. *Ecology*, 86:3289-3295.
- Costanzo, K.S., Muturi, E.J., Lampman, L.R., and Alto, R.W. 2011. The effects of resource type and ratio on competition with *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology*, 48:29-38.
- Darsie, R.F., and Ward, A.R. 2005. Identification and geographical distribution of the mosquitos of North America, North of Mexico. Gainesville, University Press of Florida.
- Davidson, A., and Belbin, L. 2002. Exposure of natural Antarctic marine microbial assemblages to ambient UV radiation: effects on the marine microbial community. *Aquatic Microbial Ecology*, 27:159:174.
- De Lange, H.J., Morris, D.P., and Williamson, C.E. 2003. Solar ultraviolet photodegradation of DOC may stimulate freshwater food webs. *Journal of Plankton Research*, 25:111-117.
- Estrada-Franco, J.G., Craig, G.B. 1995. Biology, disease relationships and control of *Aedes albopictus*. Technical paper No. 42, Pan American Health Organization, Washington, D.C.
- Evans, J.J. 1997. Rubber tire leachates in the aquatic environment. *Reviews in Environmental Contamination and Toxicology*, 151:67-115.
- Evans, A., and Evans, R. 2006. UK waste tyre management best practice: Handling of post-consumer tyres – collection & storage. Tyres report, The Waste & Resources Action Programme.

- Fausser, P., Tjell, J.C., Mosbaek, H., Pileegard, K. 1999. Quantification of tire-tread particles using extractable organic zinc as tracer. *Rubber Chemistry and Technology*, 72:969-977.
- Fish, D..1985. An analysis of adult size variation within natural mosquito populations. In L.P. Lounibos, J.R. Rey, and J.H. Frank, (eds.) *Ecology of mosquitoes: Proceedings of Florida Medical Entomology Workshop*, pg, 419-30.
- Fisher, I.J., Bradshaw, W.E., and Kammeyer, C. 1990. Fitness and its correlates assessed by intra- and interspecific interactions among tree-hole mosquitoes. *Journal of Animal Ecology*, 59:819-829.
- Fonseca, D., M., Keyghobadi, N., Malcolm, C.A., Mehmet, C, Schaffner, F., Mogi, M., Fleischer, R.C., and Wilkerson R.C. 2004. Emerging vectors in the *Culex pipiens* complex. *Science*, 303: 1535-1538.
- Freed, T.Z., and Leisnham, P.T. 2014. Roles of spatial partitioning, competition, and predation in the North American invasion of an exotic mosquito. *Oecologia*, 175:601-611.
- Gao, K., Li, P., Watanabe, T., and Helbling, E.W. 2008. Combined effects of ultraviolet radiation and temperature on morphology, photosynthesis, and DNA of *Arthrospira* (*Spirulina*) *Platensis* (*Cyanophyta*). *Journal of Phycology*, 44:777-786.
- Gualtieri, M., Andrioletti, M., Vismara, C., Milani, M. and Camatini, M. 2005a. Toxicity of tire debris leachates. *Environment International*, 31:723-730.
- Gualtieri, M., Andrioletti, M., Mantecca, P., Vismara, C., and Camatini, M. 2005b. Impact of tire debris on in vitro and in vivo systems. *Particle and Fibre Toxicology*, 2:1-14.
- Hadapad, A.B., Vijayalakshmi, N., Hire, R.S., Dongre, Tk. 2008. Effect of ultraviolet radiation on spore viability and mosquitocidal activity of an indigenous ISPC-8 *Bacillus sphaericus* Neide strain. *Acta Tropica*, 107:113-116.
- Hard, J.J., and Bradshaw. 1993. Reproductive allocation in the western tree-hole mosquito, *Aedes sierrensis*. *OIKOS journal*. 66:55-65.
- Hader, D.P., Kumar, H.D., Smith, R.C., Worrest, R.C. 2007. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical & Photobiological Sciences*, 6:267-285.
- Hedberg, C.W., Washburn, J.W., and Sjogren, R.D. 1985. The association of artificial containers and LaCrosse encephalitis cases in Minnesota 1979. *Journal of the American Mosquito Control Association*, 1:89-90.

- Himeidan, Y.E., Temu E.A., ElRayah E.A., Munga, S., and Kweka, E. 2013. Chemical cues for Malaria vectors oviposition site selection: challenges and opportunities. *Journal of insects*, 2013:1-9.
- Horner, J.M. 1996. Environmental health implications of heavy metal pollution from car tires. *Reviews on Environmental Health*, 11:175-178.
- Juliano, S.A., and Lounibos, L.P. 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecology letters*, 8:558-574.
- Juliano, S.A. 2009. Species interactions among larval mosquitoes: context dependence across habitat gradients. *Annual Review of Entomology*, 54:37-56.
- Kesavaraju, B., Leisnham, P.T., Keane, S., Delisi, N., Pozatti, R. 2014. Interspecific competition between *Aedes albopictus* and *Ae. sierrensis*: Potential for competitive displacement in the Western United States. *PLoS ONE*, 9(2): [e89698](https://doi.org/10.1371/journal.pone.0089698). [doi:10.1371/journal.pone.0089698](https://doi.org/10.1371/journal.pone.0089698)
- Kilpatrick, A.M., Meola, M.A., Moudy, R. M., and Kramer, L.D. 2008. Temperature, viral genetics, and the transmission of West Nile Virus by *Culex pipiens*. *PLoS Pathogens*, 4:1-7.
- Kim, H. C., Wilkerson, R., Pecor, J., Lee, W., Lee, J., O'Guinns, M., and Klein, T. 2005. New records and reference collection of mosquitoes (*Diptera: Culicidae*) on Jeju Island, Republic of Korea. *Entomological Research*, 35:55-56.
- Kreider, M.L., Panko, J.M., McAtee, B.L., Sweet, and Finley, B.L. 2010. Physical and chemical characterization of tire-related particles: Comparison of particles generated using different methodologies. *Science of the Total Environment*, 408:652-659.
- Lounibos, L.P. 2002a. Invasions by insect vectors of human disease. *Annual Review of Entomology*, 47:233-266.
- Lounibos, L.P., Suarez, S., Menendez, Z., Nishimura, N., Escher, R.L., O'Connell, S.M., and Rey, J.R. 2002b. Does temperature affect the outcome of larval competition between *Aedes aegypti* and *Aedes albopictus*? *Journal Vector Ecology*, 27:86-95.
- Lounibos, L.P., Escher, R.L., and Nishimura, N. 2011. Retention and adaptiveness of photoperiodic egg diapause in Florida populations of invasive *Aedes albopictus*. *Journal of American Mosquito Control Association*, 27:433-436.
- Macia, A. 2006. Differences in performance of *Aedes aegypti* larvae raised at different densities in tires and ovitraps under field conditions in Argentina. *Journal of Vector Ecology*, 37:371-377.

- MacGregor, M.E. 1932. Certain pathological effects of ultra-violet radiation on mosquito larvae and pupae. *Proceedings of the Royal Society of London*, 112:27-38.
- Markowski, D. 2015. Mosquito of the month: *Culex pipiens* – Northern House Mosquito. Vector Disease Control International. Mosquito of the month series. www.vdci.net/blog/topic/mosquito-of-the-month-series
- McKenzie, R.L., Bjorn, L.O., Bais, A., Ilyasd, M. 2003. Changes in biologically active ultraviolet radiation reaching the Earth surface. *Photochemical and Photobiological Sciences*, 2:5-15.
- McMahon, T.J., Galloway, T.D., and Anderson, R.A. 2008. Tires as larval habitats for mosquitoes (*Diptera: Culicidae*) in southern Manitoba, Canada. *Journal of Vector Ecology*, 33:198-204.
- McMichael, A.J., Lucas, R., Ponsonby, A.L., and Edwards, S.J. 2003. Stratospheric ozone depletion, ultraviolet radiation and health. In: McMichael et al. (Ed.), *Climate change and human health, risk and responses*. WMO library cataloguing, Geneva, pp. 159-178.
- Mazza, C.A., Izaguirre, M.M., Curiales, J., and Ballare, C.L. 2010. A look into the invisible: ultraviolet-B sensitivity in an insect (*Caliothrips phaseoli*) revealed through a behavioural action spectrum. *Proceedings Biological Science*, 277:367-373.
- Merritt, R.W., Dadd, R.H., Walker, E.D. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual Review of Entomology*, 37:349-376.
- Mitchell, C.J., Haramis, L.D., Karabatsos, N., Smith, G.C., and Starwalt, V.J. 1998. Isolation of La Crosse, Cache Valley, and Potosi viruses from *Aedes* mosquitoes (*Diptera: Culicidae*) collected at used tire-sites in Illinois during 1994-1995. *Journal Medical Entomology*, 35:573-577.
- Moore, G.C., and Mitchell, C.J. 1997. *Aedes albopictus* in the United States: Ten year presence and public health implications. *Emerging Infectious Diseases*, 3:39-334.
- Murrel, E.G., and Juliano S.A. 2008. The role of detritus type in interspecific competition and population distributions of *Aedes aegypti* and *Aedes albopictus*. *Journal of Medical Entomology*, 45:375-383.
- Nelson, S.M., Mueller, G., and Hemphill, D.C. 1994. Identification of tire leachate toxicants and a risk assessment of water quality effects using tire reefs in canals. *Bulletin of Environmental Contamination and Toxicology*. 52:574-581.
- Newman, P.A., Oman, L.D., Douglas, A.R., Fleming, E.L., Frith, S.M., Hurwitz, M.M., Kawa, S.R., Jackman, C.H., Krotkov, E.R., Nash, E.R., Nielsen, J.E., Pawson, S., Stolarski, R.S., and Velders, J.M. 2009. What would have happened to the ozone layer if

chlorofluorocarbons (CFCs) had not been regulated? Journal of Atmospheric Chemistry and Physics. 9:2113-2128.

Ngoagouni, C., Kamgang, B., Nakoune, E., Paupy, C., and Kasanji, M. 2015. Invasion of *Aedes albopictus* (Diptera: Culicidae) into central Africa: what consequences for emerging diseases? Parasites & Vectors, 8:1-7.

Pelz-Stelinski, K., Kaufman, M.G., and Walker, E.D. 2011. Beetle (Coleoptera: Scirtidae) facilitation of larval mosquito growth in tree hole habitats is linked to multitrophic microbial interactions. Microbial Ecology, 3:690-703.

Rubber Manufacturers Association-RMA. 2017. 2015 U.S. scrap tire management summary. Rubber Manufacturers Association, Washington, DC.

Scott, J.G. 1999. Cytochromes P450 and insecticide resistance. Insect Biochemistry and Molecular Biology, 29:757-777.

Scotto, J., Cotton, G., Urbach, F., Berger, D., and Fears, T. 1988. Biologically effective ultraviolet radiation: surface measurements in the United States, 1974 to 1985. Science, 239:762-764.

Smith, C., Baldwin, A.H., Sullivan, J., and Leishnam, P.T. 2013. Effects of Elevated Atmospheric CO₂ on Competition between the Mosquitoes *Aedes albopictus* and *Ae. triseriatus* via Changes in Litter Quality and Production. Journal of Medical Entomology, 50:521-532.

Sprenger, D. and Wuithiranyagool, T. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. Journal of the American Mosquito Control Association, 2:217-219.

Stephensen, E., Adolfsson-Erisi, M., Celander, M., Hulander, M., Parkkonen, J., Hegelund, T., Sturve, J., Hasselberg, L., Bengtsson, M., and Forlin, L. 2003. Biomarker responses and chemical analysis in fish indicate leakage of polycyclic aromatic hydrocarbons and other compounds from car tire rubber. Environmental Toxicology, 22:2926-2931.

Suwanchaichinda, C., and Brattsten, L.B. 2002. Induction of microsomal cytochrome P450s by tire-leachate compounds, habitat components of *Aedes albopictus* mosquito larvae. Archives of Insect Biochemistry and Physiology, 49:71-79.

Vinogradova, E.B. 2000. *Culex pipiens* mosquitoes; taxonomy, distribution, ecology, physiology, genetics, applied importance and control. Pensoft, Sofia, Bulgaria.

Walker, E.D., Lawson, D.L., Merrit, R.W., Morgan, W.T., and Klug, M.J. 1991. Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. Ecology, 72:1529-1546.

Wik, A., and Dave, G. 2005. Environmental labeling of car tires - toxicity to *Daphnia magna* can be used as a screening method. *Chemosphere*, 58:645-651.

Wik, A., and Dave, G. 2006. Acute toxicity of leachates of tire material to *Daphnia magna* – Variability and toxic components. *Chemosphere*, 64:1777-1784.

Wik A., and Dave, G. 2009. Occurrence and effects of tire wear particles in the environment – A critical review and an initial risk assessment. *Environmental Pollution*, 157:1-11.

Wu, H., Gao, K., and Wu, H. 2009. Responses of a marine red tide alga *Skeletonema costatum* (*Bacillariophyceae*) to long-term UV radiation exposures. *Journal of Photochemistry and Photobiology*, 94:82-86.

Yee, D.A. 2008a. Tires as habitats for mosquitoes: A review of studies within the eastern United States. *Journal of Medical entomology*, 45:581-593.

Chapter 2: Effects of ultraviolet radiation on metabolic rate and fitness

of *Aedes albopictus* and *Culex pipiens* mosquitoes

Oswaldo C. Villena^{1,5}, Edward Landa², Bahram Momen², Joseph Sullivan³, Paul T. Leisnham⁴

1. Marine Estuarine & Environmental Science, University of Maryland, College Park, MD 20742
2. Environmental Science & Technology, University of Maryland, College Park, MD 20742
3. Department of Plant Science & Landscape Architecture, University of Maryland, College Park, 20742
4. Marine Estuarine & Environmental Science and Environmental Science & Technology, University of Maryland, College Park, MD 20742
5. Corresponding author: oswaldo.villena@gmail.com

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Abstract

Environmental changes will alter many environmental factors in the coming years including temperature, precipitation, humidity, and the amount of solar radiation reaching the earth's surface, which in turn will have an impact on living organisms like invertebrates. In this study, we assessed the effect of UV-B radiation upon the metabolic rate and upon three fitness parameters (survival, development time, and body size) of the mosquitoes *Aedes albopictus* and *Culex pipiens*, and upon the production of microbial resources on which mosquito larvae feed in aquatic microcosms. We set up three UV-B radiation treatments mimicking levels typically measured in full-sun (FS) and shade (S) conditions, as well as a control group with no UV-B radiation (NUV). The metabolic rate expressed as heat production (μ watts/ml) for larvae and microbial community was measured at days 1, 8, and 15. Our results indicated that UV-B radiation affected the metabolic rate of both *Cx. pipiens* and *Ae. albopictus* larvae; metabolic rates were significantly higher in full-sun (FS) compared to shade (S) and no-UV condition (NUV), at days 8 and 15 compared to day 1 (Figures 1A and 1B). *Culex pipiens* metabolic rates were significantly higher than *Ae. albopictus* at day 15 compared to days 1 and 8 (Figure 1B). Metabolic rates were significantly lower in microbial communities from vials with *Ae. albopictus* larvae, *Cx. pipiens* larvae, and no larvae in FS conditions compared to vials from S and NUV conditions, especially at day 8 (Figure 2A and 2B). There was a major effect of UV-B conditions only on the survival of *Ae. albopictus* and *Cx. pipiens* mosquitoes, with significantly lower survival in FS compared to S and NUV conditions. UV-B radiation at levels found in aquatic environments in open fields showed a negative impact on the metabolic rate of *Ae. albopictus* and *Cx. pipiens* larvae and on the microbial

communities on which they feed. These negative impacts could have important implications for the distribution and abundance of these mosquitoes and for the transmission rate of illness caused by the pathogens that these two broadly distributed mosquitoes transmit.

Key words: Ultraviolet radiation, metabolic rate, *Ae. albopictus*, *Cx. pipiens*, microbial communities

Introduction

Environmental changes (e.g., global warming, climate change) will trigger major changes in environmental factors (e.g., temperature, solar radiation) in coming years. These changes are likely to have profound impacts on insect ecology and physiology, including survival, development time and underlying metabolic processes (Helbling and Zagarese, 2003). Environmental changes will differ with geographical regions, and the impacts on the ecology and physiology of insects will vary with the extent of temperature increase, amounts and patterns of precipitation and humidity, and changes in incoming solar radiation, especially UV-B radiation. Despite many uncertainties, there is consensus that environmental changes have had and will have impacts upon insect metabolic processes, fitness variables, geographical ranges, and abundance; upon species extinction; upon activity and abundance of natural enemies; and upon the transmission of vector-borne diseases (Shuman, 2011; Gray, 2013).

Temperature is the most well-studied environmental factor that affects insect biology and ecology. Most insects are ectothermic, meaning that their bodily heat source is primarily sourced from the environment; through thermoregulation, they regulate their body temperature to optimally support survival and reproduction (Klowden, 2007; Terblanche et al. 2005, 2009; Klowden, 2007). Although in the context of environmental changes much of the focus has been on changing temperatures, precipitation and humidity also have important impacts on insects. Terrestrial insects lose water through their cuticle, and aquatic insects require water for habitat. Water availability could affect insect activity, distribution patterns, and species richness, especially for those insects that inhabit

ephemeral habitats (e.g., mosquitoes). Other environmental variables may also have subtle yet important effects upon insects; perhaps the most interesting of these is UV radiation.

Ultraviolet radiation (UVR) is part of the electromagnetic spectrum emitted by the sun, with a wavelength range between 400 and 100 nm (Andrady et al., 1998); it is subdivided into three subtypes: UV-A (400-315 nm), UV-B (315-280 nm) and UV-C (280-100 nm). Of these three subtypes UV-C, the most harmful, does not reach the earth's surface (Dyer, 2001; Caldwell et al., 2003). Of the UVR that reaches the earth's surface, around five percent corresponds to UV-B radiation and ninety five percent to UV-A radiation. Of these two, UV-B radiation is more harmful to biotic and abiotic environments because of its shorter wavelength, which means higher energy levels (Andrady et al., 1998). Variation in exposure to radiation throughout the landscape, because of varying shade conditions, can moderate the direct and indirect effects of UVR upon insects. Relatively few studies have examined the effects of UVR, and the few that have been published have focused on insect control through the use of UVR traps (Shimoda and Honda, 2013; Sliney et al., 2016) or, the use of UVR to affect insect physiology (e.g., flight behavior, orientation, visual ecology) in greenhouse facilities (Johansen et al., 2011).

Mosquitoes are blood-feeding insects of the order Diptera. They are medically important because they transmit vector-borne diseases. *Aedes albopictus* and *Cx. pipiens* are two common mosquitoes in urban areas of the eastern United States (Joy et al., 2003; Joy 2004; Costanzo et al., 2005). *Aedes albopictus* is an important vector for the transmission of many viral pathogens, including yellow fever, dengue, and Chikungunya (Lambrechts et al., 2010). *Aedes albopictus* is also capable of hosting the Zika virus and it is therefore considered a potential vector for Zika virus (Wong et al., 2013). *Culex pipiens*

is an important vector for the transmission of West Nile virus, Japanese encephalitis, and meningitis (Gerhardt et al., 2001; Kim et al., 2005; Molaei et al., 2006). *Aedes albopictus* and *Cx. pipiens* are also capable of transmitting the dog heartworm (*Dirofilaria immitis*), which not only affects dogs but also cats, foxes, coyotes, and other animals (Cancrini, 2007).

Mosquitoes have a complex life cycle; they lay eggs in aquatic environments where the larvae and pupae develop in several weeks until adults emerge into the terrestrial environment where they can freely move (Juliano, 2009). Mosquito larvae feed on microbial communities (Juliano, 2009). Environmental effects on larval stages have important consequences for some adult traits (Terblanche and Chown, 2007). Larval ecology affects distribution and abundance of adults, by modulating survival as well as adult fitness parameters, such as body size, that can affect adult survival, biting rate, and ultimately the ability to vector and transmit pathogens. There is little information on the effect of UV-B radiation on mosquito metabolic rate and survival and on the microbial community on which mosquitoes feed. One of the few studies that has assessed the effects of UVR on mosquitoes dates back to the 1930's (MacGregor, 1932); the author, demonstrated clear negative effects on larvae and pupae of *Ae. aegypti* and *Cx. pipiens* mosquitoes. However, a significant limitation of the study is that the UVR levels used were not comparable to field conditions.

Other studies have demonstrated that UV-B radiation has effects on microbial communities (Pancotto et al., 2003), but none have examined how these effects may impact mosquito populations. Future variations in UV-B, resulting from climate change and anthropogenic activities (e.g., change in land use, pollution), may have more important

consequences for microbial communities and for decomposition of dead plant and animal material than the changes in UV-B caused by ozone depletion, thereby affecting the food chains that depend on microbial communities (Ballare et al., 2011). The goal of this chapter is to test the effect of field-relevant UV-B radiation on the metabolic rate, larval survival, development time, and adult body size of *Ae. albopictus* and *Cx. pipiens* mosquitoes, and on the production of the microbial communities on which the larvae feed.

Materials and methods

Collection and maintenance of mosquitoes

Aedes albopictus and *Cx. pipiens* larvae were collected from multiple locations in College Park, Baltimore, and Towson, Maryland. Neither *Ae. albopictus* or *Cx. pipiens* are endangered, and collection sites were either on publically accessible lands or on private lands where consent for collections was granted at the time of collection; thus, no field permits were required to collect them. Field collected *Ae. albopictus* and *Cx. pipiens* larvae were reared to adulthood at 25°C at 16:8 (L:D) h photoperiod, and then released into 1-m² single-species cages. Adults were kept in an insectary at 25°C and >85% RH, 16:8 (L:D) h photoperiod. Both colonies were supplied 20% sugar solution. Females from both colonies were fed horse or rooster blood once a week via an artificial feeder (Hemotek, Accrington, UK) to ensure egg production and experimental larvae. *Aedes albopictus* females oviposited on seed paper in 500 ml black cups covered filled with 200 ml of deionized (DI) water. Eggs were collected over multiple weeks and stored at >80% RH and 16:8 hours (L:D) photoperiod until hatching for the experiment. *Culex pipiens* oviposited egg rafts into a 500 ml black bowl filled with 400 ml of DI water. *Culex pipiens* eggs cannot be held without hatching; thus, egg rafts were collected within 24 h of oviposition, hatched

in a lactobumina: yeast solution, and larvae were transferred into the experiment after being rinsed. *Ae. albopictus* eggs that had been stored were also hatched in a lactobumina: yeast solution and transferred into the experiment after being rinsed and within 24 h of hatching. Experimental larvae were of both species were F₁₋₃ generation.

Experiment set up

The experimental design was a split plot-randomized complete block design (RCBD) with UV-B radiation condition as the main plot, mosquito larvae cohorts (*Ae. albopictus*, *Cx. pipiens*, or no larvae) as the sub-plots, and three replicate blocks. Individuals of *Ae. albopictus* and *Cx. pipiens* were sorted into single species cohorts of 10 newly hatched individuals and added to 20 ml vials with 17 ml of DI water that were inoculated with 1 ml of water containing a microbial community that was collected from discarded tires. A total of 45 vials were prepared. Ten newly hatched *Ae. albopictus* or ten newly hatched *Cx. pipiens* were randomly added to 15 vials each. 15 vials only contained microbial community; no larvae were placed on these vials. Fifteen of the total vials (5 of each treatment) were randomly allotted to one of three Percival reach-in environmental chambers, model I-36 VL, located in the Aqua Engineering laboratory in the Environmental Science and Technology Program (ENST). Each chamber was kept at 25° C, 16:8 (L: D), and 80- 90 % of humidity, to mimic typical summer conditions in the northeastern U.S. (Day et al., 1993; Li et al., 2006). At the end, each environmental chamber had 5 vials containing 10 first instar larvae of *Ae. albopictus*, 5 vials containing 10 first instar larvae of *Cx. pipiens*, and 5 vials containing no larvae, only the microbial community. Vials represent sub-samples and each experimental unit was a group of 5 vials in one of the three environmental chambers. Each vial was checked daily to collect pupae

and place them in individual vials with water from that vial until adults emerged. I recorded the following information for each adult: date of emergence, sex, species, and replicate (notebook for the experiment). On the day of emergence, the adults were killed by placing them on a drying oven for further analysis such as wing length measurements. The Experiment was run until all larvae had died or eclosed.

One of three UV-B radiation conditions mimicking full-sun (10.82 $\mu\text{mol}/\text{m}^2/\text{s}$), shade (6.1 $\mu\text{mol}/\text{m}^2/\text{s}$), and a no-UV control group (0 $\mu\text{mol}/\text{m}^2/\text{s}$), were applied to each chamber. To achieve the required UV-B levels in the full-sun and shade treatment chambers, cellulose diacetate filters were applied on four UV-B-313 lamps (Q Panel Lab Products, Cleveland, OH) in each chamber, and vials were placed 5 cm and 20 cm from the bulbs, respectively. For the control group, I used regular Phillips 32 watts bulbs, model 205047, which simulate a visible range of sunlight (400 nm - 700 nm). To assure uniform exposure to UV radiation, vials were rotated daily. I ran the experiment three times (blocks) and applied a different UV-B treatment to each incubator each time to minimize incubator-treatment confounding effect.

Measurement of metabolic rate

Metabolic rate was measured as the rate of heat production ($\mu\text{watts}/\text{ml}$) by a heat conduction, multicell differential scanning calorimeter (MC-DSC model 4100, Calorimetry Sciences Corp.). The multicell differential calorimeter was set up in isothermal mode, at a temperature of 25 °C, which allows concurrent measurements of two samples using two 1 cm^3 ampoules. For larvae, five were selected randomly from each sub-sample of each of the three treatments. Before being place in the ampoule, they were washed in sterilized water and placed inside the ampoule with 1 ml of deionized water. The heat

production was monitored for 60 minutes to allow for temporal equilibration and consistency of final readings. Previous to this step, I ran a blank sample (just deionized water) for 60 minutes. After obtaining the reading in μwatts (μW) I subtracted the blank reading from the sample reading, and the result was the final metabolic rate value in $\mu\text{watts/ml}$ (Zhang et. al., 2009). Metabolic rates were measured when larvae were first instars (within 2 to 24 hours of hatching); the second measurement was when larvae were 8 days old; and the final measurement was made when larvae were 15 days old. For mosquito larvae metabolic rate measurements, from each species, five larvae were collected randomly from each vial, together with 5 ml of water from the same vial and placed in a sterile bottle (5 ml) and transported to the laboratory for metabolic rate measurements. Vials in chambers were refilled with deionized water as needed.

To measure the metabolic rate of the microbial community I followed the same procedure described for mosquito larvae metabolic rate measurements. The only difference was that I placed 1 ml of water sampled from the vials in the ampoules. We measured the microbial metabolic rate 24 hours after inoculation of vials with a 1 ml of microbial inoculum from tires (day 1). After completing microbial metabolic measurements for day 1, we placed the first instar larvae in the corresponding vials as described in previous paragraph. The metabolic rate of the microbial community was also measured on days 8 and 15. Before placing samples in the ampoules, they were washed in sterilized water and sterilized with ethanol before each run (Zhang et al., 2009). To place larvae and water microbial samples into the ampoules, we used sterile pipettes, tips, and forceps to avoid any kind of sample contamination. To collect microbial community samples to measure metabolic rate, we mixed the liquid content in each vial with a manual stirrer and took a 2

ml sample and placed it in a sterile bottle (5 ml) for its transportation to the laboratory for metabolic rate measurements.

Analyses

All the data were analyzed using linear models using the SAS 9.4 software (SAS Institute Inc. 2013). The metabolic rate of *Ae. albopictus* and *Cx. pipiens* larvae and microbial community from containers with *Ae. albopictus* larvae, *Cx. pipiens* larvae and no larvae were analyzed as a three-way analysis of variance (ANOVA) containing one repeated factor (day of sampling) using the PROC MIXED procedure, with UV-B condition, species and days as fixed effects, day as the repeated variable, and block as a random effect. Metabolic rate was measured on days 1, 8, and 15. To account for assumptions of normality and homogeneity of variances, data were $\log_{10}(y)$ transformed.

For vials with larval mosquito cohorts, fitness parameters were calculated (proportion survival, development time, and wing length). To determine survival rate, the number of adults were compared with the initial number of larvae placed in the experimental units; to measure mean development time, we considered the days from hatching to adulthood; and to measure wing length, we used a dissecting microscope and the image analysis system called Image Pro Plus 6.0. These fitness parameters were analyzed as a two way ANOVA using the PROC MIXED procedure; we considered UV conditions and species as fixed effects, and block as a random effect in the model. To account for assumptions of normality and homogeneity of variances, data were $\log_{10}(y+1)$ transformed. We did a pairwise mean comparison in the mixed procedures using the LSMEANS statement with tukey adjustment. For all analyses experiment-wise $\alpha = 0.05$; marginal significance was defined $\alpha = 0.05-0.10$.

Results

Metabolic rate of mosquito larvae

There was an interaction between UV-B condition and day of sampling (Table 1). There was also an interaction between species and day of sampling for larval metabolic rate, indicating differences in metabolic rate depending on the day of sampling for both *Ae. albopictus* and *Cx. pipiens* (Table 1, Figure 1B). At day 15, metabolic rate of *Cx. pipiens* was significantly higher compared to *Ae. albopictus* in FS conditions; this was not seen on days 1, and 8 (Figure 1B). Main effects of UV-B condition, species, and days were detected on larval metabolic rates (Table 1). Metabolic rates of both *Ae. albopictus* and *Cx. pipiens* were significantly higher under FS condition compared to NUV condition (Figure 1A). Metabolic rates of both *Ae. albopictus* and *Cx. pipiens* were higher at days 8 and 15 compared to day 1, with *Cx. pipiens* metabolic rates being higher than *Ae. albopictus* metabolic rates (Figure 1B).

Metabolic rate of microbial community

There was an interaction between UV-B condition and day of sampling for microbial metabolic rate (Table 2). Main effects of UV-B condition and day of sampling were detected on microbial metabolic rates (Table 2, Figures 2A and 2B). Metabolic rates of microbial communities from vials with *Ae. albopictus*, *Cx. pipiens* larvae and no larvae were significantly lower in FS condition compared to S and NUV conditions (Figure 2A). Metabolic rates of microbial communities from vials with *Ae. albopictus*, *Cx. pipiens* larvae and no larvae were significantly lower at day 8 compared to days 1 and 15 (Figure 2B).

Mosquito Fitness parameters

There was no an interaction between UV-B condition and species for *Ae. albopictus* and *Cx. pipiens* fitness parameters: survival, development time, and body size (Table 3, Figure 3). UV-B condition affected the survival of *Ae. albopictus* and *Cx. pipiens* mosquitoes similarly, with significantly lower survival of both species under FS conditions compared to S and NUV conditions (Figure 3A and 3B). There was also a main effect of species on body size between *Ae. albopictus* and *Cx. pipiens*, with *Cx. pipiens* being the larger on average (Figure 3E and 3F).

Discussion

Ultraviolet radiation may have important effects on the development of mosquitoes, effects that could have important implications for the distribution and abundance of pathogen-transmitting species and their microbial food resources. This is the first study that has assessed the effect of UV-B radiation comparable to that which reaches water bodies in open fields (full-sun), shaded areas (shade), and no-UV radiation conditions (control group) upon the fitness (survivorship, development time, and body size) and metabolic rates of two of the most broadly distributed mosquito species in the world: *Ae. albopictus* and *Cx. pipiens*; and upon the microbial communities on which they feed. In previously published field studies, resting metabolic rate increased in mosquito larvae between emergence and day 4 to 5 (Gray and Bradley, 2003). Our results showed that larval metabolic rate of both *Cx. pipiens* and *Ae. albopictus* mosquitoes significantly increased in FS conditions compared to NUV conditions (Figure 1A). In addition, larval metabolic rate is significantly higher at day 8 and day 15, indicating a direct effect of UV-B radiation on

mosquito metabolism. Furthermore, at day 15, metabolic rate was significantly higher for *Cx. pipiens* compared to *Ae. albopictus* (Figure 1B).

The increase of metabolic rate in *Cx. pipiens* larvae from day 8 to day 15 under FS conditions compared to *Ae. albopictus* presumably reflects that UV-B radiation had a greater negative effect on *Cx. pipiens* larvae. It has been shown in many insect species that metabolic rate is strongly related to the physical and biological factors that influence metabolism (Gray and Bradley, 2003). The *Culex pipiens* mosquito larvae probably increases its metabolic rate under FS conditions, especially at day 15, in response to negative effects of UV-B radiation. These effects could cause larvae to increase energy expenditure in order to perform physical and biological activities such as getting food, growing, or competing with conspecifics, which is reflected in greater metabolic rates. It has been shown previously that UV-B radiation has a greater negative effect on the larval stage than the pupae stage, as the pupae stage is more resistant to damage by UV radiation (MacGregor, 1932). In that study, larvae of *Cx. pipiens* exposed to UV-B radiation were affected when exposed for long periods of time (more than 48 hours). Larvae lost movement coordination and increased swimming rates after 24 hours of exposure but still 60 % of larvae were able to pupate. However, none were able to become adults. Histological analyses showed that larvae suffered damage in the cuticle, there was disintegration of the abdominal segments, that the peristaltic-wave no longer travelled between the 7th and 8th segment, and that the pulse rate was lowered (MacGregor, 1932).

Natural and artificial container aquatic habitats (e.g., puddles, tree holes, tires) are inhabited by a specialized community of macroinvertebrates (e.g., mosquitoes) that feed on microbial communities associated with decaying organic matter from insect carcasses

and leaf litter (Walker et al., 1988; Walker et al., 1991). Microbial abundance and diversity could be affected by environmental stressors (e.g., contaminants, UV radiation). In this study, we assessed how microbial communities from microcosms that contain *Ae. albopictus* larvae, *Cx. pipiens* larvae, or no larvae (just microbial community), were affected by UV-B radiation. We used microbial community metabolic rate expressed as heat production ($\mu\text{watts/ml}$) as an indicator of the quantity of microbial community. Microbial community metabolic rate in the three different microcosms was significantly lower in FS compared to S and NUV conditions, especially for microbial communities from microcosms that do not contain larvae; metabolic rate decreased in 35.73 % from NUV conditions and in 33.93 % from S conditions compared to FS conditions. This showed that UV radiation levels reaching water bodies on open fields had a negative effect in the metabolic rate of microbial community compared with water bodies in shade areas. This could be a mechanism leading to low reproduction and even dying of bacterial community, which would indirectly affect larvae that feed on them. At day 8, microbial community metabolic rate in the three different microcosms was significantly lower compared to days 1 and 15. Increase of metabolic rate from day 8 to day 15 is probably due to the input of dead carcasses from larvae that were dying in the vials due to the negative effect of UV-B radiation; this material probably served as a nutrient source that increased microbial community size and metabolic rate.

In regard to the effect of UV radiation on microbial and algae communities that serve as food resources for mosquito larvae (Pelz-Stelinski et al., 2011), there are different points of view. Some authors indicate that UV-B radiation has negative effects on microorganisms and algae (Wu et al., 2009; Hader et al., 2007). Gao et al (2008) reported

that UV-B radiation damages the DNA, proteins, membranes, and photochemical efficiency of photosynthetic prokaryote organisms like *Arthrospira platensis* (cyanobacteria), affecting photosynthesis and biomass production. The spiral structure of *A. platensis* is broken, and there is inhibition of photosynthetic activity with exposure to UV-B radiation in a temperature range of 18 to 20 °C, and this results in low biomass densities. The damage to these cells is temperature and density dependent (Gao et al., 2008). Wu et al. (2005) also found that exposure to 6 hours of UV-B radiation breaks the spiral filaments of *A. platensis* into small pieces and it also affects photosynthesis activity. UV-B radiation specifically affects the photosynthetic electron transport and pigment-protein complexes of *A. platensis* (Wu et al., 2005). Hader et al. (2007) showed that UV radiation affects negatively algae and microbial communities in aquatic ecosystems on which mosquito larvae feed. UV radiation penetrates significant depths in aquatic systems, depending on water transparency, with effects ranging from effects on major biomass producers such as phytoplankton to effects on consumers in the food web such as mosquito larvae. Davidson and Belbin (2002) found that marine phytoplankton and protozoan community assemblages exposed to UV radiation at less than 2 meters depth for more than a day suffer a reduction in biomass and concentration per cubic meter of water, which would represent less availability of food for mosquito larvae that feed on this microbial assemblage. On the other hand, other authors have suggested that UV radiation could be beneficial to microbial communities because of increased availability of dissolved organic carbon; this would promote bacterial growth and bacterial abundance, leading to the increase of food resources for mosquito larvae (De Lange et al., 2003).

Of the fitness parameters we assessed, only survival was significantly negatively affected by UV-B radiation, in FS conditions in both species (Figure 3A, 3B). Lower survival rates in FS conditions were probably a consequence of the direct effect of UV-B radiation, but also of the indirect effect of lower amounts of food resources in microcosms in FS conditions compared to microcosms from S and NUV conditions. UV-B exposure probably stressed larvae, which was reflected in greater metabolic rates. Greater metabolic rates were probably due to greater expenditures of energy to keep with biological processes (e.g., growth) and searching for food resources, which provide the calories needed for biological processes, considering that food resources decline in habitats exposed to high UV-B radiation. Stress in insects had been detected through the release of stress hormones such as cortisol, epinephrine, octopamine (Peric-Mataruga et al., 2006; Farooqui, 2012). Furthermore, Development time showed a trend toward greater development time under FS conditions compared to NUV conditions in both species. Also, for body size of *Ae. albopictus* and *Cx. pipiens* there was a trend toward smaller body size in FS condition compared to NUV conditions. These results are similar to those of Sang et al. (2017), who found a negative effect of UV-B on survival, development time and reduced size in *tribolium castaneum*, which is not a mosquito, but it has a similar life cycle. The study of Hori et al. (2014) showed that not only could UV radiation have a negative impact on mosquito survival, but also that wavelengths in the violet and blue range could cause pupae mortality as high as 60 percent. Understanding the effects of ultraviolet radiation on larval metabolic rate and fitness parameters of mosquitoes could lead to the development of new ways to control mosquitoes, to predict future geographic distribution due to changes in

solar radiation, and to prevent outbreaks of illness caused by viruses transmitted by these mosquitoes.

In Summary, we observed that larval metabolic rate of *Ae. albopictus* and *Cx. pipiens* were significantly higher in full-sun conditions compared to no-UV conditions, especially at day 15, and that negative effects upon these mosquitoes were expressed in lower survival rates, greater development time, and smaller sizes of both species under FS conditions compared to NUV conditions. Also, we observed that the bacterial communities of container aquatic habitats demonstrated lower metabolic rates in response to disturbance by UV-B radiation in FS conditions. These findings enhance the understanding of how changes in UV-B radiation could affect mosquito fitness and the microbial communities on which mosquitoes feed; and they suggest impacts upon some key ecological processes such as decomposition, nutrient cycling, and microbial diversity, processes that should be evaluated in future studies.

List of tables and figures

Tables

Table 1. Three-way ANOVA of the effects of UV-B conditions (FS, S, and NUV) and species (*Ae. albopictus* and *Cx. pipiens*) at three different times (days 1,8, and 15) on the larvae metabolic rate of *Ae. albopictus* and *Cx. pipiens* mosquitoes.

Variable	Larval metabolic rate		
	dfs	F	P
UV conditions	2,10	5.50	0.0245
Species	2,10	6.08	0.0333
UV conditions x Species	2,10	0.58	0.5799
Days	2,24	350.85	<0.0001
UV conditions x Days	2,24	13.96	<0.0001
Species x Days	2,24	14.79	<0.0001
UV conditions x Species x Days	2,24	0.87	0.4975

Table 2. Three-way ANOVA of the effects of UV-B conditions (FS, S, and NUV) and the species that inhabit the vials where microbial samples come from (*Ae. albopictus*, *Cx. pipiens*, and no larvae) at three different times (days 1, 8, and 15) in the metabolic rate of microbial community.

Variable	Microbial metabolic rate		
	dfs	F	P
UV conditions	2,16	10.74	0.0011
Species	2,16	1.13	0.3483
UV conditions x Species	4,16	0.79	0.5502
Days	2,36	5.69	0.0071
UV conditions x Days	4,36	3.65	0.0135
Species x Days	4,36	0.47	0.7562
UV conditions x Species x Days	8,36	0.54	0.8203

Table 3. Two-way ANOVA of the effects of UV-B conditions (FS, S, and NUV) and specie (*Ae. albopictus* and *Cx. pipiens*) on the fitness parameters (survival, developmental time, and body size) of *Ae. albopictus* and *Cx. pipiens* mosquitoes.

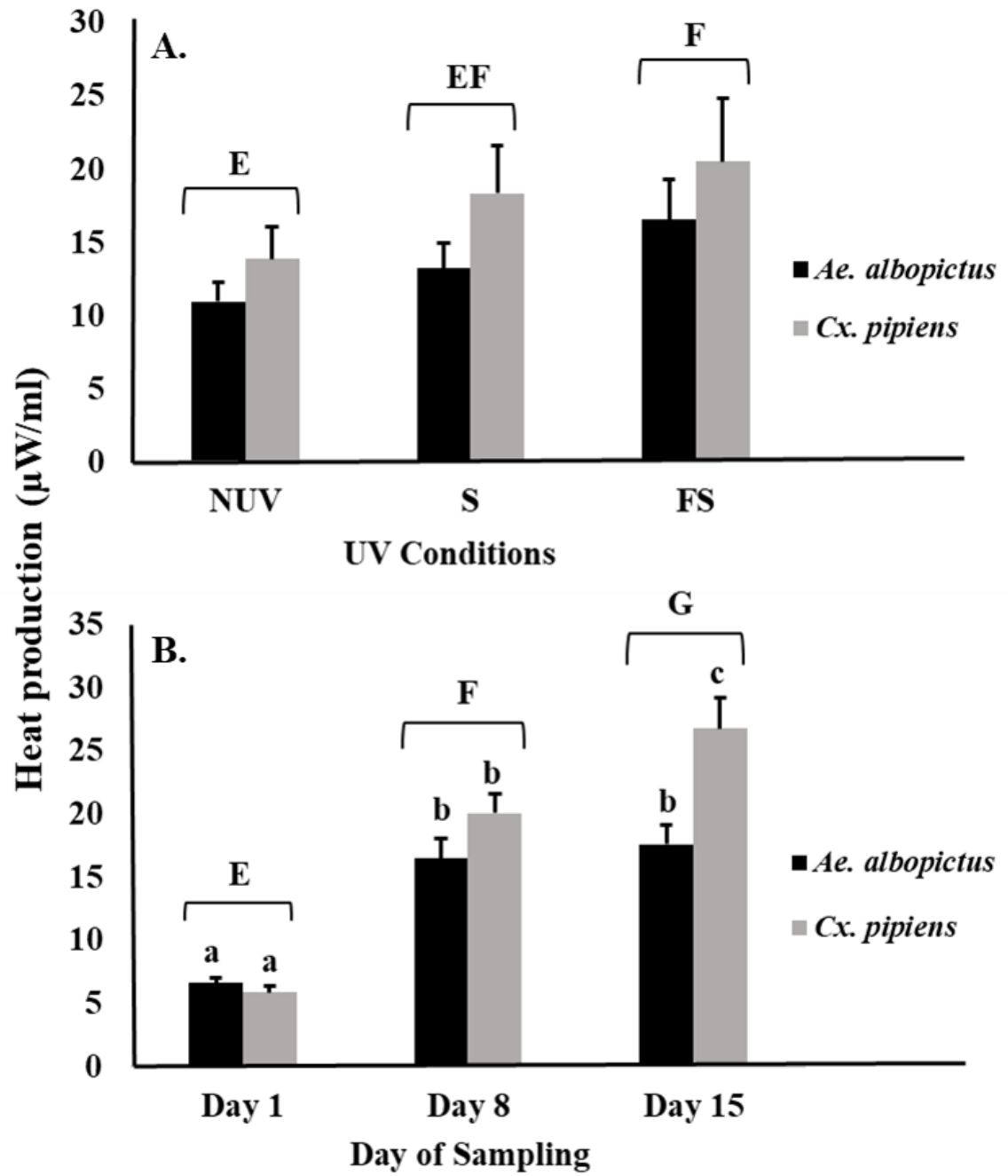
Variable	Survival			Development time			Body size-wing length		
	dfs	F	P	dfs	F	P	dfs	F	P
UV conditions	2,11	7.11	0.0104	2,11	0.80	0.4773	2,11	1.05	0.3857
Species	1,11	0.01	0.9963	1,11	1.50	0.2491	1,11	16.36	0.0023
UV conditions x species	2,11	0.59	0.5717	2,11	0.30	0.7456	2,11	0.05	0.9518

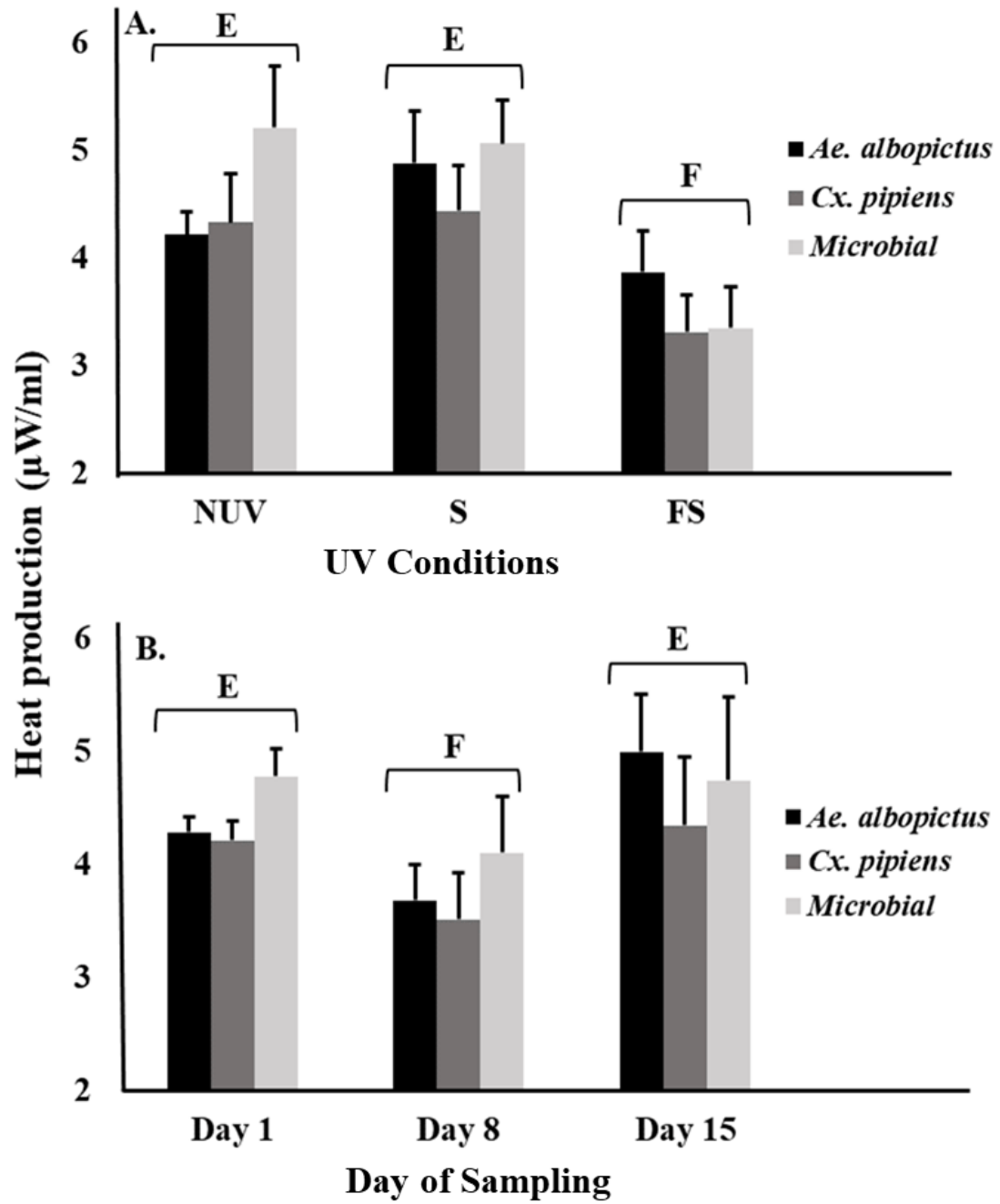
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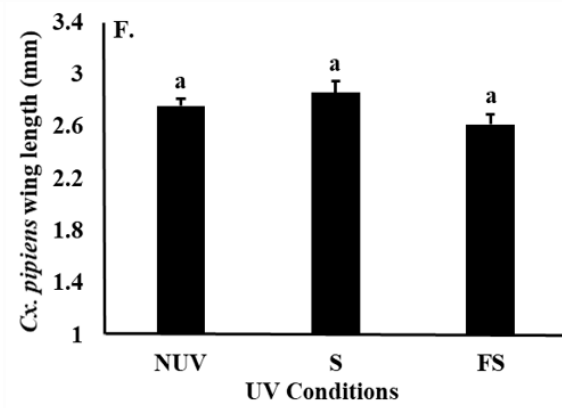
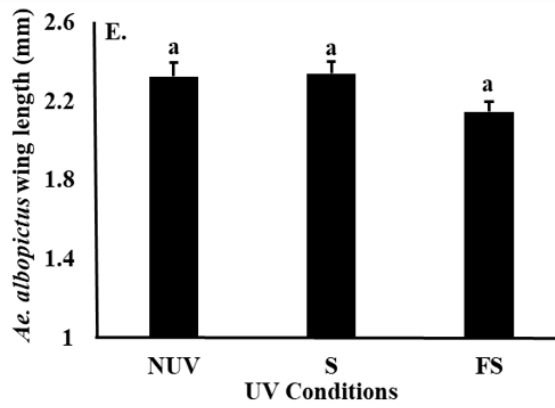
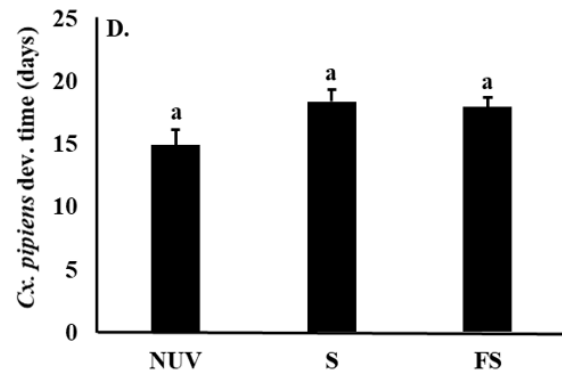
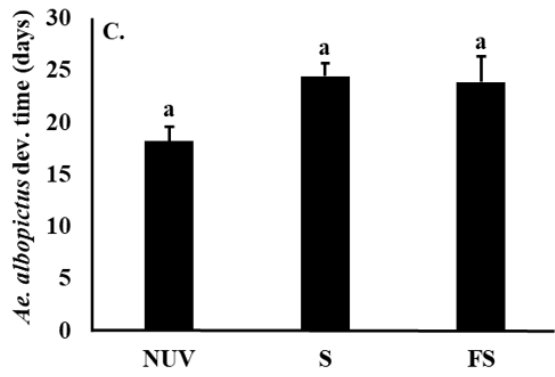
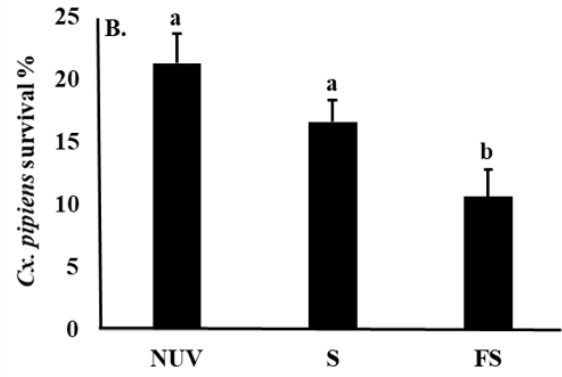
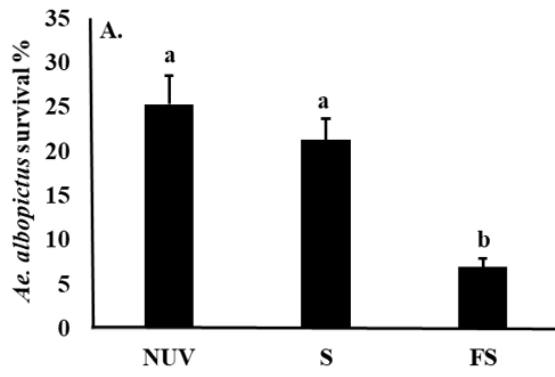
Figure 1. Least squares means (\pm SE) for metabolic rate expressed as heat production (μ W/ml) of larvae of *Ae. albopictus* and *Cx. pipiens* in response to (a) UV-B conditions (NUV, S, and FS) and (b) day of metabolic rate measurement (days 1, 8, and 15). Data were statistically tested using ANOVA. Significant pairwise comparisons among treatment levels for main effects of (a) UV-B conditions and (b) day of sampling are indicated by capitalized letters, and interaction effects of UV-B conditions and day of sampling are indicated by lower case letters.

Figure 2. Least squares means (\pm SE) for metabolic rate expressed as heat production (μ W/ml) of microbial community from vials that contain *Ae. albopictus* larvae, *Cx. pipiens* larvae, and no larvae (just microbial community) in response to (a) UV conditions (NUV, S, and FS) and (b) day of metabolic rate measurement (days 1, 8, and 15). Data were statistically tested using ANOVA. Significant pairwise comparisons among treatment levels for main effects of (a) UV-B conditions and (b) day of sampling are indicated by capitalized letters.

Figure 3. Least squares means (\pm SE) for fitness parameters of *Ae. albopictus* and *Cx. pipiens* mosquitoes. (a) survival percentage of *Ae. albopictus* (b) survival percentage of *Cx. pipiens* (c) development time of *Ae. albopictus* (d) development time of *Cx. pipiens* (e) body size of *Ae. albopictus*, and (f) body size of *Cx. pipiens* in response to UV-B conditions (FS, S, and NUV). Data were statistically tested using ANOVA. Significant pairwise comparison among treatment levels for main effects are indicated by different letters above bars.







Appendices

Appendix 1: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) and species (*Ae. albopictus* and *Cx. pipiens*) at three different times (days 1, 8, and 15) on the larvae metabolic rate of *Ae. albopictus* and *Cx. pipiens* mosquitoes

```
PROC IMPORT OUT= WORK.LmetrateRM
    DATAFILE= "C:\Documents\Data\MRLrepcon.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
proc print data=LmetrateRM;
run;
proc sort data=LmetrateRM;
key trt sp blk/ascending;
run;
data b;
set LmetrateRM;
mr1=log10(mr1);
mr8=log10(mr8);
mr15=log10(mr15);
run;
proc print data=b;
run;
data l;
set b;
day= 1; mr=mr1; output;
day= 8; mr=mr8; output;
day= 15; mr=mr15; output;
drop mr1 mr8 mr15;
proc print data=l;
run;
proc mixed data=l covtest;
class vial trt sp blk day;
model mr = trt|sp|day / residual ddfm=kr;
repeated day/ subject= trt*sp*blk type=cs;
random blk;
lsmeans trt sp day trt*day sp*day / pdiff adjust=tukey;
run;
```

Appendix 2: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) and species (*Ae. albopictus* and *Cx. pipiens*) at three different times (days 1, 8, and 15) on the metabolic rate of the microbial communities.

```
PROC IMPORT OUT= WORK.MbMetrate
    DATAFILE= "C:\Documents\Data\MRMbrepcon.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
Proc print data=MbMetrate;
run;
proc sort data=Mbmetrate;
key trt sp blk/ascending;
run;
data e;
set MbMetrate;
mr1=log10(mr1);
mr8=log10(mr8);
mr15=log10(mr15);
run;
proc print data=e;
run;
data k;
set e;
day= 1; mr=mr1; output;
day= 8; mr=mr8; output;
day= 15; mr=mr15; output;
drop mr1 mr8 mr15;
proc print data=k;
run;
proc mixed data=k covtest;
class vial trt sp blk day;
model mr = trt|sp|day / residual ddfm=kr;
repeated day/ subject=trt * sp * blk type=cs;
random blk;
lsmeans trt sp day trt*day / pdiff adjust=tukey;
run;
```

Appendix 3: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) and species (*Ae. albopictus* and *Cx. pipiens*) on the fitness parameters (survival, development time, and body size) of *Ae. albopictus* and *Cx. pipiens* mosquitoes

```
PROC IMPORT OUT= WORK.UpSurvLarva
    DATAFILE= "C:\Documents\Data\SurLarvae.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
Proc print data=UpSurvLarva;
run;
proc sort data=UpSurvlarva;
key sp trt blk rep/ascending;
run;
proc means data=UpSurvlarva n mean stderr stddev;
by sp trt blk;
var eclo;
output out=sural n=count nmiss=nmiss;
run;
proc print data=sural;
run;
data surA2;
set sural;
ss=log10(count+1);
run;
Proc print data=surA2;
run;
*survival;
proc mixed data=surA2;
class trt sp;
model ss = trt|sp /residual ddfm=sat;
random blk;
LSMEANS trt sp trt*sp/pdiff adjust=tukey;
run;
*development time;
proc means data=Survlarvae n mean stderr stddev;
by trt sp blk;
var eclo;
output out=devtime1 mean=mean;
run;
data devtime2;
set devtime1;
dt=log10(mean+1);
run;
proc mixed data=devtime2;
```

```

class trt sp blk;
model dt = trt|sp /residual ddfm=kr;
random blk;
lsmeans trt sp trt*sp /adjust=tukey;
run;
*wing length;
proc means data=Survlarvae n mean stderr;
by trt sp;
var wingL;
output out=wing1 mean=mean stderr=stderr;
run;
proc print data=wing1;
run;
data wing3;
set Survlarvae;
ss=log10(wingL+1);
run;
proc means data=wing3 n mean stderr;
by trt sp blk;
var wingL;
output out=wing1 mean=mean stderr=stderr;
run;
proc mixed data=wing1;
class trt sp blk;
model mean = trt|sp/ residual ddfm=sat;
random blk;
lsmeans trt sp trt*sp /adjust=tukey;
run;

```

References

- Andrady, A.L., Hamid, H.S., Hu, X., and Torikai, A. 1998. Effects of increased solar ultraviolet radiation on materials. *Journal of Photochemistry and Photobiology*, 46:96-103.
- Ballare, C.L., Caldwell, M.M., Flint, S.D., Robinson, S.A., and Bornman, J.F. 2011. Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. *Photochemical and Photobiological Sciences*, 10, 226. [doi: 10.1039/c0pp90035d](https://doi.org/10.1039/c0pp90035d)
- Caldwell, M.M., Ballare, C.L., Bornman, J.F., Flint, S.D., Bjorn, L.O., Teramura A.H., Teramura, A.H., Kulandaivelu, G., and Tevini, M. 2003. Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climatic change factors. *Photochemical and Photobiological Sciences*, 2:29-38.
- Cancrini, G., Scaramozzino, P., Gabrielli, S., Di Paolo, M., and Romi, R. 2007. *Aedes albopictus* and *Cx. pipiens* implicated as natural vectors of *Dirofilaria repens* in Central Italy. *Journal of Medical Entomology*, 44:1064-1066.
- Costanzo, K.S., Mormann, K., and Juliano, S.A. 2005. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology*, 42:559-570.
- Day, K.E., Holtze, K.E., Metcalfe-Smith, J.L., Bishop, and C.T., Dutka, B.J. 1993. Toxicity of leachate from automobile tires to aquatic biota. *Chemosphere*, 27:665-675.
- Davidson, A., and Belbin, L. 2002. Exposure of natural Antarctic marine microbial assemblages to ambient UV radiation: effects on the marine microbial community. *Aquatic Microbial Ecology*, 27:159:174.
- De Lange, H.J., Morris, D.P., and Williamson, C.E. 2003. Solar ultraviolet photodegradation of DOC may stimulate freshwater food webs. *Journal of Plankton Research*, 25:111-117.
- Dyer, A.G. 2001. Ocular filtering of ultraviolet radiation and the spectral spacing of photoreceptors benefit von Kries colour constancy. *The Journal of Experimental Biology*, 204:2391-2399.
- Farooqui, T. 2012. Review of octopamine in insect nervous system. *Open Access Insect Physiology*, 4:1-17.
- Gao, K., Li, P., Watanabe, T., and Helbling, E.W. 2008. Combined effects of ultraviolet radiation and temperature on morphology, photosynthesis, and DNA of *Arthrospira* (*Spirulina*) *Platensis* (*Cyanophyta*). *Journal of Phycology*, 44:777-786.

- Gerhardt, R., Gottfried, K., Apperson, C., Davis B., Erwin, P., Smith A., Panella, N., Powell E., and Nasci, R. 2001. First isolation of LaCrosse virus from naturally infected *Aedes albopictus*. *Emerging Infectious Diseases*, 7:807-811.
- Gray, E.M., and Bradley, T.J. 2003. Metabolic rate in female *Culex tarsalis* (Diptera: Culicidae): Age, Size, Activity, and Feeding effects. *Journal of Medical Entomology*, 40:903-911.
- Gray, E.M., 2013. Thermal acclimation in a complex life cycle: The effects of larval and adult thermal conditions on metabolic rate and heat resistance in *Culex pipiens* (Diptera: Culicidae). *Journal of Insect Physiology*, 59:1001-1007.
- Hader, D.P., Kumar, H.D., Smith, R.C., Worrest, R.C. 2007. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical & Photobiological Sciences*, 6:267-285.
- Helbling, E.W., and Zagarese, H. 2003. UV effects in aquatic organisms and ecosystems. *Journal of Plankton Research*, 26:255-256.
- Hori, M., Shibuya, K., Sato, M., and Saito Y. 2014. Lethal effects of short-wavelength visible light on insects. *Scientific Reports*, 4:7383, [doi: 10.1038/srep07383](https://doi.org/10.1038/srep07383)
- Johansen, N.S., Vanninen, I., Pinto, D.M., Nissinen, A.I., and Shipp, L. 2011. In the light of new greenhouse technologies: 2. Direct effects of artificial lighting on arthropods and integrated pest management in greenhouse crops. *Annals of Applied Biology*, 159:1-27.
- Joy, J.E., Hanna, A.A., and Kenned, B.A. 2003. Spatial and Temporal Variation in the Mosquitoes (Diptera: Culicidae) inhabiting waste tires in Nicholas County, West Virginia. *Journal of Medical Entomology*, 40:73-77.
- Joy, J.E. 2004. Larval mosquitoes in abandoned tire pile sites from West Virginia. *Journal of the American Mosquito Control Association*, 20:12-17.
- Juliano, S.A. 2009. Species interactions among larval mosquitoes: context dependence across habitat gradients. *Annual Review of Entomology*, 54:37-56.
- Kim, H. C., Wilkerson, R., Pecor, J., Lee, W., Lee, J., O'Guinns, M., and Klein, T. 2005. New records and reference collection of mosquitoes (Diptera: Culicidae) on Jeju Island, Republic of Korea. *Entomological Research*, 35:55-56.
- Klowden, M.J. 2007. *Physiological Systems in Insects*. Academic press Elsevier, London, UK.
- Lambrechts, L., Scott, T.W., and Gubler, D.J. 2010. Consequences of the expanding global distribution of *Aedes albopictus* for Dengue Virus Transmission. *PLoS ONE*, 4(5):e646. [doi:10.1371/journal.pntd.0000646](https://doi.org/10.1371/journal.pntd.0000646)

- Li, W., Seifert, M., Xu, Y., and Hock, B. 2006. Assessment of estrogenic activity of leachate from automobiles tires with two in vitro bioassays. *Fresenius Environmental Bulletin*, 15:74-79.
- MacGregor, M.E. 1932. Certain pathological effects of ultra-violet radiation on mosquito larvae and pupae. *Proceedings of the Royal Society of London*, 112:27-38.
- Molaei, G., Andreadis, T.G., Armstrong, P.M., Anderson, J.F., and Vossbrinck, C.R. 2006. Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, northeastern United States. *Emerg. Infect. Dis.*, 12:468-474.
- Pancotto, V.A., Sala, O.E., Cabello, M., Lopez, N.I., Robson, T.M., Ballare, C.L., Caldwell, M.M., and Scopel, A.L. 2003. Solar UV-B decrease decomposition in herbaceous plant litter in Tierra del Fuego, Argentina: potential role of an altered decomposer community. *Global Change Biology*, 9: 1465-1474.
- Pelz-Stelinski, K., Kaufman, M.G., and Walker, E.D. 2011. Beetle (*Coleoptera: Scirtidae*) facilitation of larval mosquito growth in tree hole habitats is linked to multitrophic microbial interactions. *Microbial Ecology*, 3:690-703.
- Peric-Mataruga, V., Nenadovic, V., and Ivanovic, J. 2006. Neurohormones in insect stress: A review. *Arch. Biol. Sci.*, 58:1-12.
- Sang, W., Yu, L., He, L., Ma, W.H., Zhu, Z.H., Zhu, F., Wang, X.P., and Lei, C.L. 2016. UVB radiation delays *Tribolium castaneum* metamorphosis by influencing ecdysteroid metabolism. *PLoS ONE*, 11(3): [e0151831](https://doi.org/10.1371/journal.pone.0151831)
- SAS institute Inc. 2013. *Base SAS 9.4 Procedures Guide: Statistical Procedures*, Second Edition. Cary, NC: SAS Institute Inc.
- Shimoda, M., and Honda, K-I. 2013. Insects reaction to light and its application to pest management. *Applied Entomology and Zoology*. 48:413-421.
- Shuman, E.K. 2011. Global Climate Change and Infectious Diseases. *International Journal of Occupational and Environmental Medicine*, 2(1). www.theijoem.com
- Sliney, D.H., Gilbert II, D.W., and Lyon, T. 2016. Ultraviolet safety assessment on insect light traps. *Journal Occupational Environmental Hygiene*, 13:413-424.
- Terblanche, J.S., Sinclair, B.J., Klok, C.J., McFarlane, M.L., and Chown, S.L. 2005. The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica chalconota* (*Coleoptera: Chrysomelidae*). *Journal of Insect Physiology*, 51:1013-1023.

- Terblanche, J.S., and Chown, S.L. 2007. Physiological diversity in insects: ecological and evolutionary contexts. *Advances in Insects Physiology*, 33:50-152.
- Terblanche, J.S., Clusella-Trullas, S., Deere, J.A., Van Vuuren, B.J., and Chown, S.L. 2009. Directional evolution of the slope of metabolic rate-temperature relationship is correlate with climate. *Physiological and Biochemical Zoology*, 82:495-503.
- Walker, E.D., and Merrit, R.W. 1988. The significance of leaf detritus to mosquito (*Diptera, Culicidae*) productivity from treeholes. *Environmental Entomology*, 17:199-206.
- Walker, E.D., Lawson, D.L., Merrit, R.W., Morgan, W.T., and Klug, M.J. 1991. Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosm. *Ecology*, 72:1529-1546.
- Wong, P-SJ., Li, M.I., Chong, C-S., Ng L-C., Tan, C-H., 2013. *Aedes* (*Stegomyia*) *albopictus* (Skuse): A potential vector of Zika Virus in Singapore. *PLoS Neglected Tropical Diseases*, 7(8): e2348. [doi:10.1371/journal.pntd.0002348](https://doi.org/10.1371/journal.pntd.0002348)
- Wu, H., Gao, K., Villafañe, V.E., Watanabe, T., Helbling, E.W. 2005. Effects of solar radiation on morphology and photosynthesis of filamentous cyanobacterium *Arthrospira platensis*. *Applied Environmental Microbiology*, 71:5004-5013.
- Wu, H., Gao, K., and Wu, H. 2009. Responses of a marine red tide alga *Skeletonema costatum* (*Bacillariophyceae*) to long-term UV radiation exposures. *Journal of Photochemistry and Photobiology*, 94:82-86.
- Zhang, H., Liu, J., Li, C., Momen, B., Kohanski, R.A., and Pick, L. 2009. Deletion of *Drosophila* insulin-like peptides causes growth defects and metabolic abnormalities. *Proceedings of the National Academy of Sciences of the United States of America*, 106:19617-19622.

Chapter 3: The effect of ultraviolet radiation on the release and fate of zinc from discarded tires

Oswaldo C. Villena^{1,6}, Bahram Momen², Joseph Sullivan³, Edward Landa², Alba Torrens⁴, Paul T. Leisnham⁵

1. Marine Estuarine & Environmental Science, University of Maryland, College Park, MD 20742
2. Environmental Science & Technology, University of Maryland, College Park, MD 20742
3. Department of Plant Science & Landscape Architecture, University of Maryland, College Park, MD 20742
4. Department of Civil & Environmental Engineering, University of Maryland, College Park, MD 20742
5. Marine Estuarine & Environmental Science and Environmental Science & Technology, University of Maryland, College Park, MD 20742
6. Corresponding author: oswaldo.villena@gmail.com

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Abstract

Each year 280 million scrap tires are generated and around 67 million tires are in stockpiles in the United States. When tires degrade, metals and organic compounds are released to the environment where they could have toxic effects on cells and organisms. We assessed the behavior and fate of discarded tire pollutants using zinc as an indicator. Whole discarded tires filled and maintained with 4 L of deionized water were exposed to three different levels of UV-B radiation that mimic full-sun conditions, 10.82 $\mu\text{mol}/\text{m}^2/\text{s}$ (FS), shade conditions, 6.1 $\mu\text{mol}/\text{m}^2/\text{s}$ (S), and no UV radiation, 0.6 $\mu\text{mol}/\text{m}^2/\text{s}$ (NUV) for 150 days in a controlled greenhouse facility. Water samples from tires were collected on days 1, 50, 100, and 150, and biofilm was harvested at the end of the experiment (day 210) to assess the concentration of dissolved and total zinc concentrations. Zinc concentration was on average higher in tires exposed to FS conditions compared to tires exposed to S and NUV conditions over the duration of the experiment for both dissolved and total zinc in the water and for total recoverable zinc in biofilm. In FS and S conditions, dissolved zinc concentrations in water rose to a peak on day 100, with the highest concentration in FS (2.301 mg/L), but then declined to day 150; and total zinc concentration in water rose to a peak at day 150, with the highest concentration in FS (4.11 mg/L). Amount of biofilm was significantly higher in FS condition compared to NUV condition and total zinc concentration in tire biofilm was higher in the FS condition (11.54 mg/g) than in the S (7.75 mg/g) and NUV (2.92 mg/g) conditions. These results showed that UV-B radiation affects the degradation of tires, and that zinc may shift from the water to the biofilm layer. Increased leaching of zinc and other contaminants with UV-B degradation, and shifts of these contaminants from the water column into the biofilm may have important

environmental impacts, especially for aquatic systems that may be vulnerable to contaminant toxicity.

Key words: Zinc, tire leachate, UV-B radiation, tire contaminants

Introduction

The Rubber Manufacturers Association (RMA, 2017) estimates that around 280 million automobile tires are discarded annually in the United States. Approximately 67 million discarded tires are stored in American stockpiles while illegal dumping adds additional tires into the environment. Discarded tires present a substantial challenge to discard and recycle (RMA 2017). Discarded tires are very durable and persist in the environment, unless they are processed with mechanical, thermal or a combination of these treatments (Kwon and Castaldi, 2009). Discarded tires could have a range of adverse environmental effects including threats to public health (Horner, 1996). When tires breakdown and the chemicals they contain are released into the environment from tire decomposition, incineration or accidental fires; each of these processes could pollute the major earth components (e.g., atmosphere, lithosphere, hydrosphere) and negatively impact the living organism that inhabit these earth components. Discarded tires represent a problem in landfills because of their volume and its slow degradability. Landfill buried tires represent a potential risk due to the release of contaminating leachates to surrounding groundwater when infiltration of rainwater occurs (Evans, 1997). Another major, but less studied, concern of discarded tires is their ability to collect rainwater and provide a potential developmental habitat for pestiferous and disease-vector mosquitoes (Yee et al., 2010).

Tires are made of a conglomerate of organic (e.g., polycyclic aromatic hydrocarbons PAHs, benzothiazoles, 4-tert-butylphenol) and inorganic (e.g., zinc, copper, cadmium, lead) contaminants that can leach and persist in the environment, and accumulate overtime (Peterson et al., 1986; Day et al., 1993, Selbes et al., 2015). Many tire

contaminants are bioavailable and persistent in the aquatic environment. Contaminants from tire leachate have been documented to affect a range of biota, including fish (Spies et al., 1987; Day et al., 1993; Hartwell et al., 2000), algae (Gualteri et al., 2005), bacteria (Day et al., 1993; Hartwell et al., 2000; Crampton, et. al., 2014), small planktonic crustaceans such as *Daphnia magna* and *Ceriodaphnia dubia* (Wik and Dave, 2005; Wik et al., 2009; Marwood et al., 2011), and insects (Villena et al., 2017). Tire leachate can have both direct or indirect effects. Some examples of direct effects could be detrimental effects on growth, survival, weight, and reproduction (Day et al., 1993). Indirect effects could be an interference in the outcome of competition between two or more similar species that are competitors, especially if one is more susceptible to specific pollutants (Rohr and Crumrine, 2005; De Hoop et al., 2013); or negative effects on biota, making them more susceptible to predators (Sanchez-Bayo et al., 2012).

Tire deterioration is influenced by a number of environmental factors, including temperature, ozone, humidity, and ultraviolet radiation (UVR) (Andrady et al., 1998; Andrady et al., 2003). Once tires crack and split, water can reach the steel belts of tires, causing oxidation (Andrady et al., 1998), and an acceleration in the leaching of compounds into the environment. Perhaps the most important factor affecting tire deterioration and contaminant leaching is UVR. Not only does UVR promote the deterioration of tire structure, tire leachate that is continually exposed to UVR undergoes a further photochemical reaction called phototoxicity whereby leachate molecules absorb light photons to be made even more toxic to biotic organisms; in some cases 10 times more toxic (Wik and Dave, 2005). Ultraviolet radiation has a wavelength range between 100-400 nm (Andrady et al., 1998) and is subdivided into three subtypes: UV-A (400-315 nm), UV-B

(315-280 nm) and UV-C (280-100 nm). Only UV-A and UV-B reach the earth's surface (Caldwell et al., 2003). Although UV-B constitutes only approximately 5% of the total UVR that reaches the earth's surface, has higher energy levels due to its shorter wavelength and therefore causes almost all of the negative UVR effects on tire and other materials (Andrady et al., 1998).

Tire exposure to UVR can vary with shade and tire orientation (Turner, 2011). Despite the importance of tire deterioration on the rate of contaminant release into the environment there is a paucity of data on effects of different field-relevant UVR levels on the rate and accumulation of contaminants in the environment. In this chapter, we assess the effect of three different UV-B radiation conditions that mimic a range of circumstances in the field (full-sun, shade, and no-UV radiation) on the leaching of zinc from whole discarded tires in a controlled laboratory experiment. Zinc is commonly used as a marker of tire leachate and in prior studies has been shown to be toxic at concentrations commonly detected in the field across a range of species, is common across almost all tire types, and is relatively easy to detect (Nelson et al., 1994; Wik and Dave, 2009; Marwood et al., 2011).

Materials and methods

Ultraviolet radiation treatments

In a controlled greenhouse facility at University of Maryland College Park, 90 Goodyear brand tires (Model: Assurance, P215/60R16), organized in 6 blocks of 5 tires per treatment, yielding 18 experimental units, were randomly assigned to one of three UV-B conditions that mimicked three common conditions in the field: (1) full-sun: 10.82 $\mu\text{mol}/\text{m}^2/\text{s}$ (FS); (2) shade: 6.1 $\mu\text{mol}/\text{m}^2/\text{s}$ (S); and (3) no UV radiation: 0.6 $\mu\text{mol}/\text{m}^2/\text{s}$ (NUV). UV-B radiation was provided by 12 UVB-313 lamps (Q Panel Lab Products, Cleveland, OH) suspended over each of three benches that were assigned one of the three UV-B conditions. UV-B lamps for FS and S conditions were wrapped with cellulose diacetate (CA) biofilm, which transmitted UV-B radiation down to 290 nm at a height of 1.2 and 0.6 meters, respectively. For the NUV radiation treatment, polyester filters that block almost all UV-B radiation below 316 nm were used (Grant et al., 2010; Sullivan et al., 2010). FS and S lamps were adjusted to specific heights above tires to attain the appropriate UV-B radiation levels, and all UV-B levels were confirmed by an ultraviolet meter (UVM-SS, Apogee Instruments Inc.) (Sullivan et al., 2010). Tires were obtained from the Motor Transportation Service of the University of Maryland College Park. The tires were 70 cm high, 18.89 cm wide, and 42 cm in diameter and four liters in volume in the base.

Zinc concentrations in water

The experiment design was a repeated measure randomized complete block design (RCDB). Tires were exposed to UV-B radiation in 6 blocks of 15 tires, and blocks were run for 210 days in sequential temporal order. In each block, five tires were randomly

assigned to each of the three benches, making one experimental unit the group of 5 discarded tires on a bench. To assure uniform exposure to UV radiation, tires were rotated every 4 days. Tires were filled with 4 L deionized water and were routinely topped up throughout the duration of the experiment (Macia, 2006).

Water samples were taken from each tire at day 1, 50, 100, and 150 after tire set-up, and analyzed for total recoverable and dissolved zinc concentrations following the U.S. EPA 3015A method for microwave assisted acid digestion of aqueous samples and extracts (U.S. EPA, 2007). For total recoverable zinc, 100 ml of water was extracted from each tire and 150 μ l of concentrate nitric acid was added; samples were then refrigerated until analysis. For dissolved zinc, I collected 19 ml of filtered water and added 1 ml of concentrated nitric acid in Polypropylene conical centrifuge tubes, and refrigerated until analysis. 45 ml of water sampled for total recoverable zinc plus 5 ml of concentrated nitric acid (10% v/v) were placed in a 100 ml digestion vessel. For the digestion process, a full set of 12 vessels was placed in an Ethos D Milestone Microwave equipment; if there were not enough samples to make a full set, I prepared a full set by filling remaining digestion vessels with 45 ml deionized water and 5 ml of concentrated nitric acid (method blanks). The vessel with the temperature sensor always contained a normal sample (not a method blank). For the digestion process, we followed the lab program # 11 to provide digestion with 10 minute ramp to 160 °C followed by 10 minute ramp to 165 °C. Then, digested samples were transferred to 50 ml Polypropylene conical centrifuge tubes and stored at 5 °C.

Analysis of water samples for total and dissolved zinc concentrations was conducted with an Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)

with autosampler following the U.S. EPA Method 200.7 for the analysis of water and wastes using ICP-AES (U.S. EPA, 1994). Standardization of equipment occurred after daily calibration and after every 11 samples. Calibration standards were 0, 0.4, 2, 4, and 6 mg zinc/L in 5% (v/v) nitric acid. Standardization regression was linear up to 100 mg zinc/L. Samples for total zinc concentration were diluted to 5% (v/v) nitric acid before analysis. Water samples for dissolved zinc were already at 5% (v/v) nitric acid. Before analyzing a water sample, a rinse/wash was done using 5% nitric acid (v/v) for one minute, the sample was pre-flushed for 30 seconds at pump speed 4 followed by 30 seconds at pump speed 2, followed by 30 second integration; each time a sample probe was moved took an additional 8 seconds; total sample turn-around time is 2 minutes 47 seconds.

Zinc concentration in biofilm

On day 210, each tire was destructively sampled for biofilm. All water was removed, and total biofilm was extracted with a plastic spatula into 30 ml glass beakers and the wet weight was measured. Biofilm samples were dried for 24 hours at 95 °C and then at 105 °C for four hours in a Blue M Stabil-Therm Oven. After samples were dried, they were placed in a glass vacuum desiccator and weighted to obtain dry weight. Digestion of biofilm samples was performed with Ethos D Milestone Microwave with a carousel of 12 digestion vessels. Samples were prepared for digestion by placing up to 0.3 g of oven-dried biofilm and 10 ml of concentrate nitric acid into a 100 ml closed digestion vessel; then a full set of 12 vessels containing nitric acid was placed into the Ethos D Milestone Microwave following U.S. EPA 3015A method (U.S. EPA, 2007). If there were not enough samples to make a full set, then a full set was prepared by filling the remaining digestion vessels with 10 ml of concentrated nitric acid (method blanks). The vessel with the

temperature sensor should always contain a normal sample (not a method blank). For the digestion process, we followed the lab program # 14 that included 10 steps to provide digestion with a slow ramp to 175 °C followed by 9.5 minutes at 175 °C. After biofilm was digested it was brought to 100 ml with deionized water and transferred to 50 ml Polypropylene conical centrifuge tubes for storage at 5 °C (10 % nitric acid v/v).

Analyses of biofilm samples for total recoverable zinc were conducted with Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) with autosampler following the U.S. EPA Method 200.7 for the analysis of water and wastes using ICP-AES (U.S. EPA, 1994). Standardization of equipment occurred after daily calibration and after every 11th sample following U.S. EPA Method 200.7. Calibration standards were 0, 0.4, 2, 4, and 6 mg Zn/L in 5% (v/v) nitric acid. Standardization regression was linear up to 100 mg zinc/l. For analyses, digested samples were further diluted by mixing 5 ml of digested sample with 5 ml deionized water to provide a solution that contained nitric acid at about 5% (v/v) concentration, which is the limit for the equipment. These diluted solutions were analyzed for total recoverable zinc.

Analyses

Differences of total and dissolved zinc concentrations in the tire water column between UV-B conditions were tested using linear models using the SAS PROC MIXED procedure (SAS 9.4, SAS Institute Inc. 2013), with separate analyses by sample day to meet the assumptions of normality and homogeneity of variances. Models included UV-B radiation condition as a fixed effect, and block as a random effect. Measurements at day 1 were used as a covariate. Each experimental unit was a group of 5 tires on one of the three benches, where each individual tire was treated as a sub-sample. The response variables

were dissolved and total zinc concentrations from tire water columns (mg/L). Significant differences of amounts of biofilm (g) per tire and total recoverable zinc concentration in tire biofilm (mg/g) between UV-B conditions were tested using a nested linear model, using PROC MIXED procedure (SAS 9.4, SAS Institute Inc. 2013). The model included UV-B condition as a fixed effect, and tires nested in blocks as a random effect. We did a pairwise mean comparison in the mixed procedure using LSMEANS statement with tukey adjustment. Experiment-wise $\alpha = 0.05$ for all analyses.

Results

Zinc concentration in water

Leachate from discarded tires exposed to FS condition generally had the highest dissolved and total zinc concentrations, followed by those exposed to S condition and then NUV condition at days 50, 100, and 150 (Table 4; Figures 4A and 4B). Dissolved zinc in both FS and S peaked on day 100 before declining, while dissolved zinc in NUV remained consistently lower throughout the experiment. In contrast, total zinc concentration rose over the course of the experiment in FS, peaking on day 150, and was similar on days 100 and 150 in the S condition. Consistent with dissolved zinc, total zinc concentrations in NUV were lower than other conditions and similar throughout the experiment.

Amount and zinc concentration in biofilm

At the conclusion of the experiment, tires exposed to FS condition had the greatest biofilm, followed by those exposed to S condition and then NUV condition ($F_{2,25} = 7.95$, $P = 0.0019$; Figure 5A). Biofilm from FS also showed higher concentrations of total recoverable zinc per gram dry biomass compared to S and NUV conditions ($F_{2,25} = 22.13$,

$P = <0.0001$; Figure 5B). The mean concentration of total recoverable zinc in tire biofilm was 11.54 mg/g, 7.75 mg/g, and 2.92 mg/g for FS, S, and NUV conditions respectively.

Discussion

This study showed that longer exposure to increasing UV-B radiation promoted the leaching of zinc, and likely other contaminants, from whole discarded vehicle tires. Tires exposed to full-sun (FS) conditions consistently leached higher zinc concentrations compared to those exposed to shaded (S) or no-UV (NUV) conditions. Under FS, dissolved zinc peaked on sample day 100 after first exposure and then declined by day 150, while total zinc continued to increase. This result appears to be because much of the zinc was retained in biofilm, which was harvested in considerably greater amounts and had higher zinc concentration in FS conditions. A similar but less pronounced trend was seen in S conditions, while NUV conditions showed consistently low zinc concentrations.

Over 280 million vehicle tires are discarded in the United States each year, approximately one tire per person per year (RMA, 2017). Despite the U.S. EPA and individual states' efforts to reduce stockpiles of scrap tire through the establishment of waste tire management and recycling acts and the waste tire and recycling management fund, which is dedicated to the cleanup of scrap tire stockpiles and market development programs for scrap tires (U.S. EPA, 1993; Miller, 2014), many thousands of tires are still illegally discarded or are stored for extended periods. Despite the potential for tires to deteriorate from UV-B radiation exposure over extended periods and to release leachate into watersheds, there are relatively few studies assessing the rate of tire decay over time under varying environmental conditions. This is one of the few studies that have addressed the rate of tire decay using whole tires and UV-B radiation levels similar to those found

under different sun and shade conditions in the natural environment. In the literature, most studies have used crumb rubber particles (Wik and Dave, 2005; Marwood et al., 2011; Rhodes et al., 2012; Villena et al. 2017) or tire chips (Selbes et al., 2015) to obtain tire leachate and assess its toxicity. Few studies have used whole tires, under laboratory conditions, however in these studies, tires were totally submerged in water (Kellough, 1991; Day et al., 1993; Abernethy et al., 1994). Furthermore, our study measured zinc concentrations in water that was retained in whole tire casings over time and in the biofilm that formed in the bottom of the tires. Such measurements are particularly important because tire casings provide an important habitat for a number of Dipteran insects, including mosquitoes that can transmit a number of vector-borne disease agents to animals and humans (e.g., zika, chikungunya, and West Nile virus).

Our results showed a maximum average dissolved zinc concentration of 2.3 (SE±0.5) mg/L (0.18-6.91 mg/L) which was reached at day 100 in FS conditions. Interestingly, these results are consistent with a previous study by Villena et al. (2017) that found an average of 2.39 (SE±1.17) mg/L (0.05-7.26 mg/L) of dissolved zinc concentration in 42 whole discarded tires in five sun exposed field locations in the state of Maryland, suggesting that my experimental conditions in the greenhouse are generally realistic to those in the field.

The most well documented environmental impacts of tire leachate are in aquatic organisms, and perhaps the most interesting one is in vector-borne disease mosquitoes. Villena et al. (2017) tested the effect of experimentally-derived tire leachate on two mosquito species, *Aedes triseriatus* and *Aedes albopictus* and found that leachate with zinc concentrations at as low as 0.05 mg/L and 44.50 mg/L could have severe negative effects

on the population performance of each species, respectively, and ultimately lead to their complete mortality at higher concentrations. Although, other factors likely contributed to mortality in this experimental study, including low resource levels, the findings in the study here suggest that tires may not need to be exposed to UVR conditions for long to have toxic conditions for *Ae. triseriatus*. In our experiment, we found levels at day 100 in FS conditions that can be lethal to *Ae. triseriatus* especially under FS, but not to *Ae. albopictus*.

A limitation of the Villena et al. (2017) was that it only tested the effects of dissolved zinc concentrations rather than total zinc concentrations within the tire environment, which include zinc associated with fine particulate matter (0.45 μm) and with biofilm. Mosquito larvae filter-feed from the water column and browse surfaces to feed on detritus and associated microbial organisms (Merritt et al., 1992). Our study showed much higher zinc concentrations in unfiltered water samples (i.e., total recoverable zinc) and biofilm, peaking at 4.11 (SE \pm 0.93) mg/L (0.50 - 20.02 mg/L), at day 150 from water samples in FS conditions and in FS tires as biofilm among all three experimental conditions at 11.54 (SE \pm 1.85) mg/g (4.17-19.13 mg/g). Furthermore, tires exposed to FS conditions had greater amounts of biofilm compared to S and NUV conditions. Therefore, it is highly likely that mosquito larvae would experience negative impacts from tires that degrade in the field, especially under FS conditions, which would likely have much lower dissolved zinc concentrations than those reported in Villena et al (2017).

Our findings are broadly consistent with studies of tires in natural water bodies, which find concentrations of tire particles higher in sediment compared to the column water (Kumata et al., 2002; Marwood et al., 2011). The tire particle concentration in the sediment portion ranges from 0.3 to 155 g/kg (Kumata et al., 2002; Wik et al., 2006), while the

concentration of tire particles in the water column ranges from 0.0003 to 0.197 g/L (Kumata et al., 1997). In our study, we found almost three times higher zinc concentration in the biofilm of the discarded tires than in the water column under FS conditions and twice higher concentration for tires exposed to S conditions. Under NUV radiation there is no significant difference between zinc concentration in the biofilm or in the water column. Of the released zinc and likely other contaminants from tires, some would be dissolved in the water column, but some would be bound to inorganic or fine particulate organic matter (FPOM) in the water column. Other tire contaminants would precipitate to the sediment fraction of the tire casings (biofilm layer) where they will be attached or be absorbed into the biofilm layer at the bottom of the tire casings (Cuong et al., 2008; Huang et al., 2012). Mosquitoes that filter-feed on the water column (e.g., *Cx. pipiens*) will be exposed mainly to the dissolved fraction of tire contaminants or contaminants attached to FPOM. On the other hand, mosquitoes that browse-feed (e.g., *Ae. albopictus*) will be exposed to tire contaminants attached or absorbed into the biofilm layer (Merritt et al., 2012).

The zinc concentrations we found within tire water and tire biofilm, especially under conditions that mimic full-sun exposure, are well within the level that might negatively affect a range of living organisms, besides mosquitoes. The study of Day et al. (1993) showed a 96-hour median lethal concentration (LC₅₀) for *Oncorhynchus mykiss* (rainbow trout) that ranged from 11.8% to 19.3% (v/v) for leachate from used tires and 52.1% to 80.4% (v/v) for leachate from new tires, showing that leachate from discarded tires is more toxic than leachate from new tires. This is probably because antioxidants and antiozonants have been removed from the surface of the tires through wear of tire tread. This in turn could cause cracking of tires which could expose fillers and metals inside the

tire to humidity and heat, producing higher leaching of toxicants. Kellough (1991) exposed *O. mykiss* to tire leachate from tire cuts and whole tires that were submerged in water for 30 and 60 days. In both cases, 100 % mortality was observed. Zinc concentration in the fish tissue was 81 mg/kg in fish exposed to tire leachate from whole tires and 120 mg/kg in fish exposed to tire leachate from cut tires (Kellough, 1991). Wik and Dave (2005) found a median effective concentration (EC₅₀) range between 0.125 to 2.4 g/L for *Daphnia magna* after 48 hours of exposure to tire leachate obtained from grated rubber. Marwood et al. (2001) found an EC₅₀ of 4,3 g/L and a 1.25 g/L of no observed adverse effect concentration (NOAEC) for *D. magna* using tire leachate extracted at 44°C. Gualtieri et al. (2005) found a LC₅₀ range from 50 to 100 g/L for the African clawed frog (*Xeno Dus laevis*) after 120 hours of exposure to tire leachate.

Consistent with past studies, this study used zinc as a marker of tire leachate because it has been shown to be toxic at concentrations commonly detected in the field across a range of species, is common across almost all tire types, and is relatively easy to detect (Nelson et al., 1994; Wik and Dave, 2009; Marwood et al., 2011). Nelson et al. (1994) identified zinc from tire leachate as the toxicant that caused acute toxicity to *Ceriodaphnia dubia* with a 24-hour LC₅₀ of 147.1 ug/L. To isolate zinc, they used ethylene diamine tetraacetic acid (EDTA) and sodium thiosulfate to remove the toxicity of copper, cadmium, and lead from the solution. To reconfirm these results, they added zinc chloride to deionized water and found it was toxic to *C. dubia*. Nelson et al. (1994) found concentrations of 0.75 mg/L of zinc, 0.0067 mg/L of copper, 0.0067 mg/L of lead, and 0.0006 mg/L of cadmium from the leachate of 29 tire plugs of 10.25 cm in diameter and 100 g of weight. All the tire leachate samples tested for organic compounds were under the

detection limit, which was 0.001 mg/L (Nelson et al., 1994). Further, Marwood et al. (2011) suggest that zinc and aniline are probably the most important toxic chemicals in tire leachate, tire particle leachate extracted at 44 °C was analyzed and found zinc as the most abundant heavy metal in the samples with a concentration of 0.056 mg/L, and they also found two organic compounds at quantifiable levels, aniline (16mg/L) and N,N'-bis(1,4-dimethylpentyl)-p-phenylenediamine (26mg/L). We showed that the concentrations of zinc within the water and biofilm after 150 days would almost certainly have negative impacts on exposed communities of organisms. However, in addition to zinc a suite of other compounds are integral to tire material, have negative effects on living organisms, and may leach at different rates than zinc (e.g., benzothiazoles). Future studies should also aim to characterize the fate of these other contaminants to better understand the impact of tire deterioration on living systems.

This study is among the first to test the effects of different field-relevant UV-B levels on the rate and accumulation of zinc in used tires, and there is considerable scope to further study this environmental issue. Our findings in combination with prior studies (Villena et al., 2017) suggest that tire leachate is likely to have negative effects on at least some mosquito species through direct toxicity. Perhaps more ecologically important may be indirect effects that leachate may have by moderating species interactions, particularly among mosquito communities. Tire habitats are typically resource limited and mosquito larvae usually engage in strong intra- and inter-specific competition for microbial and detrital food resources. If competing mosquito species are differentially affected by tire leachate, the outcome of hetero-specific competition may be altered or even reversed,

leading to changes in the distribution and abundance of vector species and risk of disease transmission to human, wildlife, and livestock. In chapter 4, we test these hypotheses.

List of tables and figures

Tables

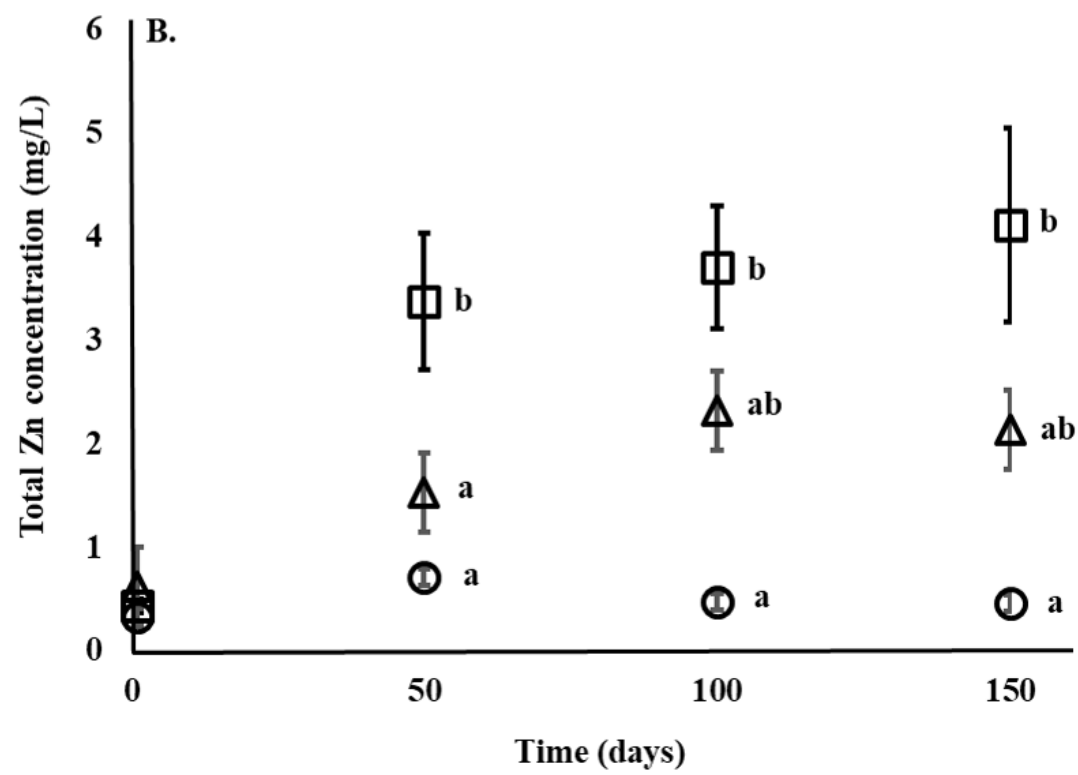
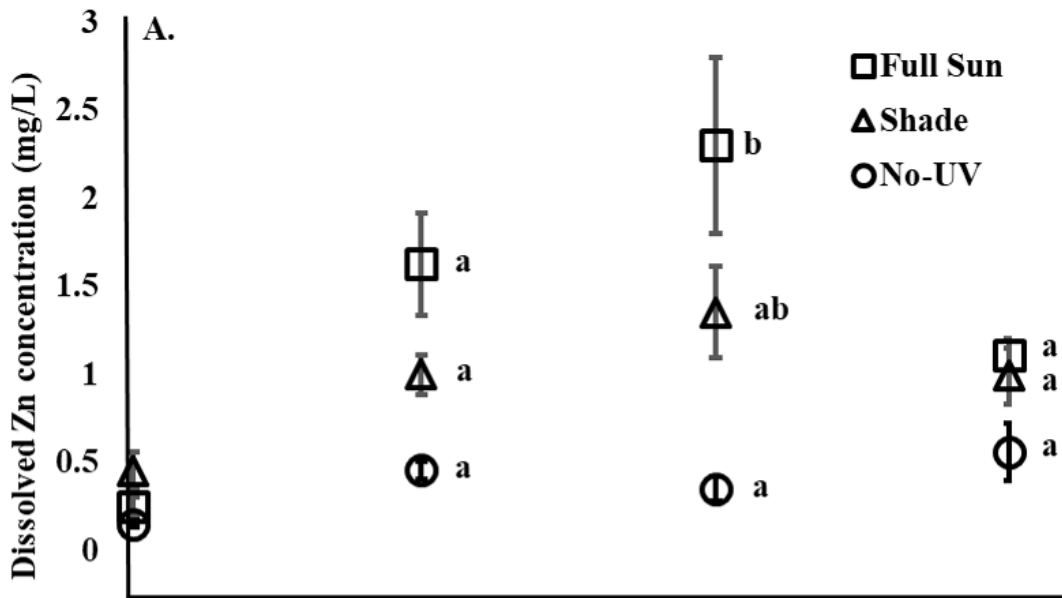
Table 4. ANOVA results of the effects of UV-B radiation conditions (full-sun, shade, and no-UV) on the concentrations of dissolved and total zinc concentrations in tire water.

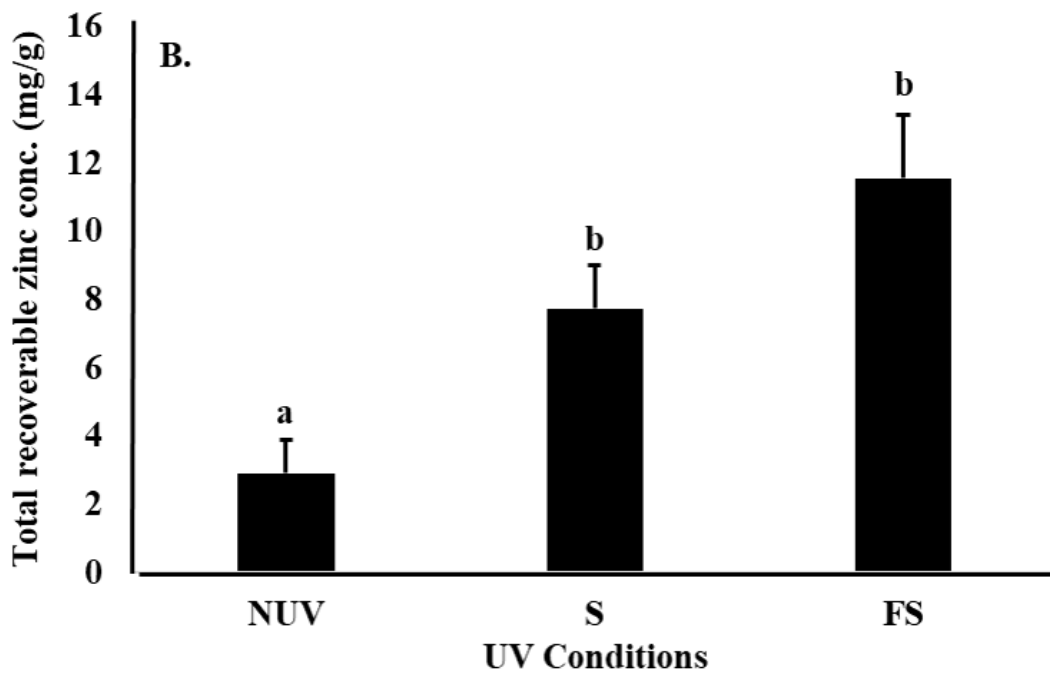
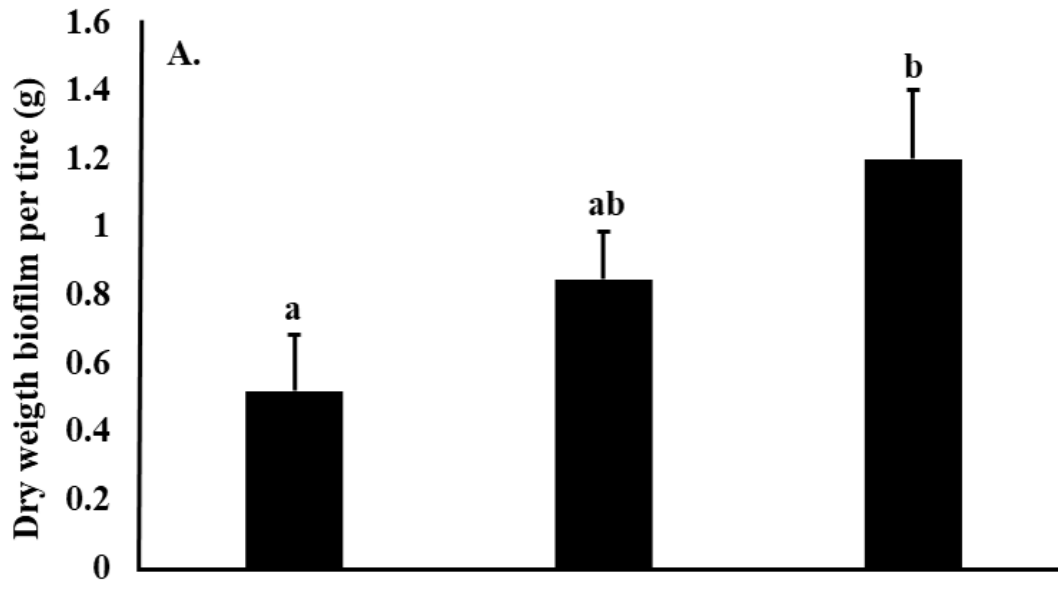
Source of Variation	Day 50			Day 100			Day 150		
	dfs	F	P	dfs	F	P	dfs	F	P
Dissolved zinc	2,11	3.75	0.0573	2,11	4.50	0.0473	2,11	2.54	0.1175
Total zinc	2,11	7,21	0.0100	2,11	11.81	0.0041	2,11	4.24	0.0452

Figures

Figure 4. Least square means (\pm SE) for (a) dissolved and (b) total zinc concentrations in water samples from discarded tires exposed to UV radiation conditions that mimicked full-sun (FS), shade (S) and no ultraviolet radiation (NUV) conditions at days 50, 100, 150 (expressed in mg/l). Data were statistically tested using ANOVA. Significant pairwise comparison among day of sampling are indicated by different letters.

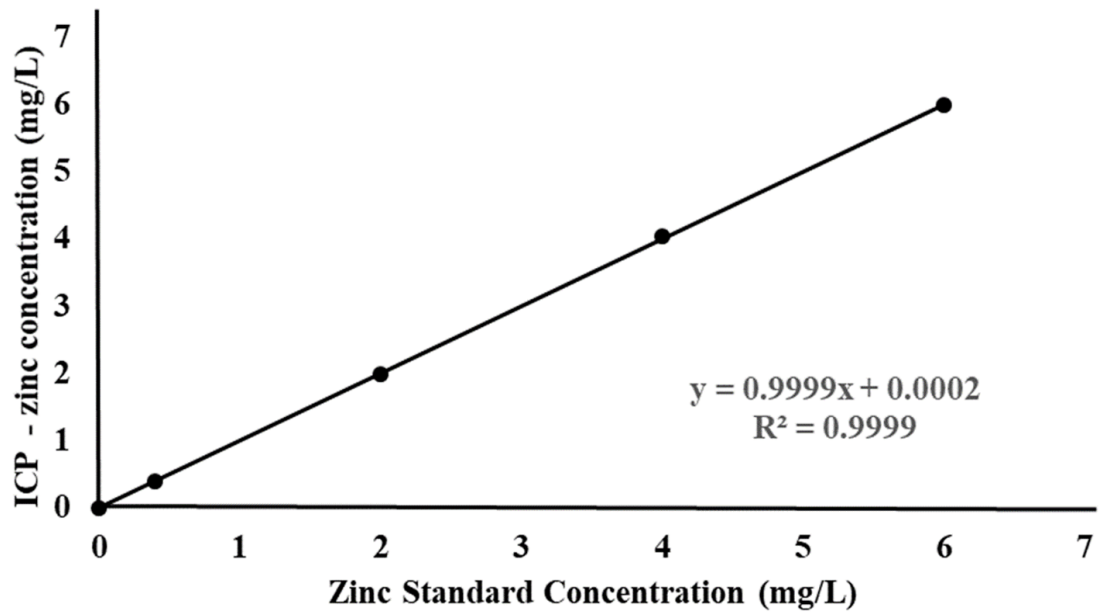
Figure 5. Least square means (\pm SE) for (a) dry weight of tire biofilm expressed in g and (b) total recoverable zinc concentrations in biofilm samples expressed in mg/g from discarded tires exposed to UV radiation conditions that mimicked full-sun (FS), shade (S) and no ultraviolet radiation (NUV) conditions for 150 days. Data were statistically tested using ANOVA. Significant pairwise comparison among UV-B conditions are indicated by different letters above bars.





Appendices

Appendix 4: Calibration of the Inductively coupled plasma atomic emission spectroscopy (ICP-AES), using the following zinc concentrations: 0, 0.4, 2, 4, 6 mg/L.



Appendix 5: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) on the dissolved and total zinc concentration in discarded tires.

```
PROC IMPORT OUT= WORK.zinc
  DATAFILE= "C:\Documents\Data\Zinc\waterZnCov.csv"
  DBMS=CSV REPLACE;
  GETNAMES=YES;
  DATAROW=2;
RUN;
PROC PRINT DATA=zinc;
RUN;
proc sort data=zinc;
key trt day /ascending;
run;
proc means data=zinc;
var ivdzn ivtzn DZn TZn;
by trt day block;
output out=a mean=ivdzn ivtzn DZn TZn;
run;
proc print data=a;
run;
proc sort data=a;
by day trt block;
run;
proc mixed data=a;
class block day trt;
model DZn= ivdzn trt/residual ddfm=sat;
random block;
lsmeans trt/ adjust=tukey;
by day;
run;
proc sort data=a;
by day trt block;
run;
proc mixed data=a;
class block day trt;
model TZn= ivtzn trt/residual ddfm=sat;
random block;
lsmeans trt/ adjust=tukey;
by day;
run;
```


Appendix 6: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) on the total recoverable zinc concentration in biofilm from discarded tires.

```
PROC IMPORT OUT= WORK.Znbio2
  DATAFILE= "C:\Documents\Data\Zinc biofilm2.csv"
  DBMS=CSV REPLACE;
  GETNAMES=YES;
  DATAROW=2;
run;
Proc print data=Znbio2;
run;
proc sort data=Znbio2;
  by trt blk;
  run;
*Amount of biofilm per trt**;;
proc means data=Znbio2 n mean median stderr;
  by trt blk;
  var Drybiofilm;
  output out=biofilm1 n=count mean=meanwbio median=medianwbio stderr=stderrwbio
  nmiss=nmiss;
  run;
*Amount of Zn per trt mg/g**;;
proc means data=Znbio2 n mean median stderr;
  by trt blk;
  var TZnGram;
  output out=zn1 n=count mean=meanzn median=medianzn stderr=stderrzn nmiss=nmiss;
  run;
*data transformation**;;
data b;
set Znbio2;
TZnGram=log10(TZnGram);
DryBiofilm=log10(DryBiofilm);
run;
proc mixed data=b;
class trt Tire blk;
model TZnGram = trt /ddfm=sat residual;
random blk;
LSMEANS trt/pdiff adjust=tukey;
run;
proc mixed data=b;
class trt blk;
model DryBiofilm = trt /ddfm=sat residual;
random blk;
LSMEANS trt/pdiff adjust=tukey;
run;
```

References

- Abernethy, S.G., Montemayor, B.P., and Penders, J.W. 1996. The Aquatic toxicity of scrap automobile tires. Ontario Ministry of Environment and Energy. pp 1-112.
- Andrady, A.L., Hamid, H.S., Hu, X., and Torikai, A. 1998. Effects of increased solar ultraviolet radiation on materials. *Journal of Photochemistry and Photobiology*, 46:96-103.
- Andrady, A.L., Hamid, H.S., and Torikai, A. 2003. Effects of climate change and UV-B on materials. *Journal of Photochemistry and Photobiology*, 2:68-72.
- Caldwell, M.M., Ballare, C.L., and Bornman, J.F., Flint, S.D., Bjorn, L.O., Teramura A.H., Teramura, A.H., Kulandaivelu, G., and Tevini, M. 2003. Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climatic change factors. *Photochemical and Photobiological Sciences*, 2:29-38.
- Crampton, M., Ryan, A., Eckert, C., Baker, K.H., and Herson, D.S. 2014. Effects of leachate from crumb rubber and zinc in green roofs on the survival, growth, and resistance characteristics of *Salmonella enterica* subsp. *Enterica* Serovar *Typhimurium*, *Applied and Environmental Microbiology*, 80:2804-2810.
- Cuong, D.T., Karuppiah, S., Obbard, J.P. 2008. Distribution of heavy metals in the dissolved and suspended phase of the sea-surface microlayer, seawater column, and in sediments of Singapore's coastal environment. *Environmental Monitoring and Assessment*, 138:255-272.
- Day, K.E., Holtze, K.E., Metcalfe-Smith, J.L., Bishop, and C.T., Dutka, B.J. 1993. Toxicity of leachate from automobile tires to aquatic biota. *Chemosphere*, 27:665-675.
- De Hoop, L., De Troch, M., Hendriks, A.J., and De Laender. 2013. Modeling toxic stress by atrazine in a marine consumer-resource system. *Environmental Toxicology Chemistry*, 32:1082-1095.
- Evans, J.J. 1997. Rubber tire leachates in the aquatic environment. *Reviews of Environmental Contamination and Toxicology*, 151:67-115.
- Grant, R.H., Apostol, K.G., and Schmitz, H.F. 2010. Physiological impacts of short-term UV irradiance exposures on cultivars of *Glycine max*. Book *UV radiation in global climate change*, pp 458-487.
- Gualteri, M., Andrioletti, M., Vismara, C., Milani, M., and Camatini, M. 2005. Toxicity of tire debris leachates. *Environment International*, 31:723-730.
- Hartwell, S.I., Jordahl, D.M., and Dawson, C.E. 2000. The effect of salinity on Tire Leachate Toxicity. *Water, Air, and Soil Pollution*, 121:119-131.

- Horner, J.M. 1996. Environmental health implications of heavy metal pollution from car tires. *Reviews on Environmental Health*, 11:175-178.
- Huang, J., Ge, X., and Wang, D. 2012. Distribution of heavy metals in the water column, suspended particulate matters and the sediment under hydrodynamic conditions using an annular flume. *Journal of Environmental Science (China)*, 24:2051-2059.
- Kellough, R.M. 1991. The effects of scrap automobile tires in water. Waste Management Branch, Ontario Ministry of the Environment, Ontario, pp 54.
- Kumata, H., Takada, H., and Ogura, N. 1997. 2-(4-Morpholinyl) benzothiazole as an indicator of tire-wear particles and road dust in the urban environment. In: *Molecular markers in environmental geochemistry*. American Chemical Society Symposium Series, vol. 671, Washington, DC, pp. 291-305.
- Kumata, H., Yamada, J., Masuda, K., Takada, H., Sato, Y., Sakurai, T., and Fujiwara, K. 2002. Benzothiazolamines as tire-derived molecular markers: sorptive behavior in street runoff and application to source apportioning. *Environmental Science and Technology*, 36:702-708.
- Kwon, E., and Castaldi, M.J. 2009. Fundamental understanding of the thermal degradation mechanisms of waste tires and their air pollutant generation in a N₂ atmosphere. *Environmental Science and Technology*, 43:5996-6002.
- Macia, A. 2006. Differences in performance of *Aedes aegypti* larvae raised at different densities in tires and ovitraps under field conditions in Argentina. *Journal of Vector Ecology*, 37:371-377.
- Marwood, C., McAtee, B., Kreider, M., Scott Ogle, R., Finley, B., Sweet, L., and Panko, J. 2011. Acute aquatic toxicity of tire and road wear particles to alga, daphnid, and fish. *Ecotoxicology*, 20:2079-209.
- Merrit, R.W., Dadd, R.H., and Walker, E.D. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual Review of Entomology*, 37:349-376.
- Miller, K.L. 2014. Waste tire recycling programs. Office of Legislative Research. Research Report 2014-R-0276, Hartford, CT, U.S.
- Nelson, S.M., Mueller, G., and Hemphill, D.C. 1994. Identification of tire leachate toxicants and a risk assessments of water quality effects using tire reefs in canals. *Bulletin Environmental Contamination and Toxicology*, 52:574-581.
- Peterson, J.C., Clark, D.R., and Sleevi, P.S. 1986. Tire fire oil: Monitoring a new environmental pollutant. *Analytical Chemistry*, 58:70-75.

- Rhodes, E.P., Zhiyong, R., and Mays, D.C. 2012. Zinc leaching from tire crumb rubber. *Environmental Science & Technology*, 46:12856-12863.
- Rohr J.R., and Crumrine, P.W. 2005. Effects of an herbicide and an insecticide on pond community structure and processes. *Ecological Applications*, 15:1135-1147.
- Rubber Manufacturers Association-RMA. 2017. 2015 U.S. scrap tire management summary. U.S. Tire Manufacturers Association, Washington, DC.
- Sanchez-Bayo, F., Van Den Brink, P.J., and Mann, R.M. 2012. Ecological impacts of toxic chemicals. Benthan Science Publisher, Sidney, Australia. 250 pp.
- SAS institute Inc. 2013. Base SAS 9.4 Procedures Guide: Statistical Procedures, Second Edition. Cary, NC: SAS Institute Inc.
- Selbes, M., Yilmaz, O., Khan, A.A., and Karanfil, T. 2015. Leaching of DOC, DN, and inorganic constituents from scrap tires. *Chemosphere*, 139:617-623.
- Spies, R.B., Anderson, B.D., and Rice, D.W. 1987. Benzothiazoles in estuarine sediments as indicators of street runoff. *Nature*, 327:697-699.
- Sullivan, J.H., Pope, L.C., Sutherland, B.M., Bennett, P.V., Blum, J.E., Stapleton, A.E., and Gitz, D.C. 2010. Assessment of DNA damage as a tool to measure UV-B tolerance in soybean lines differing in flavonoid composition. Book *UV Radiation in global change*, pp 437-457.
- Turner, J. 2011. Ultraviolet radiation reflection from building materials: characterization, quantification and the resulting effects. Thesis for a PhD degree, University of Southern Queensland, Australia, pp. 282.
- United States Environmental Protection Agency (U.S. EPA). 1993. Scrap Tire Handbook. U.S Environmental Protection Agency Region 5. 77 West Jackson Blvd, Chicago, IL.
- United States Environmental Protection Agency (U.S. EPA). 1994. Method 200.7, Revision 4.4: Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry. Cincinnati, Ohio.
- United States Environmental Protection Agency (U.S. EPA). 2007. SW-846 test method 3015A: microwave assisted acid digestion of aqueous samples and extracts. <https://www.epa.gov/sites/production/files/2015-12/documents/3015a.pdf>
- Villena, O., Terry, I, Kayoko, I., Landa, E., LaDeau, S., and Leisnham, P. 2017. Effect of tire leachate on the invasive mosquito *Aedes albopictus* and the native congener *Aedes triseriatus*. *PeerJ Preprints* 5:e3065v1 <https://doi.org/10.7287/peerj.preprints.3065v1>

Wik, A., and Dave, G. 2005. Environmental labeling of car tires - toxicity to *Daphnia magna* can be used as a screening method. *Chemosphere*, 58:645-651.

Wik, A., and Dave, G. 2006. Acute toxicity of leachates of tire material to *Daphnia magna* – Variability and toxic components. *Chemosphere*, 64:1777-1784.

Wik A., and Dave, G. 2009. Occurrence and effects of tire wear particles in the environment – A critical review and an initial risk assessment. *Environmental Pollution*, 157:1-11.

Yee, D.A., Kneitel, J.M., and Juliano, S.A. 2010. Environmental correlates of abundances of mosquito species and stages in discarded vehicle tires. *Journal of Medical Entomology*, 47: 53-62.

**Chapter 4: The role of tire leachate in condition-specific competition
and the persistence of a resident mosquito from a competitively superior
invader**

Oswaldo C. Villena^{1,4}, Edward Landa², Joseph Sullivan³, Bahram Momen², Paul T. Leisnham²

1. Marine Estuarine & Environmental Science, University of Maryland, College Park, MD 20742
2. Department of Environmental Science & Technology, University of Maryland, College Park, MD 20742
3. Department of Plant Science & Landscape Architecture, University of Maryland, College Park, MD 20742
4. Corresponding author: oswaldo.villena@gmail.com

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Abstract

Competitive interactions between mosquitoes depend on environmental conditions. Tire leachate, released by tire degradation under three different UV-B radiation conditions (full-sun, shade, and no-UV conditions), may promote condition-specific competition for limited food resources between the invasive *Aedes albopictus* and resident *Culex pipiens* mosquito in tire habitats. We tested the hypothesis that exposure to tire leachate alters heterospecific resource competition, diminishing the superior competitiveness of *Ae. albopictus* over *Cx. pipiens*, thereby facilitating species coexistence. Our results showed some evidence to support this hypothesis. Although negative effects of *Ae. albopictus* competition on the population performance of *Cx. pipiens* were consistent across all UV-B conditions, there were increased competitive effects of *Cx. pipiens* on *Ae. albopictus* population performance and survival in shade and full-sun conditions. In NUV condition, per capita rate of population change (λ') and survival of *Ae. albopictus* were greater under *Cx. pipiens* competition (50:50) compared to conspecific competition (100:0 and 50:0), a trend that was not apparent under S and FS conditions. *Culex pipiens* development time was statistically significantly negatively affected by *Ae. albopictus* competition, increasing on average by 10 days compared with low conspecific competition (50:0). *Culex pipiens* and *Ae. albopictus* body size were affected by UV condition, showing statistically significantly greater body size when adults emerged from tires exposed to S and FS conditions compared to NUV condition. Larger mosquitoes can represent a threat to public health because of their higher vectorial capacity, longer life expectancy, greater flying range, and better fecundity rates compared to smaller mosquitoes. Furthermore, overall zinc concentration in mosquitoes was higher in *Ae. albopictus* compared to *Cx.*

pipiens in tires with higher tire leachate concentration (from FS and S conditions). These findings suggest that UV-B exposure and tire degradation that *Ae. albopictus* and *Cx. pipiens* are likely to experience under shade and full-sun conditions in the field are likely to facilitate their coexistence and produce larger adults of both species, a finding that could have health consequences due to the higher vectorial capacity of larger mosquitoes.

Key words: Tire leachate, condition-specific competition, *Ae. albopictus*, *Cx. pipiens*

Introduction

Understanding the ecological mechanisms that govern the establishment and spread of exotic insect species and their impacts on resident communities is of fundamental importance to the field of invasion biology. Traditional niche theory and empirical research indicate that superior competitive ability is often a critical trait that determines the success and impacts of exotic species (Juliano, 2009; Davis, 2009; Lockwood et al., 2009). With one limiting resource in a constant environment, interspecific competition should result in competitive exclusion or local extinction (Tilman, 1982; Chase and Leibold, 2003). There are numerous instances where a competitively superior invader has displaced a resident species (Lounibos et al., 2016). However, there is also evidence of competitively inferior residents escaping competitive exclusion or local extinction via a number of mechanisms, including differential resource use (e.g., Tilman, 1982; Blaustein and Chase, 2007), temporal and spatial habitat segregation (Costanzo et al., 2005a; Leisham et al., 2014), Oviposition patterns and asynchrony in hatching (Costanzo et al., 2005a), and trade-offs between competitive ability and environmental tolerances (Dunson and Travis, 1991; Hemphill, 1991; Chesson and Huntly, 1997; Chesson, 2000). Condition-specific competition, whereby abiotic conditions reduce or reverse the outcome of competition between two species can facilitate coexistence when there are temporal or spatial changes in abiotic conditions (Lawton and Hassell, 1981; Juliano, 2009; Costanzo et al., 2011). Condition-specific competition is perhaps most interesting in invaded systems when it facilitates the incursion of an exotic species or persistence of a resident species despite the presences of a superior competitor.

A convenient model to investigate the role of condition-specific competition in biological invasions and the impacts on native communities is container dwelling mosquitoes that utilize discarded tires. Discarded tires that collect rain water are a common habitat for the developmental stages (eggs, larval, pupae) of many mosquito species worldwide (Yee, 2008b). In the United States, approximately 280 million scrap tires are generated each year and around 67 million tires are stored in stockpiles (Rubber Manufacturers Association, 2017). Tires have been particularly instrumental in the establishment and spread of invasive mosquito species (Lounibos, 2002a; Benedict et al., 2007). The best documented mosquito invasion is that of *Ae. albopictus* in North America via used tires in Texas in the mid-1980s (Sprenger and Wuithranyagool, 1986; Benedict et al., 2007). Since then, *Ae. albopictus* has spread throughout the eastern part of the United States, reaching New York state (CDC, 2016), commonly utilizing tire habitats, to become the most dominant human-biting species in many states (Juliano and Lonibos 2005).

In the Mid-Atlantic region of the United States, *Ae. albopictus* often co-occurs with *Culex pipiens* in tire habitats where larvae of both species feed on microorganisms in the water column and on surfaces (Vinogradova, 2000; Costanzo et al., 2005a, Yee et al., 2010). Microbial resources within tire habitats are associated with allochthonous inputs of plant and animal detritus, and are therefore limiting. *Aedes albopictus* and *Cx. pipiens* likely engage in strong interspecific competition in the field yet only four studies have rigorously tested competition between them. All four studies have confirmed strong competitive superiority of *Ae. albopictus* over the resident mosquitoes (Carrieri et al., 2003; Costanzo et al., 2005a; Costanzo et al., 2011; Marini et al., 2017). Of the four studies, only one study has rigorously tested the effects of environmental conditions on altering the

outcome of interspecific competition between *Ae. albopictus* and *Cx. pipiens*. A laboratory competition trial by Costanzo et al. (2011) showed that although *Ae. albopictus* was competitively superior to *Cx. pipiens* over a range of resource types, its competitive advantage was reduced in treatments with mainly grass, which is a high nutrient resource, and which, if combined with other mechanisms that relaxed the competitive impacts from *Ae. albopictus*, could ensure *Cx. pipiens* persistence.

Collectively, *Ae. albopictus* and *Cx. pipiens* are vectors for a range of human and animal pathogens, including West Nile virus (WNV), dengue, Eastern Equine encephalitis, La Crosse encephalitis, malaria, St. Louis encephalitis, Japanese encephalitis, and dog heartworm (Savage and Miller, 1995; Gerhardt et al., 2001; Kim et al., 2005), thus the distribution and abundance of each species is of medical and veterinary importance. Coexistence of *Ae. albopictus* and *Cx. pipiens* may be particularly important for the spread of human WNV because *Cx. pipiens* is the main WNV vector among avian populations where the virus amplifies, while *Ae. albopictus* can bridge the virus into human populations (Tiawsirisup et al. 2005; Rizzoli et al., 2015). These two species also show high preference for urban and suburban residential areas (Sawabe et al., 2010).

Despite being competitively inferior, *Cx. pipiens* has persisted in tire habitats in many urban and suburban areas after the invasion of *Ae. albopictus*, and factors controlling their coexistence within a tire are largely unknown. Tires are made of a conglomerate of organic (e.g., styrene-butadiene, polybutadiene), and inorganics (e.g., zinc, copper, cadmium, lead) compounds (Day et al., 1993; Selbes et al., 2015). Once tires are discarded and exposed to the effect of rain and ultraviolet radiation, organic and inorganic contaminants can leach into water in the tire casing (Andrady et al., 1998). Tire

contaminants have shown lethal (Kellough, 1991; Day et al., 1993) and sub-lethal effects on biota (Wik and Dave, 2005; Gualtieri et al., 2005a; Gualtieri et al., 2005b), including mosquitoes, in concentrations found in the field; such effects of these contaminants may allow condition-specific competition to play a role in coexistence of *Ae. albopictus* and *Cx. pipiens* (Villena et al., 2017). Two aspects of the life histories of *Ae. albopictus* and *Cx. pipiens* suggest that tire leachate may modify the outcome of interspecific competition between these two species. First, *Cx. pipiens* is well-documented to utilize a wider range of habitats than *Ae. albopictus*, including those that are likely highly contaminated by organic and inorganic pollutants that *Ae. albopictus* and most other mosquito species do not utilize (Vinogradova, 2000; Dehghan et al., 2010), suggesting that *Cx. pipiens* shows a higher tolerance to environmental contaminants. On the other hand, *Ae. albopictus* has shown reduced performance in habitats with excessive nutrient pollutants (WHO, 2003). Second, *Cx. pipiens* and *Ae. albopictus* have different feeding behaviors that may expose the invader species to higher concentrations of tire contaminants than the resident species. *Aedes albopictus* spends much more time feeding than *Cx. pipiens* and a greater proportion of its feeding time browsing surface biofilm compared with *Cx. pipiens*, which tends to exclusively filter-feed in the water column (Merritt et al. 1992), suggesting that *Ae. albopictus* may be exposed to more contaminants that may leach from a tire casing wall.

In this chapter, I tested the effects of tire leachate on competition between the competitively superior *Ae. albopictus* and resident *Cx. pipiens*. Specifically, I tested the hypothesis that tires that have been exposed to greater UV-B radiation, and that therefore contain tire leachate with higher concentration of pollutants produce a condition-specific advantage for *Cx. pipiens* by relaxing or even reversing the outcome of competition with

Ae. albopictus. From this hypothesis, I derived the following predictions: 1. *Aedes albopictus* will have a greater competitive impact on *Cx. pipiens* under UV conditions that minimize tire degradation and reduce contaminant leaching; and 2. *Culex pipiens* will demonstrate reduced or even reversed negative impact from *Ae. albopictus* competition under UV conditions that accelerate tire degradation and increase contaminant leaching.

Materials and methods

Collection and maintenance of mosquitoes

Aedes albopictus and *Cx. pipiens* larvae were collected from multiple locations in College Park, Baltimore, and Towson, Maryland. Neither *Ae. albopictus* or *Cx. pipiens* are endangered and collection sites were either on publically accessible lands or on private lands where consent for collections was granted at the time of collection; thus, no field permits were required to collect them. Field collected *Ae. albopictus* and *Cx. pipiens* larvae were reared to adulthood at 25°C at 16:8 (L:D) h photoperiod, and then released into 1-m² single-species cages. Adults were kept in an insectary at 25°C and >85% RH, 16:8 (L:D) h photoperiod. Both colonies were supplied 20% sugar solution. Females from both colonies were fed horse or rooster blood once a week via an artificial feeder (Hemotek, Accrington, UK) to ensure egg production and experimental larvae. *Aedes albopictus* females oviposited on seed paper placed inside 500 ml black cups, and filled with 200 ml of deionized (DI) water. Eggs were collected over multiple weeks and stored at >80% RH and 16:8 hours (L:D) photoperiod until hatching for the experiment. *Culex pipiens* oviposited egg rafts into a 500 ml black bowl filled with 400 ml of DI water. *Culex pipiens* eggs cannot be held without hatching; thus, egg rafts were collected within 24 h of oviposition, hatched in a lactobumina: yeast solution, and larvae were transferred into the

experiment after being rinsed. *Aedes albopictus* eggs that had been stored were also hatched in a lactobumina: yeast solution and larvae were transferred into the experiment after being rinsed and within 24 h of hatching. Experimental larvae of both species were F₁₋₃ generation.

Competition trial

The competition trial was setup as a split-plot randomized complete block design (RCBD) with ultraviolet radiation (UVR) condition as the main plot, competition treatment as the sub-plot, and four replicate blocks. Newly hatched *Ae. albopictus* and *Cx. pipiens* were added to 15 whole tires that had been exposed to one of three UV-B conditions: full-sun (FS), shade (S), and no-UV (NUV) conditions, for 150 days. Before placing newly hatched larvae, UV-B bulbs were wrapped with polyester filters that block almost all UV-B radiation below 316 nm (Sullivan et al. 2010). The experiment was housed in a greenhouse at the University of Maryland College Park at 25°C, 16:8 h (L:D) photoperiod and the set-up is described in Chapter 2. The five tires exposed to each of the three UV-B conditions were randomly assigned one of five mosquito competition treatments (*Ae. albopictus*: *Cx. pipiens*, 0:100, 0:50, 100:0, 50:0, 50:50) and provisioned with 1.0 g senescent dried white oak (*Quercus alba*) leaf litter to create a response-surface competition trial. Treatments with 100 single species larvae (i.e., 0:100, 100:0) and with 100 mixed species larvae (i.e., 50:50) were expected to exert high conspecific and heterospecific competition compared with treatments with only 50 single-species larvae which would exert little competitive pressure and represent “baseline” conditions. The experiment was set up as a blocked design in that the procedure was replicated four times as a new set of 15 tires were exposed to the UVR condition for 150 days. Each run of the

experiment was a replicate of each UVR condition x mosquito competition treatment combination, yielding 60 total experimental units. Tires were checked daily to collect pupae, which were then placed in individual vials with water until adult emergence. On the day of emergence, adults were killed by drying (>40°, > 48 h) and had their wing lengths measured. From each tire, we measured three fitness parameters for both species: proportion ♀ survivorship, median ♀ development time, and median ♀ wing length. Using these fitness parameters, we calculated λ' , a composite index of population increase based on r' , the realized per capita rate of population change ($dN/N dt = r$, the exponential growth rate) (Juliano 1998).

$$\lambda' = \exp \left[\frac{\log \left[(1/N_0) \sum_x A_x f(w_x) \right]}{D + \frac{\sum_x x A_x f(w_x)}{\sum_x A_x f(w_x)}} \right]$$

where N_0 is the initial number of females per container or microcosm (assumed to be 50 % of the larvae population); A_x is the number of females eclosing on day x ; w_x is a measure of mean female size on day x ; $f(w_x)$ is a function relating fecundity to female size, and D is the estimated time (in days) required for a newly eclosed female to mate, obtain a bloodmeal, and oviposit. D is assumed to be 10 days for *Cx. pipiens* (Vinogradova and Karpova 2006, Costanzo et al. 2011), and 14 days for *Ae. albopictus* (Juliano 1998). For *Aedes albopictus*, $f(w_x) = 78.02w_x - 121.24$ ($r^2 = 0.713$, $N = 91$, $P < 0.0001$; Lounibos et al. 2002b). There is limited information on the fecundity to size relationship for female *Cx. pipiens* as most information on this function is of autogenous *Cx. pipiens molestus*. Egg rafts from autogenous species tend to have fewer eggs than those of anautogenous species, and thus using a size-fecundity relationship based on *Cx. pipiens molestus* is undesirable

for *Cx. pipiens pipiens* (Vinogradova 2000). Therefore, we used the equation $f(wx)=148.5wx-383.82$ ($r^2=0.3724$, $N=55$, $P<0.0001$) based on a recent study following standard rearing protocols to obtain fecundity-size relationships for mosquitoes, whereby adult females of varying sizes were placed in individual cages and allowed to oviposit to obtain exact measures of fecundity across a distribution of sizes in a study at the University of Maryland (Scott et al. *unpublished*).

Zinc concentration in mosquitoes

After being dried and measured, all adults from blocks 3 and 4 were pooled by UVR condition and species to yield sufficient material to test for zinc concentration. Pooled samples were ground, microwave digested using an Ethos D Milestone Microwave following U.S. EPA 3015A method and standard protocols (EPA, 2007), and measured for total recoverable zinc using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) following the U.S. EPA Method 200.7 (EPA, 1994).

Analyses

Two-way analyses of variance (ANOVA) were used to measure the effects of UV-B radiation conditions and competition treatment on λ' and its fitness parameters: ♀ survival, median ♀ development time, median ♀ body size for both *Cx. pipiens* and *Ae. albopictus* (PROC MIXED, SAS 9.4). Significant interactions between UV-B radiation conditions and competition treatment would indicate that UV-B radiation conditions altered the impact of competition. To account for assumptions of normality and homogeneity of variances, all survivorship data were arcsin transformed and *Ae. albopictus* λ' was $\log_{10}(y+1)$ transformed. In all these analysis, we considered treatment and species as fixed effects, and block as a random effect in the model. Despite transformations, *Cx.*

pipiens λ' failed to meet parametric assumptions, and therefore we used a randomization two-way ANOVA (Randomization wrapper for SAS PROCs; Cassell 2011). Mosquito zinc concentration was also analyzed by a two-way ANOVA. To account for assumptions of normality and homogeneity of variances, zinc concentration was $\log_{10}(y+1)$ transformed. In the model, we considered UV-B radiation conditions and species as fixed effects, and block as a random effect. For all ANOVAs, we did a pairwise mean comparison in the mixed procedure using the LSMEANS statement with tukey adjustment with the exception of the *Cx. pipiens* ANOVA because main effects and interaction of effects were not statistically significant. Experiment wise $\alpha = 0.05$; marginal significance was defined $\alpha = 0.05-0.10$.

Results

There was no interaction between UV-B radiation conditions and competition treatment for *Cx. pipiens* λ' , survival, or development time (Table 5, Figure 7), indicating that there was no evidence that UV-B conditions altered the impact of competition on the *Cx. pipiens* population performance or important parameters of its fitness. But there was a marginal UV-B conditions x competition treatment interaction effect on *Cx. pipiens* body size, with the larger females eclosing from tires exposed to shade and full-sun vs. no-UV conditions, under high conspecific (0:100) and *Ae. albopictus* (50:50) competition (Table 2). Main effects of UV-B conditions were detected on *Cx. pipiens* λ' (marginal) and survival, with cohorts in tires under FS condition having the lowest population performances and survival (Figure 9A). UV-B radiation conditions also affected female body size (Table 6), with the largest median body sizes in tires exposed to S and FS conditions (Figure 9E). Main effects of competition treatment were also detected on *Cx.*

pipiens survival, development time, and body size (Table 6). *Culex pipiens* survival was lower and body size was smaller in the 0:100 and 50:50 vs. 0:50 competition treatments, indicating negative effects of both conspecific and *Ae. albopictus* competition on these fitness parameters (Figures 9B and 9F). *Culex pipiens* development time was longer in the 50:50 vs. 0:50 competition treatments, indicating a negative effect of *Ae. albopictus* competition on this fitness parameter (Figure 9D). Despite these effects of competition on *Cx. pipiens* fitness, no effect of competition was detected in its population performance.

Across almost all UV-B conditions and competition treatments, *Ae. albopictus* had higher λ' than *Cx. pipiens*, indicating that it was the superior competitor under my laboratory conditions. Consistent with *Cx. pipiens*, a main effect of UV-B conditions on *Ae. albopictus* λ' (marginal) and survival were detected, with lowest population performance and survival in tires that had been exposed to FS condition (Tables 5 and 6, Figures 6A and 8A). However, in contrast to *Cx. pipiens*, the effects of UV-B conditions were not consistent across all competition treatments, as detected by significant UV-B conditions x competition treatment interaction (Table 5). Under high *Cx. pipiens* competition (50:50), *Ae. albopictus* λ' was significantly lower in S vs. NUV conditions (Figure 6A). Also, main effects of competition treatment were detected on *Ae. albopictus* survival with lower survival in the 100:0 and 50:50 vs. 50:0 competition treatments (Figure 8B). Although effects of *Cx. pipiens* competition varied across UV conditions, *Ae. albopictus* experienced the greatest competition from conspecifics. *Ae. albopictus* λ' and survival was lower in the 100:0 vs. 50:0 competition treatment indicating a significant negative effect of conspecific competition (Figures 6A, 8B).

There was no interaction between species and UV-B conditions treatment for *Cx. pipiens* and *Ae. albopictus* body total recoverable zinc concentration expressed in mg/kg (Table 7). indicating that there was no evidence that UV-B conditions had impact on the species zinc concentration. There was a main effect of species on the total recoverable zinc concentration with *Ae. albopictus* showing greater total recoverable zinc concentration compared to *Cx. pipiens* across all UV-B conditions (Figure 10).

Discussion

Tire habitats that have been exposed to greater UV-B radiation and that therefore contain water with higher amounts of tire leachate may produce a condition-specific advantage for *Cx. pipiens* by relaxing or even reversing the outcome of competition with *Ae. albopictus*. My competition trial using tires that had been exposed to varying UV-B radiation under controlled experimental conditions showed some evidence to support this hypothesis. There were increased competitive effects of *Cx. pipiens* on *Ae. albopictus* population performance and survival in shade (S) and full-sun (FS) conditions, but not in no-UV (NUV) conditions. *Aedes albopictus* from full-sun conditions had higher amounts of bodily zinc, indicating greater exposure to tire leachate contaminants in tire habitats. Although *Ae. albopictus* had consistently higher population performance across all UV-B conditions and competition treatments, indicating competitive superiority, increased competitive impacts on *Ae. albopictus* in conditions with higher tire contaminants and reduced body ingestion of tire contaminants may facilitate the coexistence of *Cx. pipiens* with the invader.

Competition was clearly asymmetrical between *Ae. albopictus* and *Cx. pipiens*, with *Ae. albopictus* the superior competitor to *Cx. pipiens*, this finding is consistent with

the finding of Carrieri et al. 2003, Costanzo et al. 2005a, Costanzo et. al. 2011, and Marini et al. 2017. Population growth index (λ'), the finite rate of increase of a population, determines if a population is increasing or decreasing. Lambda below zero indicates that a population decreases, a λ' equal to 1.0 indicates that a population is stable, and λ' greater than 1.0 indicates population increase (Oli and Dobson, 2003). For *Ae. albopictus*, under NUV conditions, λ' was greater for heterospecific competition (50:50) compared to high conspecific competition (100:0), and marginally greater compared to low conspecific competition (50:0), which did not happen under S and FS condition, being statistically significantly smaller for 50:50 in S condition compare to NUV, indicating a negative effect of *Cx. pipiens* on *Ae. albopictus* λ' (Figure 6A). In spite of higher interspecific competition of *Cx. pipiens* on *Ae. albopictus* population performance under FS and S conditions, λ' values for *Ae. albopictus* still were above 1.0, meaning that *Ae. albopictus* population will increase in similar conditions. On the other hand, under no-UV conditions, *Cx. pipiens* λ' was lower for high conspecific and heterospecific competition, compared to low intraspecific competition, meaning that *Cx. pipiens* was equally affected by conspecific and by *Ae. albopictus* (Figure 7A). In shade conditions, both *Ae. albopictus* and *Cx. pipiens* showed similar λ' values and all values were above 1.0 for low and high conspecific competition and heterospecific competition, meaning that both populations will increase through time. In FS conditions, *Cx. pipiens* λ' values were below 1.0 meaning its population would decline through time under these conditions. These results suggest that tire leachate could be one of the mechanisms that could make coexistence possible between *Ae. albopictus* and *Cx. pipiens* under shade condition.

Culex pipiens ♀ survival was consistently lower than *Ae. albopictus* across all UV-B conditions and competition treatments (Figure 9A, 9B, 8A and 8B). However, survivorship of *Cx. pipiens* was proportionally greater than *Ae. albopictus* under S conditions compared to NUV conditions (Figure 9A, 8A). Survival of *Ae. albopictus* was affected by conspecifics, but not by *Cx. pipiens* (Figure 8B). *Culex pipiens* was affected in similar way by conspecifics and by *Ae. albopictus* (Figure 9B), suggesting that *Cx. pipiens* survival does not appear to be lower in tires with *Ae. albopictus* compared to only *Cx. pipiens*; these findings suggest that competition from *Ae. albopictus* is having no greater effect than competition from conspecifics. The increase leaching of contaminants with UV-B exposure probably played a role in the findings of these experiments. Tire leachate is probably having a sub-lethal effect on *Ae. albopictus* and *Cx. pipiens* mosquitoes in S and FS conditions. Several laboratory studies have shown that tire leachate showed sub-lethal and lethal effects to aquatic organisms of different taxonomical orders. For example, Day et al. (1993) found that tire leachate was lethal for *Oncorhynchus mykiss*, Gualtiere et al. (2005b) found that tire leachate was lethal for *Xenopus laevis* embryos, and Wik and Dave (2005) found that tire leachate was lethal for *Daphnia magna*.

High concentration of tire leachate also influenced development time and wing length. For *Cx. pipiens*, development time was statistically negatively affected by *Ae. albopictus* competition (Figure 9D), increasing on average by 10 days compared with low conspecific competition and by 6.3 days compared with high conspecific competition. Under field conditions, development time has a cost/benefit relationship (Lane and Mahony 2002). From an ecological point of view, longer development time means greater duration of mosquito larvae exposure to competition from conspecifics and other mosquito

species and longer exposure to predation, which could reduce the larval population and lead to a fewer number of adults. This scenario could be detrimental for a mosquito population. *Aedes albopictus* development time was not affected either by conspecifics or by *Cx. pipiens*.

In our study, body size of *Cx. pipiens*, was affected by UV-B conditions and competition treatment. *Cx. pipiens* showed statistically significant greater body size when adults emerged from tires exposed to S and FS conditions compared to NUV conditions (Figure 9E). *Ae. albopictus* also showed the same trend of greater body size when adults emerged from shade and full-sun conditions (Figure 8E). Bigger mosquitoes are of medical and veterinarian concern and may represent a threat to public health because greater mosquito size is positively correlated with survival, reproductive success, higher number of egg batches, higher number of eggs per batch, and greater vectorial capacity (Xue et al., 1995; McCann et al., 2010; Moller-Jacobs et al., 2014; Roux et al., 2015)

Aedes albopictus showed the capacity to absorb higher amounts of pollutants compared to *Cx. pipiens* across all UV-B conditions (Figure 10) with an average of 8 mg of zinc per kilogram of body mass in mosquitoes that were placed in tires exposed to FS condition. This is probably because compared to *Cx. pipiens*, *Ae. albopictus* spends more time feeding, and, of the time feeding, a higher proportional time browsing on surfaces (Merrit et al. 1992, Yee et al. 2008b). Even though *Ae. albopictus* were more exposed and ingested higher quantities of tire contaminants than *Cx. pipiens*, the negative effect of these contaminants did not prevent *Ae. albopictus* from having higher λ' than *Cx. pipiens*. Previous studies showed that other contaminants at field concentration (e.g., malathion) or environmental factors (e.g., temperature) have negative effects on *Ae. albopictus* that are

capable of reversing its superior competitiveness. For example, the study of Alto et al. (2013) found that field concentrations of malathion, an organophosphate insecticide, was able to eliminate the superior competitiveness of *Ae. albopictus* against *Ae. aegypti*. Alto et al. (2013) assessed the effect of malathion, widely used in agriculture, on the coexistence of *Ae. albopictus* and *A. aegypti*. In the absence of Malathion, *A. aegypti* survivorship was negatively affected by increasing densities of *Ae. albopictus*, while *Ae. albopictus* survivorship was not affected by increasing densities of *Ae. aegypti*. But in presence of malathion, the negative effect of *Ae. albopictus* competition on *A. aegypti* survivorship was eliminated. This is a good example of condition-specific competition that is mediated by a chemical contaminant. Temperature, is another example where an abiotic factor has reversed the competitive superiority of *Ae. albopictus*. Costanzo et al. (2005b) showed that competitive superiority of *Ae. albopictus* over *Ae. aegypti* under wetter environment conditions could be reversed under dryer environmental conditions. This mechanism probably explains the co-existence of *Ae. albopictus* and *A. aegypti* in Florida (Juliano et al., 2002; Juliano and Lounibos, 2004; Leishman and Juliano, 2010; Costanzo et al. 2011). In our study, tire leachate did not reverse the competitive superiority of *Ae. albopictus* over *Cx. pipiens* in tire habitats, but it showed that it can play a role in the coexistence of these two species. Coexistence of these two species may be influenced by other factors besides tire leachate or a combination of factors such as temperature, timing of hatching, or habitat segregation, each of which should be evaluated in future studies.

List of Tables and Figures

Tables

Table 5. Two-way ANOVA of the effects of UV-B conditions (full-sun, shade, and non-UV) and competition treatments (*Ae. albopictus*: *Cx. pipiens*, 100-0, 50-0, 50-50, 0-100, 050) on the per capita rate of population change (λ') from competition between *Ae. albopictus* and *Cx. pipiens* mosquitoes.

Variable	Population growth index (λ')		
	dfs	F	P
<i>Ae. albopictus</i>			
UV Condition	2, 24	2.68	0.0889
Competition treatment	2, 24	3.04	0.0665
UV condition x competition treatment	4, 24	3.10	0.0343
<i>Cx. pipiens</i>			
UV Condition	2, 24	2.97	0.0660
Competition treatment	2, 24	0.15	0.7520
UV condition x competition treatment	4, 24	0.01	0.9930

Table 6. Two-way ANOVA of the effects of UV-B conditions (full-sun, shade, non-UV) and competition treatments (*Ae. albopictus*: *Cx. pipiens*, 100-0, 50-0, 50-50, 0-100, 050) on survivorship, developmental time and body size (wing length) from competition between *Ae. albopictus* and *Cx. pipiens* mosquitoes.

Variable	Survival			Developmental time			Body size (wing length)		
	dfs	F	P	dfs	F	P	dfs	F	P
<i>Ae. albopictus</i>									
UV-B Conditions	2,24	7.29	0.0034	2,24	2.83	0.0790	2,24	0.24	0.7908
Competition treatment	2,24	8.29	0.0018	2,24	1.02	0.3752	2,24	1.45	0.2553
UV-B conditions x competition treatment	4,24	3.30	0.0274	4,24	1.74	0.1751	4,24	0.41	0.8003
<i>Cx. pipiens</i>									
UV-B Conditions	2,24	5.91	0.0082	2,21	0.37	0.6920	2,21	13.7	0.0002
Competition treatment	2,24	3.79	0.0371	2,21	5.38	0.0130	2,21	6.11	0.0081
UV-B conditions x competition treatment	4,24	0.28	0.8886	4,21	1.13	0.3708	4,21	2.61	0.0645

Table 7. Two-way ANOVA of the effects of UV-B conditions (full-sun, shade, non-UV) and species (*Ae. albopictus*, *Cx. pipiens*) on zinc concentration (mg/kg) in mosquitoes.

Variable	Zinc concentration (mg/kg)		
	dfs	F	P
Species	2,5	29.44	0.0029
UV-B conditions	1,5	1.67	0.2775
Species x UV-B conditions	2,5	2.19	0.2072

Figures

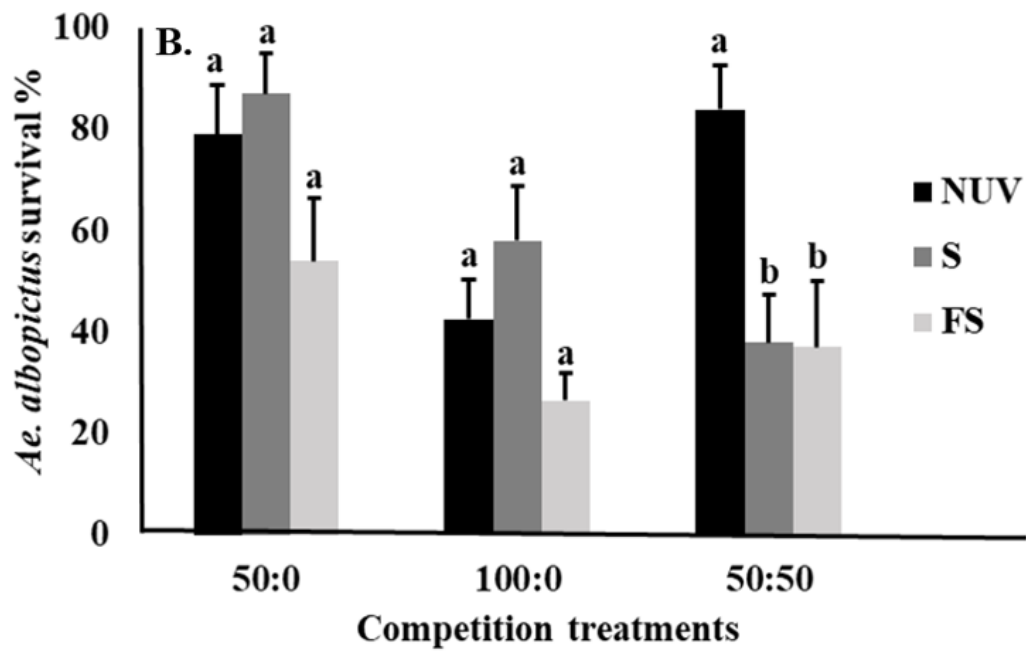
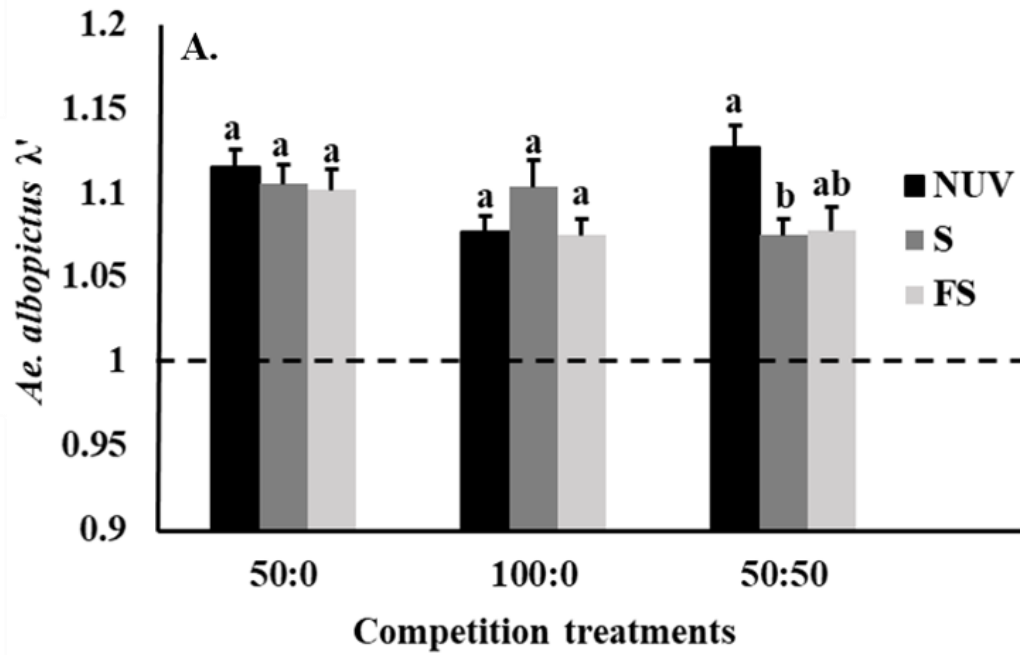
Figure 6. Least square means (\pm SE) of *Ae. albopictus* (a) λ' and (b) survival percentage in response to competition treatments (high and low conspecific, and heterospecific) and UV-B conditions (full-sun, shade, no-UV). Data were statistically tested using ANOVA. Significant pairwise comparisons among treatments for interactions effects are indicated by different letters above bars.

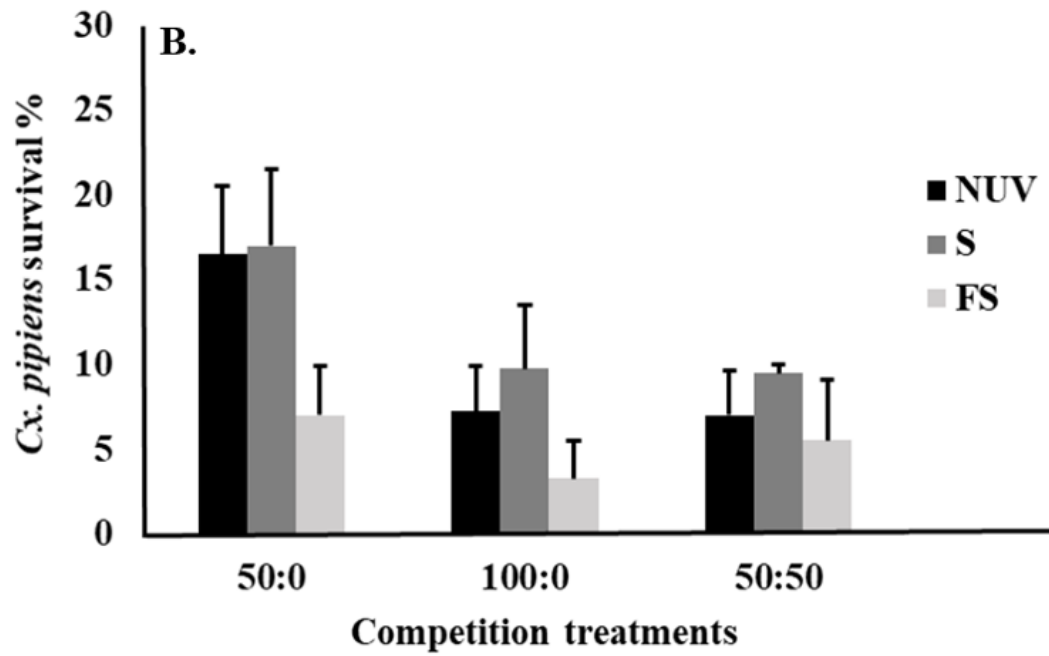
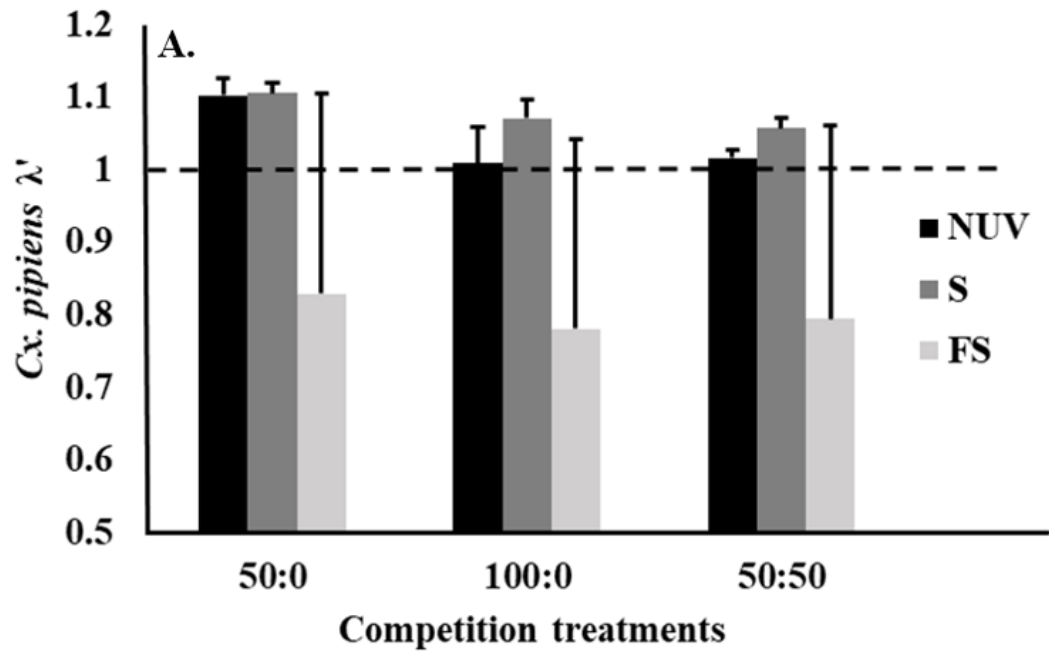
Figure 7. Least square means (\pm SE) of *Cx. pipiens* (a) λ' and (b) survival percentage in response to competition treatments (high and low conspecific, and heterospecific) and UV-B conditions (full-sun, shade, no-UV). Data were statistically tested using ANOVA. The difference between treatments is not significant.

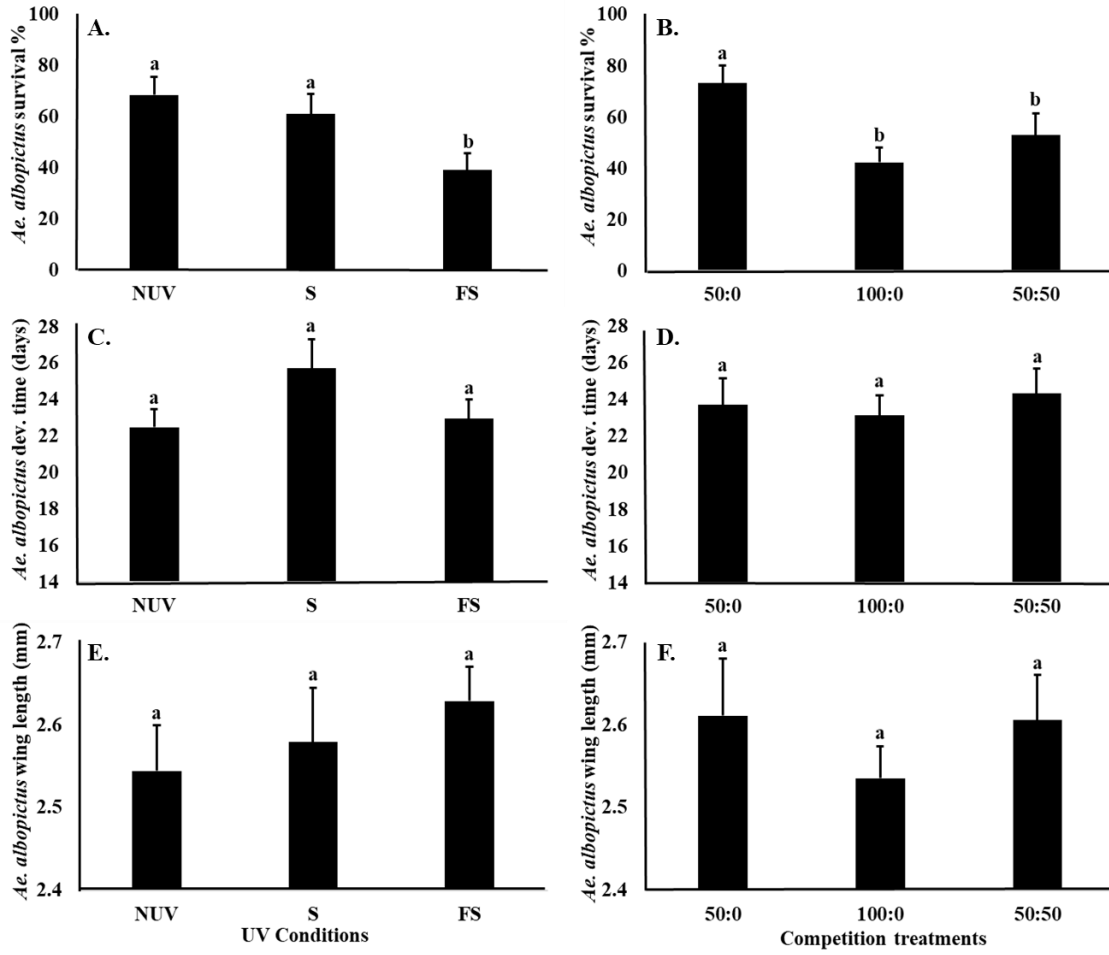
Figure 8. Least square means (\pm SE) of *Ae. albopictus* (a) survival percentage (b) development time (c) wing length in response to UV-B conditions (full-sun, shade, no-UV) and competition treatments (high and low conspecific and heterospecific). Data were statistically tested using ANOVA. Significant pairwise comparisons among treatments for main effects are indicated by different letters above bars.

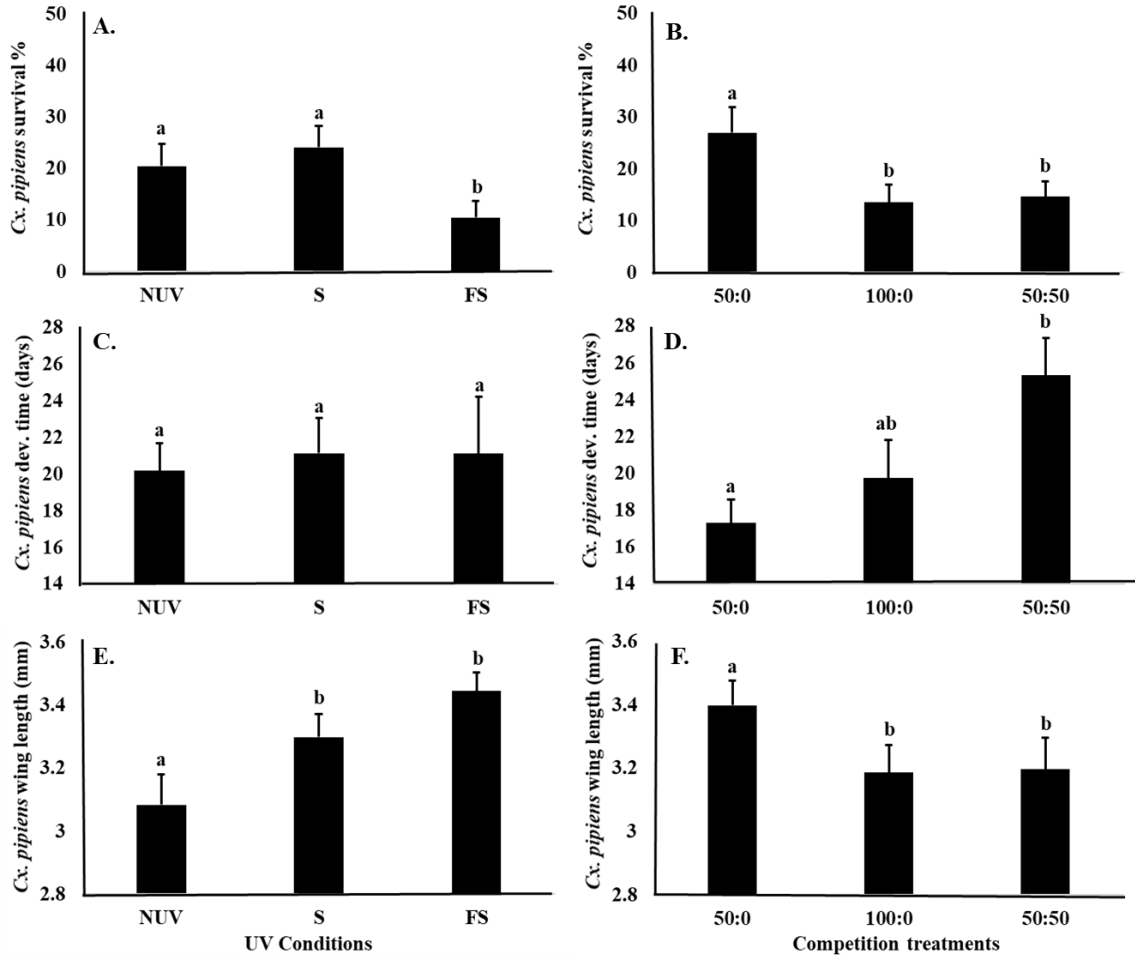
Figure 9. Least square means (\pm SE) of *Cx. pipiens* (a) survival percentage (b) development time (c) wing length in response to UV-B conditions (full-sun, shade, no-UV) and competition treatments (high and low conspecific and heterospecific). Data were statistically tested using ANOVA. Significant pairwise comparisons among treatments for main effects are indicated by different letters above bars.

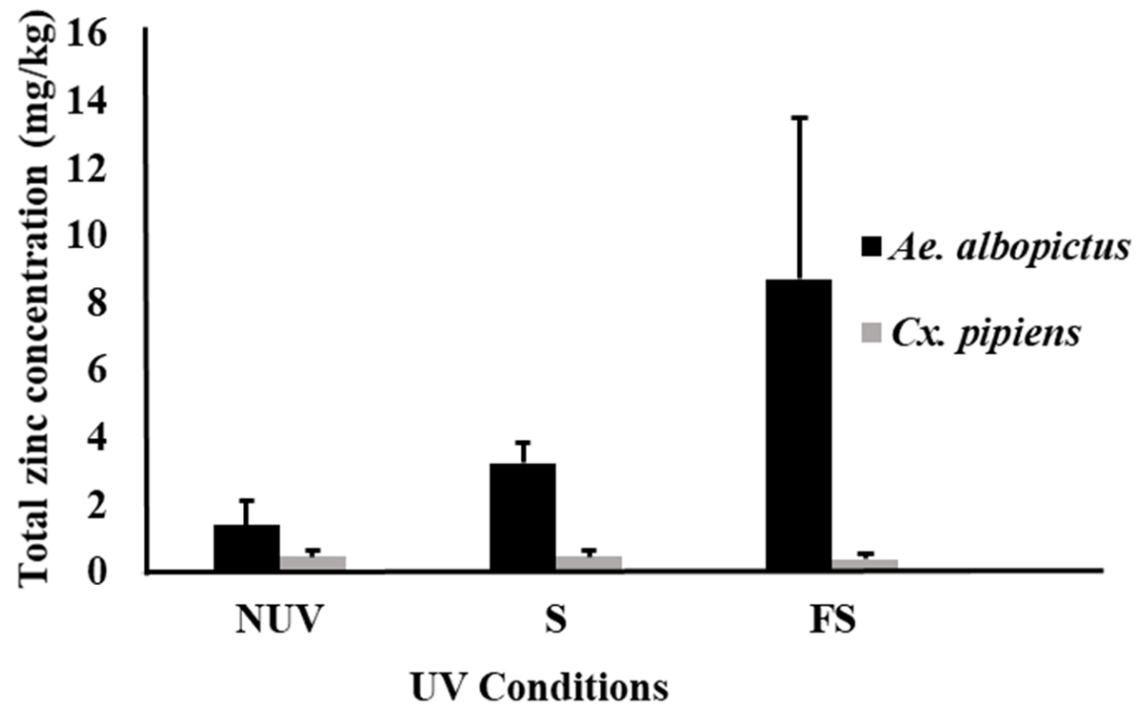
Figure 10. Least square means (\pm SE) total recoverable zinc concentration in mosquitoes (mg/kg) for *Ae. albopictus* and *Cx. pipiens* in response to UV-B conditions (full-sun, shade, non-UV). Data were statistically tested using ANOVA.











Appendices

Appendix 7: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) and competition treatments (*Ae. albopictus*: *Cx. pipiens*, 100:0, 50:0, 50:50, 0:100, 0:50) on the per capita rate of population change (λ') and fitness parameters (survivorship, development time, and body size) of *Ae. albopictus*

```
PROC IMPORT OUT= WORK.SurvAlbo
    DATAFILE= "C:\Documents\Data\SurAlbo.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
proc print data=SurvAlbo;
run;
proc sort data=SurvAlbo;
    by sex trt combAC A C blk;
run;

***survival analyses***;
proc sort data=SurvAlbo;
    by trt combAC A C blk;
run;
proc means data=SurvAlbo;
    by trt combAC A C blk;
    var eclosion;
    output out=sura1 n=count nmiss=nmiss;
run;
*Calculating proportion survival for each cohort in each tire;
data surA2;
    set sura1;
    s=count/A;
run;
data surA2;
    set surA2;
    ss = arsin(sqrt(count/A));
proc print data=surA2;
run;
proc mixed data=surA2;
    class trt combAC A C blk;
    model ss = trt|combAC /ddfm=kenwardroger residual;
    random blk;
    LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;
```



```

*survival by sex;
proc sort data=SurvAlbo;
  by sex trt combAC A C blk;
  run;
proc means data=SurvAlbo;
  by sex trt combAC A C blk;
  var eclosion;
  output out=sura1 n=count nmiss=nmiss;
  run;
*Calculating proportion survival for each cohort in each tire;
data surA2;
  set sura1;
  s=count/(A/2);
  if s>1 then s=1;
  run;
data surA2;
  set surA2;
  ss = arsin(sqrt(s));
  proc print data=surA2;
  run;
proc mixed data=surA2;
  by sex;
  class sex trt combAC A C blk;
  model ss = trt|combAC /ddfm=kenwardroger residual;
  random blk;
  LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;

***Development time analyses***;
proc sort data=SurvAlbo;
  by sex trt combAC A C blk;
  run;
proc means data=SurvAlbo;
  by sex trt combAC A C blk;
  var eclosion;
  output out=ecl2 n=count mean=meandays median=mediandays;
  run;
proc mixed data=ecl2;
  by sex;
  class sex trt combAC A C blk;
  model mediandays = trt|combAC /outp=b2;
  random blk;
  LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;
options ps=45;

```

```

proc plot data=b2;
plot resid*pred = trt;
run;

***Body Size***;
proc means data=SurvAlbo;
by trt combAC A C blk;
var wingL;
output out=wingl2 n=count mean=meanwingL median=medianwingL;
run;
proc mixed data=wingl2;
by sex;
class trt combAC A C blk;
model medianwingL = trt|combAC /outp=b2;
random blk;
LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;
options ps=45;
proc plot data=b2;
plot resid*pred = trt;
run;
data lambda;
  set SurvAlbo;
  if sex='M' then delete;
  run;
proc sort data=lambda;
  by trt combAC A C blk eclosion;
  run;

proc means data=lambda;
  by trt combAC A C blk eclosion;
  var wingL;
  output out=lambda1 mean=meanwingL median=medianwingL n=count
nmiss=nmiss;
  run;
  proc print data=lambda1;
  run;
/*calculations of lifetable stats*/;
data lambda2;
  set lambda1;
  ax=count+nmiss;
  fwx= 0.5*(-121.240+78.02*medianwingL);
  lxxmx=ax*fwx;
  xlxxmx=eclosion*ax*fwx;
  run;
/*gets sums*/;

```

```

proc means data=lambda2 noprint;
  by trt combAC A C blk;
  var lxx xlxm;
  output out=lambda3 sum=sumlxm sumxlm;
  run;
data lambda4;
  set lambda3;
  lambda=exp((log((2/A)*sumlxm))/(14+(sumxlm/sumlxm)));
  if sumxlm=. then lambda=0;
  if sumlxm<1 then lambda=0;
  r=log(lambda);
  run;
proc print data=lambda4;
  run;
proc means data=lambda4 n mean stderr;
  by trt combAC;
  var lambda;
  run;
data lambda4;
  set lambda4;
  log10lambda=log10(lambda+1);
  run;
proc print data=lambda4;
  run;
proc mixed data=lambda4;
  class trt combAC A C blk;
  model lambda = trt|combAC /outp=b2;
  random blk;
  LSMEANS trt|combAC /pdiff adjust=sidak;
  run;
  options ps=45;
proc plot data=b2;
  plot resid*pred = trt;
  run;

```

Appendix 8: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) and competition treatments (*Ae. albopictus*: *Cx. pipiens*, 100:0, 50:0, 50:50, 0:100, 0:50) on the per capita rate of population change (λ') and fitness parameters (survivorship, development time, and body size) of *Cx. pipiens* mosquitoes

```

PROC IMPORT OUT= WORK.SurvCxPip
      DATAFILE= "C:\Documents\Data\SurCulex.csv"
      DBMS=CSV REPLACE;
      GETNAMES=YES;
      DATAROW=2;
RUN;
data survcu;
set SurvCxPip;
run;
proc print data=survcu;
run;
proc sort data=survcu;
  by sex trt combAC A C blk;
run;

***survival analyses***;
proc sort data=survcu;
  by trt combAC A C blk;
run;
proc means data=survcu;
  by trt combAC A C blk;
  var eclosion;
  output out=surc1 n=count nmiss=nmiss;
run;
data surC2;
set surc1;
s=count/C;
run;
data surC2;
set surC2;
ss = arsin(sqrt(count/C));
proc print data=surC2;
run;
proc mixed data=surC2;
class trt combAC A C blk;
model ss = trt|combAC /ddfm=kenwardroger residual;
random blk;
LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;

```

```

*survival by sex;
proc sort data=survcu;
  by sex trt combAC A C blk;
run;
proc means data=survcu;
  by sex trt combAC A C blk;
  var eclosion;
  output out=surc1 n=count nmiss=nmiss;
run;
data surC2;
  set surc1;
  s=count/(C/2);
  if s>1 then s=1;
run;
data surC2;
  set surC2;
  ss = arsin(sqrt(s));
proc print data=surC2;
run;
proc mixed data=surC2;
  by sex;
  class sex trt combAC A C blk;
  model ss = trt|combAC /ddfm=kenwardroger residual;
  random blk;
  LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;

***Development time analyses***;
proc sort data=survcu;
  by sex trt combAC A C blk;
run;
proc means data=survcu;
  by sex trt combAC A C blk;
  var eclosion;
  output out=ecl2 n=count mean=meandays median=mediandays;
run;
proc mixed data=ecl2;
  by sex;
  class sex trt combAC A C blk;
  model mediandays = trt|combAC /out=b2;
  random blk;
  LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;
  options ps=45;
proc plot data=b2;
  plot resid*pred = trt;

```

```

run;
***Body Size***;
proc means data=survcu;
  by sex trt combAC A C blk;
  var wingL;
  output out=wingl2 n=count mean=meanwingL median=medianwingL;
run;
proc mixed data=wingl2;
by sex;
class sex trt combAC A C blk;
model medianwingL = trt|combAC /outp=b2;
random blk;
LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;
options ps=45;
proc plot data=b2;
plot resid*pred = trt;
run;

data lambda;
  set survcu;
  if sex='M' then delete;
run;
proc sort data=lambda;
  by trt combAC A C blk eclosion;
run;
/*determines mean wing lengths for each day and counts individuals*/;
proc means data=lambda noprint;
  by trt combAC A C blk eclosion;
  var wingL;
  output out=lambda1 mean=meanwingL median=medianwingL n=count
nmiss=nmiss;
run;
proc print data=lambda1;
run;
/*calculations of lifetable stats*/;
data lambda2;
  set lambda1;
  ax=count+nmiss;
  fwx= 0.5*(-383.82+148.05*medianwingL);
  lxxmx=ax*fwx;
  xlxmx=eclosion*ax*fwx;
run;
proc means data=lambda2 noprint;
  by trt combAC A C blk;
  var lxxmx xlxmx;

```

```

        output out=lambda3 sum=sumlxxmx sumxlxxmx;
    run;
data lambda4;
    set lambda3;
    lambda=exp((log((2/C)*sumlxxmx))/(10+(sumxlxxmx/sumlxxmx)));
    if sumxlxxmx=. then lambda=0;
    if sumlxxmx<1 then lambda=0;
    r=log(lambda);
    run;
proc print data=lambda4;
    run;
proc means data=lambda4 n mean stderr;
    by trt combAC;
    var lambda;
    run;
data lambda4;
    set lambda4;
    log10lambda=log(lambda+1);
    run;

```

*model using randomization - because data violated parametric assumptions;

```

%macro rand_gen(
indata=lambda4,
outdata=outrand,
depvar=lambda,
numreps=1000,
seed=0);
proc sql noprint;
select count(*) into :numrecs from
&INDATA;
quit;
data __temp_1;
retain seed &SEED ; drop seed;
set &INDATA;
do replicate = 1 to &NUMREPS;
call ranuni(seed,rand_dep);
output;
end;
run;
proc sort data=__temp_1;
by replicate rand_dep;
run;
data &OUTDATA ;
array deplist{ &NUMRECS } _temporary_ ;
set &INDATA(in=in_orig)

```

```

__temp_1(drop=rand_dep);
if in_orig then do;
replicate=0;
deplist{ _n_ } = &DEPVAR ;
end;
else &DEPVAR =
deplist{ 1+ mod(_n_,&NUMRECS) };
run;
%mend rand_gen;
%macro rand_anl(
randdata=outrand,
where=,
testprob=probf,
testlabel=F test,);
data _null_;
retain pvalue numsig numtot 0;
set &RANDDDATA end=endofile;
%if "&WHERE" ne ""
%then where &WHERE %str(;) ;
if Replicate=0 then pvalue = &TESTPROB ;
else do;
numtot+1;
numsig + ( &TESTPROB < pvalue );
end;
if endofile then do;
ratio = numsig/numtot;
put "Randomization test for &TESTLABEL "
%if "&WHERE" ne "" %then "where &WHERE";
" has significance level of "
ratio 6.4 ;
end;
run;
%mend rand_anl;
%rand_gen(indata=lambda4,outdata=outrand,
depvar=lambda,numreps=1000,seed=1230568)
ods output OverallANOVA=overall;
/* change model */
proc glm data=outrand outstat=outstat1;
by replicate;
class trt combAC A C blk;
model lambda = blk trt combAC trt*combAC ;
run;
%rand_anl(randdata=outstat1,
where=_source_='trt' and _type_='SS3',
testprob=probf,testlabel=main effect test)
ods output close;

```



```

%rand_ani(randdata=outstat1,
where=_source_='combAC' and _type_='SS3',
testprob=prob,testlabel=main effect test)
ods output close;
%rand_ani(randdata=outstat1,
where=_source_='blk' and _type_='SS3',
testprob=prob,testlabel=main effect test)
ods output close;
%rand_ani(randdata=outstat1,
where=_source_='trt*combAC' and _type_='SS3',
testprob=prob,testlabel=interaction test)
%rand_ani(randdata=outstat1,
run;

***proc mixed random***;
%macro rand_gen(
indata=lambda4,
outdata=outrand,
depvar=lambda,
numreps=1000,
seed=0);
proc sql noprint;
select count(*) into :numrecs from
&INDATA;
quit;
data __temp_1;
retain seed &SEED; drop seed;
set &INDATA;
do replicate = 1 to &NUMREPS;
call ranuni(seed,rand_dep);
output;
end;
run;
proc sort data=__temp_1;
by replicate rand_dep;
run;
data &OUTDATA ;
array deplist{ &NUMRECS } _temporary_ ;
set &INDATA(in=in_orig)
__temp_1(drop=rand_dep);
if in_orig then do;
replicate=0;
deplist{ _n_ } = &DEPVAR ;
end;
else &DEPVAR =
deplist{ 1+ mod(_n_,&NUMRECS) };

```

```

run;
%mend rand_gen;
%macro rand_anl(
randdata=outrand,
where=,
testprob=probf,
testlabel=F test,);
data _null_;
retain pvalue numsig numtot 0;
set &RANDDDATA end=endofile;
%if "&WHERE" ne ""
%then where &WHERE %str(;) ;
if Replicate=0 then pvalue = &TESTPROB ;
else do;
numtot+1;
numsig + ( &TESTPROB < pvalue );
end;
if endofile then do;
ratio = numsig/numtot;
put "Randomization test for &TESTLABEL "
%if "&WHERE" ne "" %then "where &WHERE";
" has significance level of "
ratio 6.4 ;
end;
run;
%mend rand_anl;
%rand_gen(indata=lambda4,outdata=outrand,
depvar=lambda,numreps=1000,seed=1230568)
ods output OverallANOVA=overall;
/* change model */
proc mixed data=outrand outstat=outstat1;
by replicate;
class trt combAC A C blk;
model lambda = trt combAC trt*combAC;
random blk;
run;
%rand_anl(randdata=outstat1,
where=_source_='trt' and _type_='SS3',
testprob=prob,testlabel=main effect test)
ods output close;
%rand_anl(randdata=outstat1,
where=_source_='combAC' and _type_='SS3',
testprob=prob,testlabel=main effect test)
ods output close;
%rand_anl(randdata=outstat1,
where=_source_='blk' and _type_='SS3',

```

```
testprob=prob,testlabel=main effect test)
ods output close;
%rand_anl(randdata=outstat1,
where=_source_='trt*combAC' and _type_='SS3',
testprob=prob,testlabel=interaction test)
%rand_anl(randdata=outstat1,
run
```

References

- Alto, B.W., Lampman, R.L., Kesavaraju, B., and Muturi, E.J. 2013. Pesticide-induced release from competition among competing *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, 50:1240-1249.
- Andrady, A.L., Hamid, H.S., Hu, X., and Torikai, A. 1998. Effects of increased solar ultraviolet radiation on materials. *Journal of Photochemistry and Photobiology*, 46:96-103.
- Benedict, M.Q., Levine, R.S., Hawley, W.A., and Lounibos, L.P. 2007. Spread of the Tiger: Global risk of invasion by the mosquito *Aedes albopictus*. *Vector Borne Zoonotic Diseases*, 7:76-85.
- Blaustein, L. and Chase, J.M. 2007. Interactions between mosquito larvae and species that share the same trophic level. *Annual Review of Entomology*, 52:489-507.
- Carrieri, M., Bacchi, M., Bellini, R., and Maini, S. 2003. On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. *Environmental Entomology*, 32:1313-132.
- Cassell, D.L. 2011. A randomization-test wrapper for SAS PROCs. *Statistics and Data Analysis*, Paper 251-27.
- Center for Disease Control – CDC. 2016. Estimated range of *Aedes albopictus* and *Aedes aegypti* in the United States, 2016. U.S. Department of Health and Human Services, Atlanta, U.S.
- Chase, J.M., and Leibold, M.A. 2003. *Ecological niches: linking classical and contemporary approaches*. University of Chicago Press, Chicago, Illinois
- Chesson, P., and Huntly, N. 1997. The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *American Naturalist*, 150:519-553.
- Chesson, P. 2000. Mechanism of maintenance of species diversity. *Annual Review of Ecology and Systematics*, 31:343-366.
- Costanzo, K.S., Mormann, K., and Juliano, S.A. 2005a. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology*, 42:559-570.
- Costanzo, K.S., Kesavaraju, B., and Juliano, S.A., 2005b. Condition-specific competition in container mosquitoes: the role of noncompeting life-history stages. *Ecology*, 86:3289-3295.

- Costanzo, K.S., Muturi, E.J., Lampman, L.R., and Alto, R.W. 2011. The effects of resource type and ratio on competition with *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology*, 48:29-38.
- Davis, M.A. 2009. *Invasion Biology*. Oxford University Press, UK. 241 pp.
- Day, K.E., Holtze, K.E., Metcalfe-Smith, J.L., Bishop, and C.T., Dutka, B.J. 1993. Toxicity of leachate from automobile tires to aquatic biota. *Chemosphere*, 27:665-675.
- Dehghan, H., Sadraei, J., Moosa-Kasemi, S.H. 2010. The morphological variations of *Culex pipiens* larvae (Diptera: Culicidae) in Yazd Province, Central Iran. *Iran Journal Arthropod-Borne Disease*, 4:42-49.
- Dunson, W.A., and Travis, J. 1991. The role of abiotic factors in community organization. *American Naturalist*, 138:1067-1091.
- Gerhardt, R., Gottfried, K., Apperson, C., Davis B., Erwin, P., Smith A., Panella, N., Powell E., and Nasci, R. 2001. First isolation of LaCrosse virus from naturally infected *Aedes albopictus*. *Emerging Infectious Diseases*, 7:807-811.
- Gualtieri, M., Andrioletti, M., Vismara, C., Milani, M. and Camatini, M. 2005a. Toxicity of tire debris leachates. *Environment International*, 31:723-730.
- Gualtieri, M., Andrioletti, M., Mantecca, P., Vismara, C., and Camatini, M. 2005b. Impact of tire debris on in vitro and in vivo systems. *Particle and Fibre Toxicology*, 2:1-14.
- Hemphill, N. 1991. Disturbance and variation in competition between two stream insects. *Ecology*, 72:864-872.
- Juliano, S.A. 1998. Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? *Ecology*, 79: 255-268.
- Juliano, S.A., O'Meara, G.F., Morrill, J.R., and Cutwa, M.M. 2002. Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. *Oecologia*, 130: 458-469.
- Juliano, S.A., and Lounibos, L.P. 2004. A field test for competitive effects of *Aedes albopictus* on *Ae. aegypti* in South Florida: differences between sites of coexistence and exclusion? *Oecologia*, 139:583-593.
- Juliano, S.A., and Lounibos, L.P. 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecology letters*, 8:558-574.
- Juliano, S.A. 2009. Species interactions among larval mosquitoes: context dependence across habitat gradients. *Annual Review of Entomology*, 54:37-56.

- Kellough, R.M.1991. The effects of scrap automobile tires in water. Report to Ontario Ministry of the Environment. Waste Management Branch, Ontario.
- Kim, H. C., Wilkerson, R., Pecor, J., Lee, W., Lee, J., O'Guinns, M., and Klein, T. 2005. New records and reference collection of mosquitoes (Diptera: Culicidae) on Jeju Island, Republic of Korea. *Entomological Research*, 35:55-56.
- Lane, S.J., and Mahony, M.J. 2002. Larval anurans with synchronous and asynchronous development periods: contrasting responses to water reduction and predator presence. *Journal of Animal Ecology*, 71:780-792.
- Lawton, J.H., and Hassell, M.P. 1981. Asymmetrical competition in insects. *Nature*, 289:793-795.
- Leisnham, P.T., and Juliano, S.A. 2010. Interpopulation differences in competitive effect and response of the mosquito *Aedes aegypti* and resistance to invasion by a superior competitor. *Oecologia*, 164:221-230.
- Leisnham, P.T., LaDeau, S.L., and Juliano, S.A. 2014. Spatial and temporal habitat segregation of mosquitoes in urban Florida. *PLoS ONE*, 9(3): e91655. [doi:10.1371/journal.pone.0091655](https://doi.org/10.1371/journal.pone.0091655)
- Lockwood, J.L., Blackburn, T.M., and Cassey, P. 2009. The more you introduce the more you get: the role of colonization pressure and propagule pressure in invasion ecology. *Diversity and Distributions*, 15:904-910.
- Lounibos, L.P. 2002a. Invasions by insect vectors of human disease. *Annual Review of Entomology*, 47:233-266.
- Lounibos, L.P., Suarez, S., Menendez, Z., Nishimura, N., Escher, R.L., O'Connell, S.M., and Rey, J.R. 2002b. Does temperature affect the outcome of larval competition between *Aedes Aegypti* and *Aedes albopictus*? *Journal of Vector Ecology*, 27:86-95.
- Lounibos, L.P., Bargielowski, I., Carrasquilla, M.C., and Nishimura, N. 2016. Coexistence of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in peninsular Florida two decades after competitive displacements. *Journal of Medical Entomology*, 53:1385-1390.
- Marini, G., Guzzetta, G., Baldacchino, F., Arnoldi, D., Montarsi, F., Capelli, G., Rizzoli, A., Merler, S., and Rosa, R. 2017. The effect of interspecific competition on the temporal dynamics of *Aedes albopictus* and *Culex pipiens*. *Parasites & Vectors*, 10:102 [DOI 10.1186/s13071-017-2041-8](https://doi.org/10.1186/s13071-017-2041-8)
- McCann, S., Day, J.F., Allan, S., and Lord, C.C. 2010. Age modifies the effect of body size on fecundity in *Culex quinquefasciatus* Say (Diptera: Culicidae). *Journal of Vector Ecology*, 34:174-181.

- Merritt, R.W., Dadd, R.H., and Walker, E.D. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual Review of Entomology*, 37:349-376.
- Moller-Jacobs, L.L., Murdock, C.C., and Thomas, M.B. 2014. Capacity of mosquitoes to transmit malaria depends on larval environment. *Parasites & Vectors*, 7:593.
<http://www.parasitesandvectors.com/content/7/1/593>
- Oli, M.K., and Dobson, F.S. 2003. The relative importance of life-history variables to population growth rate in mammals: Cole's prediction revisited. *American Naturalist*, 161:422-440.
- Rizzoli, A., Bolzoni, L., Chadwick, E.A., Capelli, G., Montarsi, F., Grisenti, M., Martinez de la Puente, J., Muñoz, J., Figuerola, J., Soriguer, R., Anfora, G., Di Luca, M., and Rosa, R. 2015. Understanding West Nile virus ecology in Europe: *Culex pipiens* host feeding preference in a hotspot of virus emergence. *Parasites and Vectors*, 8:213. DOI [10.1186/s13071-015-0831-4](https://doi.org/10.1186/s13071-015-0831-4)
- Roux O., Vantaux, A., Roche, B., Yameogo, K.B., Dabire, K.R., Diabate, A., Simard, F., and Lefevre, T. 2015. Evidence for Carry-over effects of predator exposure on pathogen transmission potential. *Proceedings of Biological Science*, 282: 20152430.
<http://dx.doi.org/10.1098/rspb.2015.2430>
- Rubber Manufacturers Association-RMA. 2017. 2015 U.S. scrap tire management summary. Rubber Manufacturers Association, Washington, DC.
- Savage, H. and Miller, B. 1995. House mosquitos of the U.S.A., *Culex pipiens*. *Wing Beats*, 6:8-9.
- Sawabe, K., Isawa, H., Hoshino, K., Sasaki, T., Roychoudhury, S., Higa, Y., Kasai, S., Tsuda, Y., Nishiumi, I., Hisai, N., Hamao, S., and Kobayashi, M. 2010. Host-feeding habits of *Culex pipiens* and *Aedes albopictus* (Diptera: Culicidae) collected at the urban and suburban residential areas of Japan. *Journal of Medical Entomology*, 47:442-450.
- Selbes, M., Yilmaz, O., Khan, A.A., Karanfil, T. 2015. Leaching of DOC, DN, and inorganic constituents from scrap tires. *Chemosphere*, 139:617-623.
- Sprenger, D. and Wuithiranyagool, T. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *Journal of the American Mosquito Control Association*, 2:217-219.
- Sullivan, J.H., Pope, L.C., Sutherland, B.M., Bennett, P.V., Blum, J.E., Stapleton, A.E., and Gitz, D.C. 2010. Assessment of DNA damage as a tool to measure UV-B tolerance in soybean lines differing in flavonoid composition. Book *UV Radiation in global change*, pp 437-457.

Tiawsirisup, S., Platt, K.B., Evans, R.B., and Rowley, W.A. 2005. A comparison of West Nile Virus transmission by *Ochlerotatus trivittatus* (COQ.), *Culex pipiens* (L.), and *Aedes albopictus* (Skuse). *Vector Borne Zoonotic Disease*, 5:40-47.

Tilman, D. 1982. *Resource competition and community structure*. Princeton University Press, Princeton, New Jersey, USA.

United States Environmental Protection Agency (EPA). 1994. Method 200.7, Revision 4.4: Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry. Cincinnati, Ohio.

United States Environmental Protection Agency (EPA). 2007. SW-846 test method 3015A: microwave assisted acid digestion of aqueous samples and extracts.
<https://www.epa.gov/sites/production/files/2015-12/documents/3015a.pdf>

Villena, O.C., Terry, I., Iwata, K., Landa, E.R., LaDeau, S.L., and Leisnham, P.T. 2017. Effects of tire leachate on the invasive mosquito *Aedes albopictus* and the native congener *Aedes triseriatus*. *PeerJ* 5:e3756; [DOI 10.7717/peerj.3756](https://doi.org/10.7717/peerj.3756)

Vinogradova, E.B. 2000. *Culex pipiens pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance, and control. PenSoft, Sofia, Bulgaria. 280 pp.

Vinogradova, E.B. and Karpova S.G. 2006. Effect of photoperiod and temperature on the autogeny rate fecundity and wing length in the urban mosquito, *Culex pipiens f. molestus* (*Diptera: Culicidae*). *International Journal Dipterological Research*, 17:3-12.

Wik, A., and Dave, G. 2005. Environmental labeling of car tires - toxicity to *Daphnia magna* can be used as a screening method. *Chemosphere*, 58:645-651.

World Health Organization-WHO. 2003. *Guidelines for dengue surveillance and mosquito control*. 2nd edition. WHO Regional office for the Western Pacific, Manila, Philippines.

Xue, R., Barnard, D.R., and Schreck, C.E. 1995. Effect of body size, parity, and age of *Aedes albopictus* on human host attack rates and the repellency of deet. *Journal of American Mosquito Control Association*, 11:50-53.

Yee, D.A., Kesavaraju, B., and Juliano, S.A. 2008b. Larval feeding behavior of three co-occurring species of container mosquitoes. *Journal of Vector Ecology*, 29:315-322.

Yee, D.A., Kneitel, J.M., and Juliano, S.A. 2010. Environmental correlates of abundances of mosquito species and stages in discarded vehicle tires. *Journal of Medical Entomology*, 47:53-62.

Chapter 5: Effects of tire pollutants on oviposition of the invasive *Aedes*

***albopictus* mosquito and the native *Culex pipiens* mosquito**

Oswaldo C. Villena^{1,5}, Bahram Momen², Edward Landa², Joseph Sullivan³, Stephanie Yarwood², Paul T. Leisnham⁴

1. Marine Estuarine & Environmental Science, University of Maryland, College Park, MD 20742
2. Environmental Science & Technology, University of Maryland, College Park, MD 20742
3. Plant Science & Landscape Architecture, University of Maryland, College Park, MD 20742
4. Marine Estuarine & Environmental Science and Environmental Science & Technology, University of Maryland, College Park, MD 20742
5. Corresponding author: oswaldo.villena@gmail.com

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Abstract

The selection of an oviposition site is a critical step in the life cycle of mosquitoes because it influences the survival of her offspring, and overall population performance. Tires are common habitats for *Ae. albopictus* and *Cx. pipiens* mosquitoes, especially in peridomestic areas. Our study hypothesis was that tire leachate with high zinc concentration and scarce levels of food resources would be a deterrent to oviposition by gravid females of *Ae. albopictus* and *Cx. pipiens* mosquitoes. We tested this hypothesis by exposing cohorts of 20 *Ae. albopictus* or *Cx. pipiens* females into four oviposition cups assigned either with two different zinc concentrations in tire leachate (high and low) and high and low amounts of food resources (filtered and unfiltered tire leachate samples) in replicated laboratory cages. The number of eggs and hatching percentage were compared between treatments for *Ae. albopictus*, and the number of egg rafts and average number of hatched larvae per egg raft were compared between treatments for *Cx. pipiens*. Our results showed no difference in the number of *Ae. albopictus* numbers of eggs between oviposition cups with high or low zinc concentration, or between the presence or absence of food resources, indicating that tire degradation and tires leachate contaminants do not provide cues. On the other hand, *Cx. pipiens* gravid females choose oviposition sites with high amounts of food resources. But, as with *Ae. albopictus*, *Cx. pipiens* mosquitoes did not show a preference/rejection for oviposition sites with high or low concentration of zinc concentration. These results indicate that neither species alters oviposition based on concentrations of tire contaminants, and thus are likely to engage in larval resource competition in the field. Understanding oviposition behavior of mosquito species could be a key factor to mosquito surveillance and control.

Key words: Tire leachate, food resources, oviposition behaviour, *Ae. albopictus*, *Cx. pipiens*

Introduction

Optimal oviposition theory predicts that female insects prefer to oviposit in locations that maximize offspring performance and reproductive success (Thompson, 1988; Spencer et al., 2002). Oviposition site selection is a response to variation in habitat quality through time and space (Wong et al., 2011; Day, 2016). During oviposition, the quality of the habitat that the females choose for their progeny affects hatching success, larval development and larval success, which could have a significant impact in the overall population growth rate (Spencer et al., 2002; Day, 2016; Yoshioka et al. 2012). Optimal oviposition site selection results in better larval performance and survival. On the other hand, oviposition in poor larval habitats can greatly reduce population growth of a specie (Spencer et al., 2002). In organisms that utilize patchy habitats, such as many amphibians and insects, developmental stages are almost always restricted to the specific site of oviposition (e.g., ephemeral pools, tree holes, tires). Oviposition site selection and larval performance have been well studied in amphibians (Volker et al., 2005; Pintar and Resetarits, 2017), and some insects, especially those that are pests for agriculture crops and forested areas (Martinez et al., 2013; Martinez et al., 2017) and on disease vectors such as mosquitoes (Himeidan et al., 2013; Afify and Galizia, 2015; Day, 2016).

Mosquito oviposition has been widely studied, especially for those species that utilize water-filled container habitats (e.g., treeholes, plant axils, artificial receptacles). Allochontous animal and leaf detritus is the main nutrient base in container habitats (Walker et al. 1991), and mosquito larvae engage in strong competition for microbial food resources associated with this detritus (Juliano, 2009). Mosquito species have shown different oviposition strategies and adaptations to assure good habitats for their offspring.

Skip oviposition strategy is the strategy whereby females scatter an egg batch between different oviposition sites, accounting for quality of the site. This spatial distribution may be a form of bet-hedging to spread the risk among the sites to cope with environmental uncertainty (e.g., lack of flooding) or the eventual case of a catastrophe event (e.g., predator presence, conspecific and heterospecific competition); in this way, it would secure the survival of at least part of the progeny (Roitberg et al., 1999). Some mosquito species are specialized and only lay eggs in specific habitats. For example, *Aedes atropalpus* only oviposit in rock pools, rock holes, and coral holes and *Deinocerites cancer* only oviposit in partially flooded holes of blue land crabs (Day, 2016). Other mosquitoes are more generalist or opportunistic and they lay their eggs in any aquatic habitat, without taking into account quality of the site; a good example is *Culex nigripalpus*, which lays eggs in salt marshes, tree holes, or artificial containers (Day, 2016). Another strategy is egg brooding where females take care of their eggs until they hatch; some examples are *Armigeres flavus* and *Trichoprosopon digitatum* (Lounibos and Machado-Allison, 1983; Okasawa et al., 1991). A case of adaptation to facilitate egg laying is the morphological adaptation of female mosquitoes in which their thorax narrows and allows them to access extremely small holes such as *Aedes angustus* (Day, 2016).

The attractiveness or repellence of oviposition sites for mosquitoes are governed by multiple physical and chemical factors (Himeidan et al., 2013; Afify and Galizia, 2015). Mosquito oviposition requires a complex integration of physical and chemical cues, which can be divided between long and short-range cues (Himeidan et al., 2013). Long-range cues would likely be sensed before physical contact with the site and are more related with macro habitat or landscape factors (Barker et al., 2003). Identification of long range cues

involves vision and olfactory systems of mosquitoes that allow mosquitoes to identify oviposition places based on habitat characteristics (e.g. presence and type of vegetation, water color, shade percentage). Short-range cues are sensed upon contact with the site, and are more related to micro habitat factors (Edgerly et al., 1998; Day, 2016). The sensing of short-range cues involves olfactory, gustatory and tactile mosquito systems to finally make a decision for an oviposition site based on temperature, presence of conspecifics, heterospecifics, or predators, and presence of volatile chemicals associated with vegetation, microbial communities, and predators (Bentley and Day, 1989; Ellis, 2008; Himeidan et al., 2013). To select an oviposition site, gravid female mosquitoes go through a complex evaluation of physical and chemical cues, which may act in two different ways. Some cues exert an attractive effect, whereas cues from unsuitable habitats exert a repulsive effect. The balance of attraction/repulsion effects of these physical and chemical cues probably guides mosquitoes to suitable habitats for their offspring.

Perhaps the most well studied set of environmental cues for mosquito oviposition are substances in container water, such as microbial and nutrient food resources or contaminants, which can be detected by an ovipositing female either in the water column or as volatile chemicals. In particular, numerous studies have shown that the quantity and species of plant detritus can influence mosquito oviposition (Pickett and Woodcock, 1996; Himeidan et al., 2013). For example, infusions of longleaf pine (*Pinus palustris*), water oak (*Quercus nigra*), and 50:50 mixture of these in container water appears to encourage greater oviposition by the Asian tiger mosquito, *Aedes albopictus* compared to St. Augustine grass (*Stenotaphrum secundatum*) infusion or just water alone (Obenauer et al., 2010). On the other hand, ghalisum (*Solenostema argel*), an herbaceous plant from West

Africa, has larvicidal effects against *Culex pipiens* and deters oviposition by adult *Cx. pipiens* females (Al-Doghairi et al., 2004). Other cues are presence or absence of con- and hetero-specific organisms. The presence of conspecifics may indicate suitable habitat for an ovipositing female or, alternatively, potential competitors for her offspring. Conspecific attraction has been observed across many taxa, such as birds, mammals, reptiles, and numerous insects (Stamps, 1988; Mokany and Shine, 2003; Ward and Schlossberg, 2004). Kiflawi et al. (2003) stated that the choice of oviposition site based on conspecifics presence represents a tradeoff between the risk of choosing an unsuitable habitat and the cost of intraspecific competition. However, high densities of conspecifics generate competition, with negative effects on larval survival, adult sizes, fecundity, and overall population performance. Presence of high densities of conspecifics or high levels of conspecifics pheromones could thus become a deterrent or repellent for the oviposition of mosquitoes (Afify and Galizia, 2015). This suggests that some mosquitoes may not only evaluate the presence of conspecifics but also their density (Chadee, 1993). Microbial communities also produce volatiles that serve as semiochemicals and could influence female mosquito oviposition (Himeidan et al., 2013). Microbial communities may serve as a direct source of food or as a modifier of organic matter on which mosquito larvae feed (Navarro et al., 2003). The influence of habitat water quality on female mosquito oviposition has mainly focused on detritus and associated microbial communities. Less research has examined the effects of contaminants in containers, especially those that may be leached from the container receptacle.

Discarded automobile tires are an important artificial habitat in peridomestic areas for the developmental stages for *Ae. albopictus* and *Cx. pipiens* mosquitoes. The Rubber

Manufacturers Association (2017) estimates that each year 280 million scrap tires are generated just in the U.S. and that around 67 million tires are in stockpiles in the U.S. Discarded automobile tires degrade under ultraviolet light and leach numerous soluble metals (e.g., zinc, copper, cadmium) and organic substances (e.g., polyaromatic hydrocarbons, benzothiazoles), which could be bioavailable and persistent in the aquatic environment (Wik and Dave, 2009). Contaminants that leach from tires affect biota including microorganisms, animals, plants, and insects, especially in the aquatic environment (Wik and Dave, 2009). Effects of tire leachate could be direct or indirect effects. Some examples of direct effects would be detrimental effects on growth, survival, weight, and reproduction (Day et al., 1993). Examples of indirect effects would be interference with the outcome of competition between two or more similar species that are competitors, especially if one is more susceptible to specific pollutants (Rohr and Crumrine, 2005). Relatively few if any studies have examined the impacts of tire leachate on the oviposition of mosquito species; previous work has only addressed the effects on insects in direct contact with the leachate in the water column.

Villena et al. 2017 showed that the abundances of *Ae. albopictus*, *Ae. triseriatus*, and *Cx. pipiens* in discarded and stockpiled field tires were all negatively associated with zinc concentration, a common marker for tire leachate. This finding could be the result of two processes. Tire leachate could affect the fitness of the immature mosquitoes developing in tires, either directly through toxicity or indirectly by altering (likely decreasing) available microbial food resources. Alternatively, the negative association between tire leachate and mosquito abundances in tire habitats could be due to ovipositional avoidance of tires with high leachate concentrations by gravid adult females. In the same study,

Villena et al. (2017) found that the survival and per capita rate of population change of larval cohorts of *Ae. albopictus* and *Ae. triseriatus* were negatively affected by increasing tire leachate concentrations in a control laboratory dose-response experiment. Moreover, in Chapters 2-4 in this dissertation, I showed that tires exposed to higher UV-B radiation under experimental conditions leached greater concentrations of zinc and likely other contaminants, and that this leachate had both direct effects on the metabolic rate and fitness of *Ae. albopictus* and *Cx. pipiens* larvae and indirect effects on the population performance of both species by moderating heterospecific and conspecific competition. Of particular importance was the finding that the effects of the competitively superior *Ae. albopictus* on the resident *Cx. pipiens* were moderately reduced in tires that had been exposed to high UV-B radiation and which contained water with higher tire leachate concentrations.

In this chapter, we report the effects of tire leachate with high and low of food resources on mosquito oviposition site selection in a controlled laboratory experiment. I used water from the tires that had been exposed to varying UV-B radiation, as reported in Chapter 2, specifically selecting the tires that exhibited the highest and lowest zinc concentrations from water tests.

Materials and methods

Tire leachate

Tire leachate was obtained from the tires that were exposed to one of three UV-B radiation treatments for 150 days (see chapter 3). I collected tire leachate from the tires with the highest and the lowest zinc concentration. The tire with the highest zinc concentration had a zinc concentration of 4.11 mg/L for dissolved zinc and 6.24 mg/L for total zinc. The tire with the lowest zinc concentration had a zinc concentration of 0.18 mg/L

for dissolved zinc and 0.2 mg/L for total zinc. Discarded tires were of the Goodyear brand, model Assurance (P215/60R16), which were obtained from the Motor Transportation Service of the University of Maryland College Park. To analyze water samples for zinc concentration we followed the U.S. EPA Method 3015A for microwave assisted acid digestion of aqueous samples and extracts (EPA, 2007), and U.S. EPA Method 200.7 for the analysis of water samples using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) with autosampler (EPA, 1994).

Food resources – tire leachate filtration

For the experiment, besides the two different zinc concentrations we had two different food resources conditions (high vs. low). To remove food resources (e.g., bacteria) on which mosquitoes feed, we filtered the tire leachate using a Corning filter system. We used the 250 ml Corning bottle filter with a pore size of 0.22 and the membrane material was cellulose acetate. The Corning bottle filters were sterilized by gamma irradiation. Tire leachate was vacuumed filtered through the in-house vacuum system of the Environmental Science and Technology Department of the University of Maryland College Park. We obtained two liters of sterile tire leachate with high zinc concentration and two liters of sterile tire leachate with low zinc concentration, which were used as the treatment without food resources. To determine that microbial community was removed by the filtration system, I measured the metabolic rate expressed as rate of heat production ($\mu\text{watts/ml}$) of both the filtered and unfiltered samples using a MSC 4100 multicell differential scanning calorimeter in isothermal mode, at 25 °C of temperature (Zhang et al. 2009).

Collection and maintenance of mosquitoes

Aedes albopictus and *Cx. pipiens* larvae were collected from multiple locations in College Park, Baltimore, and Towson, Maryland. Neither *Ae. albopictus* or *Cx. pipiens* are endangered and collection sites were either on publically accessible lands or on private lands where consent for collections were granted at the time of collection; thus, no field permits were required to collect them. Field collected *Ae. albopictus* and *Cx. pipiens* larvae were reared to adulthood at 25°C at 16:8 (L:D) h photoperiod, and then released into 1-m² single-species cages. Adults were kept in an insectary at 25°C and >85% RH, 16:8 (L:D) h photoperiod. Both colonies were supplied 20% sugar solution. Females from both colonies were fed horse or rooster blood once a week via an artificial feeder (Hemotek, Accrington, UK) to ensure egg production and the production of mosquitoes for the colony. *Aedes albopictus* females oviposited on seed paper in 500 ml black cups covered filled with 200 ml of deionized (DI) water and *Cx. pipiens* oviposited egg rafts into a 500 ml black bowl filled with 400 ml of DI water, supplemented with 3 or 4 pieces of foxtail grass as an oviposition stimulant.

Experiment set up

The experiment design was a 2-factor Randomize Complete Block Design. To assess the effect of tire leachate zinc concentration (high and low) and absence and presence of food resources (filtered and unfiltered) on *Ae. albopictus* and *Cx. pipiens* mosquito oviposition and the hatching of eggs, per block, I placed 6 bug dorm insect rearing cages inside a 3 level Percival environmental chamber model I-36LLVL. In the Percival environmental chamber humidity was set up to 85 – 92 %, temperature to 25°C,

and a photoperiod 16:8 hours (L:D). Bug dorms are built on polypropylene, with dimensions L30 x W30 x H30 cm. The mesh size of its side panels is 32 x 20 mesh/square inch. Two bug dorm insect rearing cages were placed per each of the three levels of the Percival environmental chamber, one with *Ae. albopictus* mosquitoes and the other with *Cx. pipiens* mosquitoes. Inside of each bug dorm, we set up four oviposition cups, each with one of four treatments: (1) filtered and high zinc concentration leachate, (2) unfiltered and high zinc concentration leachate, (3) filtered and low zinc concentration leachate, and (4) unfiltered and low zinc concentration leachate. Oviposition cups were built using 16 oz plastic food containers, which outside was painted with black paint. Oviposition cups were sterilized with ethanol before being filled with 180 ml of tire leachate from one of the four treatments.

Of the six bug dorm cages, each with four oviposition cups, randomly three were designed for *Ae. albopictus* and three for *Cx. pipiens* oviposition experiment. The oviposition cups placed in the cages for *Ae. albopictus* oviposition were lined with seed paper to facilitate oviposition. In the oviposition cups designated for *Cx. pipiens*, 0.1 g of foxtail grass, previously rinsed with sterile water, was added as a stimulant for oviposition. Once bug dorms cages were set up with the oviposition cups, *Ae. albopictus* and *Cx. pipiens* colonies were blood-fed with an artificial feeder (Hemotek, Accrington, UK), using rooster blood for a period of 4 hours. Next, using a mouth operated pooter aspirator we collected blood fed females from the colonies and placed them in the bug dorm cages. 20 blood-fed females were placed in each of the bug dorm cages. We also placed in each bug dorm cage 5 male mosquitos to guaranty the mating and production of eggs. Mosquitoes in the bug dorm cages were blood fed on day 3 after being placed in the bug dorm cage with an

artificial feeder (Hemotek, Accrington, UK), using rooster blood for a period of 4 hours. Also, mosquitoes were provided with sugared water (20 % v/v) during the experiment. Females were permitted to oviposit in the plastic cups for 7 days. For *Ae. albopictus*, at the end of the experiment seed germination papers were collected and eggs were counted using a dissecting microscope and recorded onto a spreadsheet. Then egg papers were dried and placed in sealable plastic bags for 3 days. Next, egg papers were hatched in a lactobumina: yeast solution and larvae were counted and recorded on a spreadsheet. For *Cx. pipiens*, every two days plastic cups were checked for egg rafts, if egg rafts were present they were removed and hatched in a lactobumina: yeast solution and larvae were counted and recorded on a spreadsheet. At the end of the experiment, cups were checked for larvae presence; if larvae were found they were added to the previous counting.

Analyses

Two-way analyses of variance (ANOVA) were used to measure the effects of tire leachate concentration and presence/absence of food resources on the oviposition site selection of *Aedes albopictus* and *Culex pipiens* mosquitoes, and to measure also the differences in metabolic rate for the filtered and unfiltered samples to assure that the microbial community had been removed from tire leachate samples (PROC MIXED procedure, SAS 9.4 Software). Number of eggs and percentage of hatched larvae were the measured variables for *Ae. albopictus* and number of egg rafts and number of larvae per egg raft for *Cx. pipiens*. To account for assumptions of normality and homogeneity of variances, all oviposition data and metabolic rate data were $\log_{10}(y+1)$ transformed. In all these analyses, we considered tire leachate concentration and presence/absence of food resources as fixed effects, and block as a random effect in the model. We did a pairwise

mean comparison in the mixed procedure using the LSMEANS statement with tukey adjustment. For all analyses experiment-wise $\alpha = 0.05$.

Results

Metabolic rate of microbial community in tire leachate samples

The metabolic rate of the microbial community in tire leachate samples was only affected by amount of food resources (filtered and unfiltered), but it was not affected by tire leachate concentration or the interaction of these two variables. After measuring metabolic rate expressed as rate of heat production (μ watts/ml), filtered tire leachate samples showed a statistically significantly lower metabolic rate compared to unfiltered samples ($F_{1,3} = 3980.96$, $P < 0.0001$; Figure 11). Filtered and unfiltered tire leachate samples with high Zn concentration had an average metabolic rate of 0.279 and 5.840 μ watts/ml respectively, and filtered and unfiltered tire leachate samples with low Zn concentration had an average metabolic rate of 0.341 and 6.295 μ watts/ml respectively.

Aedes albopictus oviposition behaviour

Aedes albopictus females did not show a preference for laying eggs between sites with high and low level of pollutants ($F_{1,3} = 0.08$, $P = 0.7990$; Table 8) or sites with presence or absence of food resources ($F_{1,3} = 1.46$, $P = 0.3133$; Table 8). *Aedes albopictus* females laid in average 56 eggs in samples with high concentration of pollutants and low levels of food resources, 80 eggs in samples with high concentration of pollutants and high levels of food resources, 75 eggs in samples with low concentration of pollutants and low levels of food resources, and 87 eggs in samples with low concentration of pollutants and high levels of food resources (Figure 12A). Percentage of larvae hatched from eggs laid by *Ae. albopictus* females was not statistically different between treatments (table 8), but a lower percentage

of larvae hatched in the low concentration of pollutants and low concentration of food resources (Figure 12B).

***Culex pipiens* oviposition behaviour**

In the case of *Cx. pipiens*, females showed a statistically significant difference for the choice of oviposition site based on the presence/absence of food resources ($F_{1,3}=35.90$, $P = 0.0093$; Table 9), but *Cx pipiens* females did not show a statistically significant difference in choice of oviposition site between sites with high or low concentrations of tire pollutants ($F_{1,3}=1.39$, $P=0.3239$; Table 9). Gravid *Cx pipiens* laid five times as many egg rafts in oviposition cups with high levels of food resources compared to oviposition cups with low food resources. *Culex pipiens* females laid an average of 0.5 egg rafts in ovicups with high levels of pollutants and low levels of food resources, 3.66 egg rafts in ovicups with high levels of pollutants and high levels of food resources, 0.83 egg rafts in ovicups with low levels of pollutants and low levels of food resources, and 4.66 egg rafts in ovicups with low levels of pollutants and high levels of food resources (Figure 13A). The number of larvae per egg raft was greatly influenced by the presence of food resources in the ovicups ($F_{1,3}=19.10$, $P=0.0222$; Figure 13B).

Discussion

Oviposition is a critical step in the reproduction process, and oviposition site selection plays a major role in egg and larvae survival and overall population growth. Oviposition site selection may be more critical when two species share the same geographical distribution, share habitat types, and are asymmetrical competitors, which is the case for *Ae. albopictus* and *Cx. pipiens* mosquitoes, where *Ae. albopictus* is a superior competitor (Carrieri et al., 2003; Constanzo et al., 2011). Oviposition site selection may

play a major role in the survival of the inferior competitor and make its coexistence possible with the superior competitor. Mosquito oviposition processes have developed through evolution into two well defined groups. One group, the specialists, only lay eggs in sites with specific physical and chemical characteristics; and the other group, the generalists, lay eggs in almost any aquatic habitat (Day, 2016). In this study, we assessed the effect of high and low zinc concentrations, as an indicator of presence of pollutants, in tire leachate, and the presence/absence of food resources in tire leachate (filtered and unfiltered samples).

Our results showed that gravid *Ae. albopictus* mosquitoes showed generalist oviposition behavior when choosing oviposition sites. In our study, *Ae. albopictus* did not show a preference for laying eggs between sites with high and low level of pollutants or sites with presence or absence of food resources. Also, the percentage of larvae hatched from eggs laid by *Ae. albopictus* females was not statistically different between treatments. Our results differ from those of Obenauer et al. (2010) that stated that *Ae. albopictus* showed skip oviposition or bet hedging oviposition behavior. In their study, they tested 6 different infusions and *Ae. albopictus* deposited higher numbers of eggs on longleaf pine, water oak-longleaf pine, water oak, longleaf pine-St. Augustine grass, and St. Augustine grass-water oak infusions compared to just St. Augustine grass infusion or well water. Probably St. Augustine grass and well water are not optimal for determining whether *Ae. albopictus* exhibits skip oviposition behavior. St. Augustine grass is considered a pasture or turf grass and probably is not a common constituent of container habitats where *Ae. albopictus* mosquitoes breed. If we remove St. Augustine grass and well water from their study, *Ae. albopictus* only shows a generalist behavior that chooses oviposition sites in a

random way. The study of Allen and Kline (1995) supports our results that *Ae. albopictus* is a generalist at the moment of selecting an oviposition site. They assessed if the presence of conspecifics had an effect at the moment of choosing an oviposition site for *Ae. albopictus* and *Ae. aegypti*. They found that gravid *Ae. aegypti* females laid more eggs in ovicups in the presence of conspecifics or *Ae. albopictus* eggs. But this is not the case for *Ae. albopictus*, which laid eggs regardless of the presence or absence of conspecifics or eggs of *Ae. aegypti*. Chadee (1993) even found that *Ae. aegypti* gravid females lay more eggs in sites with fewer than 25 eggs than in sites with more than 25 eggs of conspecifics. The presence of eggs or larvae from conspecifics or other mosquito species may be an indicator of a productive oviposition site, but the presence of too many eggs or larvae may be an indicator of high levels of competition for food resources for the coming generation.

In contrast to *Ae. albopictus*, gravid *Cx. pipiens* mosquitoes seem to be specialists at the moment of selecting an oviposition site. In ovicups with high zinc concentrations and presence of food resources *Cx. pipiens* mosquitoes lay 7.32 times more egg rafts and yield 5.22 times more larvae per egg raft compared with sites with no food resources. In a similar way, in ovicups with low zinc concentrations and presence of food resources *Cx. pipiens* mosquitoes lay 5.61 times more egg rafts and yield 3 times more larvae per egg raft compared to ovicups with no food resources. Our results showed that the *Cx. pipiens* mosquitoes search for oviposition site is not random but rather a complex process where it probably makes use of long and short range cues to approach a potential oviposition site.

Based on this study and other studies such as the one by Allen and Kline (1995) it seems that *Ae. albopictus* is a generalist at the moment of choosing an oviposition site and that it lays eggs in any aquatic environment regardless of quality. It is probable that *Ae.*

albopictus does not spend energy looking for oviposition sites with specific chemical and physical characteristics; instead it trusts its superior competitiveness against other mosquito species to assure the survival of its progeny. Numerous studies have shown that in almost all conditions *Ae. albopictus* is a superior competitor over other mosquito species, including *Cx. pipiens* (Carrieri et al., 2003; Constanzo et al., 2005; Constanzo et al., 2011), *Ae. aegypti* (e.g., Murrell and Juliano, 2008; Alto et al., 2013), *Ae. japonicus* (Freed and Leisnham, 2014), *Ae. triseriatus* (Smith et al., 2013), and *Ae. sirrensis* (Kesavaraju et al., 2014). On the other hand, *Cx. pipiens*, an inferior competitor, shows specialist behavior at the moment of looking for an oviposition site, searching for sites with high amounts of food resources that could guaranty the survival of its progeny.

When searching for oviposition sites, mosquitoes look for cues, which could be one or more of three different types of substances that have an effect on gravid female mosquitoes: attractants, repellents, and deterrents. Our results showed that gravid females of both *Ae. albopictus* and *Cx. pipiens* mosquitoes laid eggs on tire leachate regardless of high or low concentration of zinc. Tire leachate seems to be acting as an attractant to gravid females of both species, *Ae. albopictus* and *Cx. pipiens*, to lay eggs. The study of Allgood (2011) also found that gravid females of *Ae. albopictus* and *Cx. quinquefasciatus* laid significantly more eggs in tires with higher pollutants concentration.

Understanding oviposition behavior of mosquito species could be a key factor to mosquito surveillance and control. Knowledge of this critical behaviour could allow for predictions about where mosquitoes are more likely to breed. Also, knowledge about what substances or compounds attract gravid female mosquitoes could be used to bait gravid mosquito traps and oviposition traps, and become an important tool in mosquito control.

Future research should focus on testing individual tire leachate compounds to determine which specific compounds have an attractant effect on gravid female mosquitoes and use this in mosquito traps to help control mosquito populations.

List of tables and figures

Tables

Table 8. Two-way ANOVA of the effects of leachate concentrations (high and low) and the amount of food resources (filtered and unfiltered) for the number of laid eggs, number of hatched larvae, and percentage of hatching for *Ae. albopictus* mosquitoes.

Variable	Number of eggs			Number Hatched Larvae			% Hatching		
	dfs	F	P	dfs	F	P	dfs	F	P
Leachate concentration	1,3	0.08	0.7990	1,3	0.27	0.6406	1,3	6.91	0.0784
Food resources	1,3	1.46	0.3133	1,3	0.46	0.5482	1,3	1.04	0.3828
Lechate conc x Food resources	1,3	0.11	0.7660	1,3	0.43	0.5595	1,3	3.07	0.1782

Table 9. Two-way ANOVA of the effects of leachate concentrations (high and low) and the amount of food resources (filtered and unfiltered) for the number of egg rafts, number of hatched larvae, and number of hatched larvae per egg raft for *Cx. pipiens* mosquitoes.

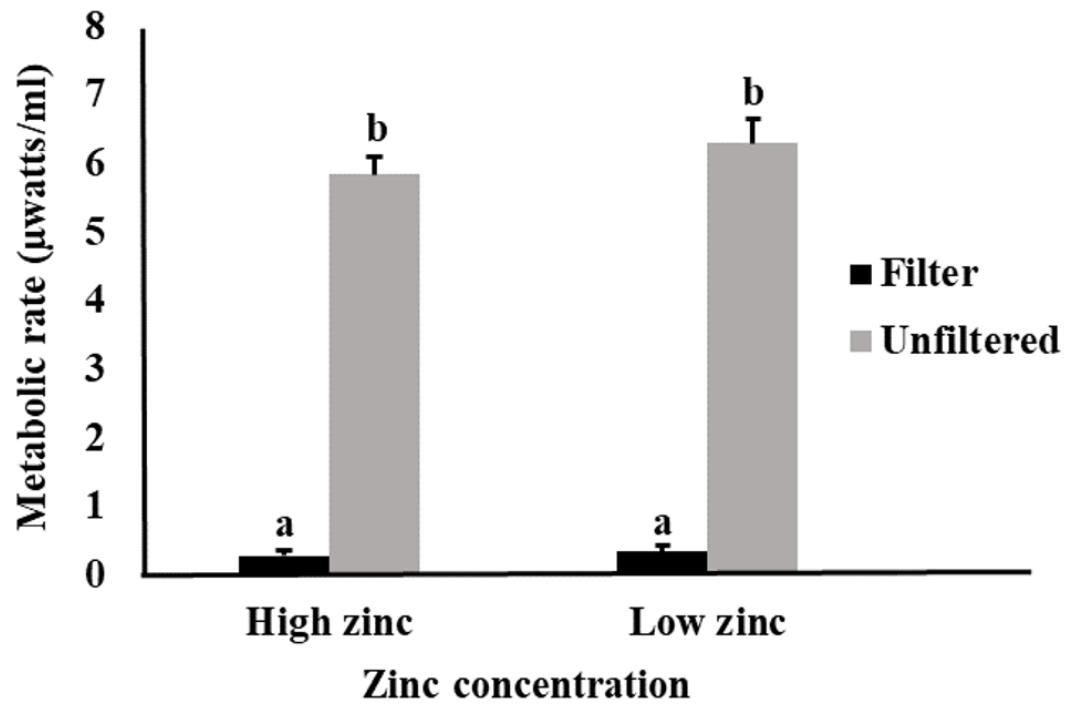
Variable	Number of egg rafts			Number Hatched Larvae			Hatched larvae/egg raft		
	dfs	F	P	dfs	F	P	dfs	F	P
Leachate concentration	1,3	1.39	0.3239	1,3	1.86	0.2664	1,3	1.84	0.2679
Food resources	1,3	35.90	0.0093	1,3	28.96	0.0126	1,3	19.10	0.0222
Lechate conc x Food resources	1,3	0.04	0.8489	1,3	0.001	0.9756	1,3	0.001	0.9856

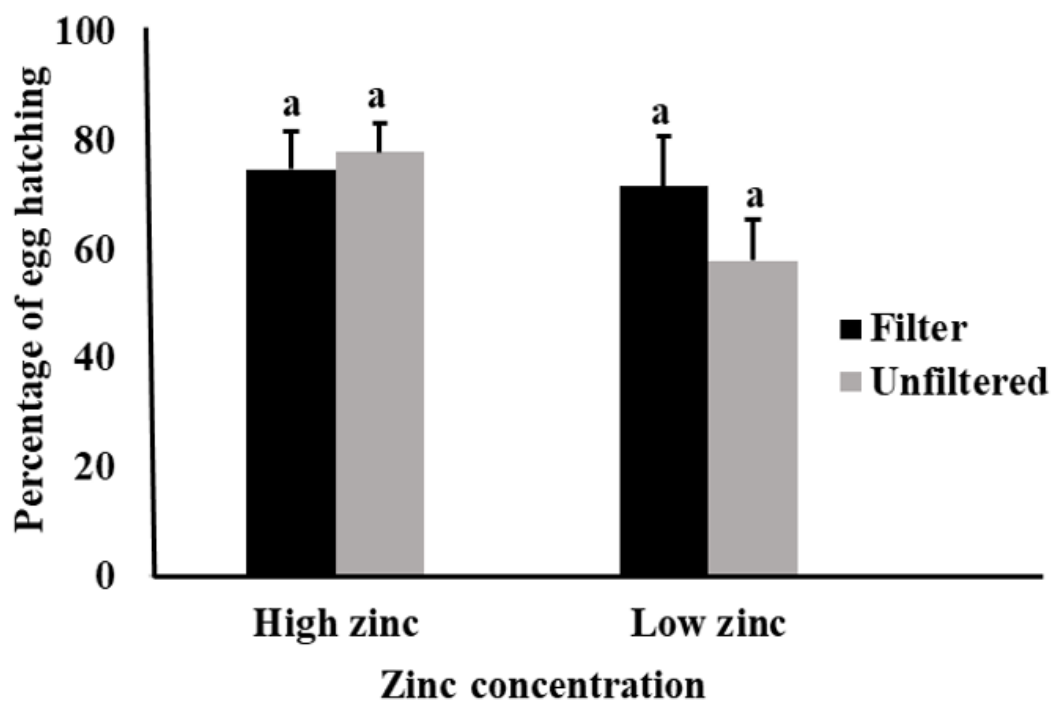
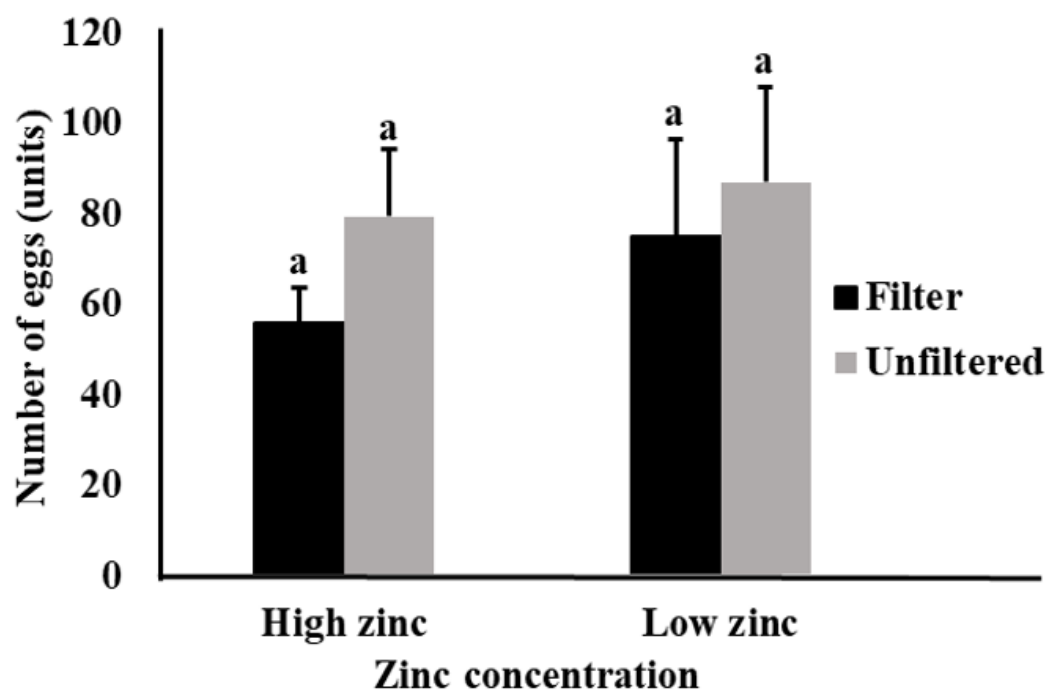
Figures

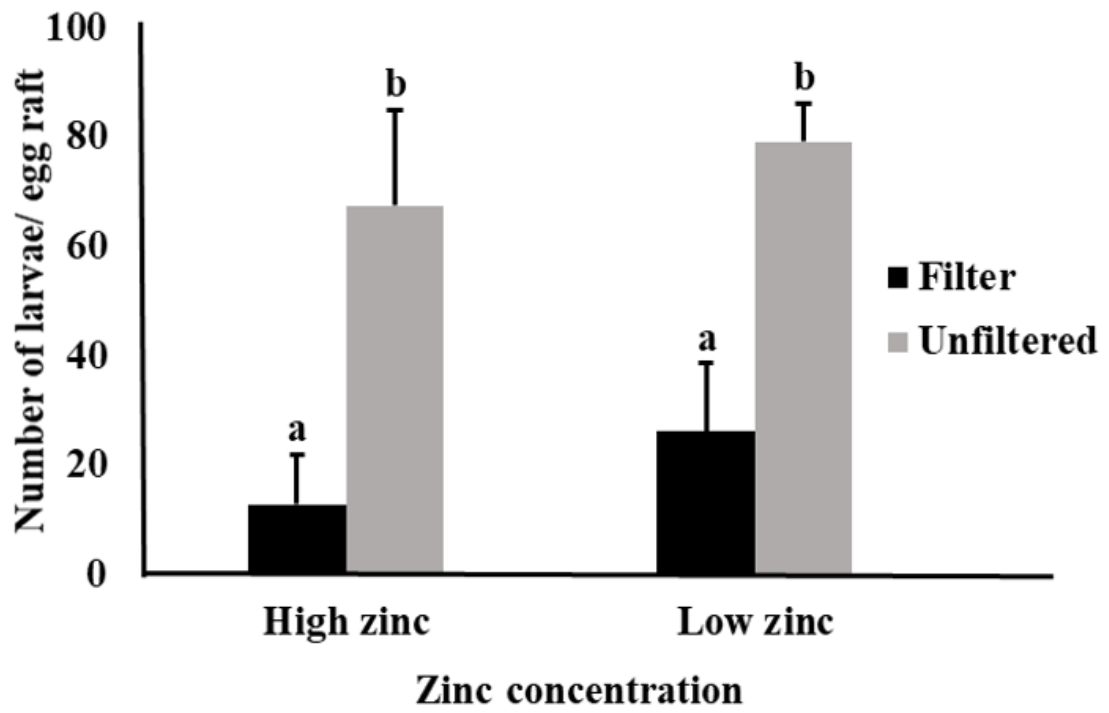
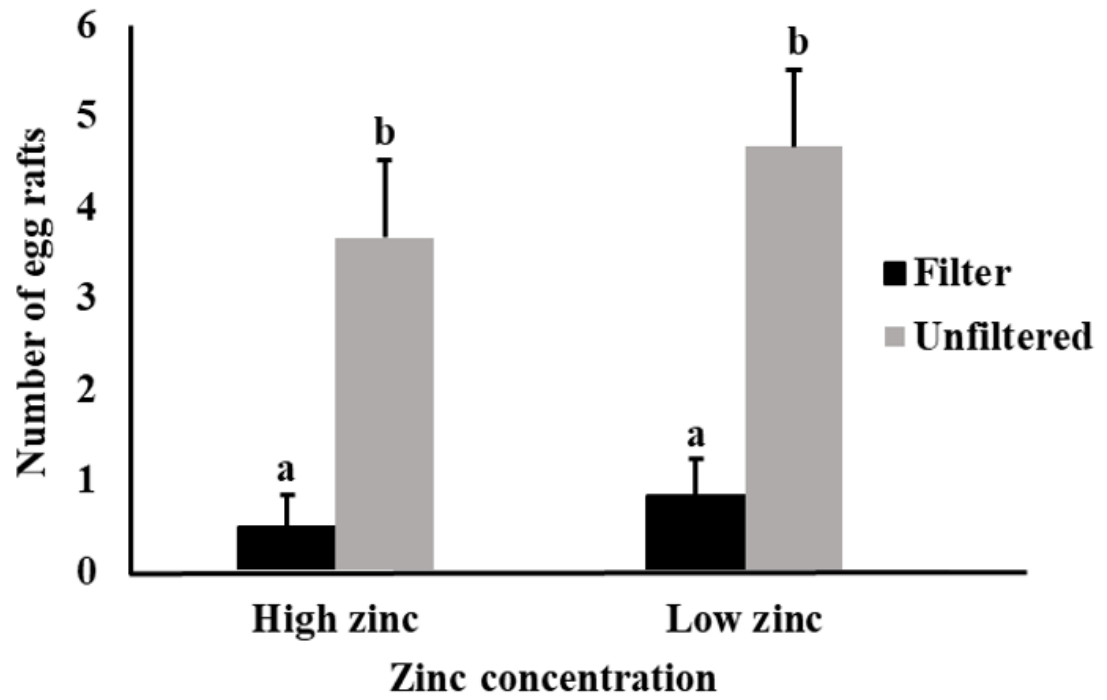
Figure 11. Least squares means (\pm SE) of microbial metabolic rate expressed as μ watts/ml for filtered and unfiltered tire leachate samples with high and low zinc concentrations. Data were statistically tested using ANOVA. Significant pairwise comparisons among treatment levels are indicated by different letters above bars.

Figure 12. Least squares means (\pm SE) for (a) number of eggs, (b) number of hatched larvae, and (c) percentage of hatching by tire leachate concentration (high and low), and two levels of food resources (filtered and unfiltered) for *Ae. albopictus* mosquitoes.

Figure 13. Least squares means (\pm SE) for (a) number of eggs rafts and (b) number of larvae per egg raft for *Cx. pipiens* mosquitoes exposed to two levels of tire leachate concentration (high and low), and two levels of food resources (filtered and unfiltered). Significant pairwise comparisons among treatment levels are indicated by different letters above bars.







Appendices

Appendix 9: SAS code to assess the amount levels of food resources (filter and unfiltered) in tire leachate with high and low zinc concentrations.

```
PROC IMPORT OUT= WORK.metrate
    DATAFILE= "C:\Documents\Data\metab rate.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
proc print data=metrate;
run;
data b;
set metrate;
metrate=log10(metrate+1);
run;
proc print data=b;
run;
proc sort data=b;
key blk LeachateConc FoodRes/ascending;
run;
proc print data=b;
run;
proc means data=b;
var metrate;
by blk LeachateConc FoodRes;
output out=a mean=metrate;
run;
proc print data=a;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model metrate= LeachateConc|FoodRes/residuals ddfm=sat;
random blk;
run;
```

Appendix 10: SAS code to assess the effects of zinc concentration (high and low) and amount of food resources (filter and unfiltered) on the oviposition behavior of *Ae. albopictus* mosquitoes

```
PROC IMPORT OUT= WORK.albovip
    DATAFILE= "C:\Documents\Data\AlboOvip.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
proc print data=albovip;
run;
data e;
set albovip;
NumEggs=log10(NumberEggs+1);
totalLarvae=log10(HatchedLarvae+1);
PercHatch=log10(PercHatching+1);
run;
proc sort data=e;
key LeachateConc FoodRes blk/ascending;
run;
proc means data=e n mean stderr;
var NumEggs totalLarvae PercHatch;
by LeachateConc FoodRes blk;
output out=a mean= NumEggs totalLarvae PercHatch;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model NumEggs = LeachateConc|FoodRes / residual ddfm=kr;
random blk;
lsmeans LeachateConc FoodRes LeachateConc*FoodRes / adjust=tukey;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model totalLarvae = LeachateConc|FoodRes / residual ddfm=kr;
random blk;
lsmeans LeachateConc FoodRes LeachateConc*FoodRes / adjust=tukey;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model PercHatch = LeachateConc|FoodRes / residual ddfm=kr;
random blk;
lsmeans LeachateConc FoodRes LeachateConc*FoodRes / adjust=tukey;
run;
```

Appendix 11: SAS code to assess the effects of zinc concentration (high and low) and amount of food resources (filter and unfiltered) on the oviposition behavior of *Cx. pipiens* mosquitoes

```
PROC IMPORT OUT= WORK.culexovip
    DATAFILE= "C:\Documents\Data\CulexOvip.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
proc print data=culexovip;
run;
data d;
set culexovip;
eggRaft=log10(eggrafts+1);
totalLarvae=log10(total_larvae+1);
larvaeRaft=log10(larvae_per_raft+1);
run;
proc sort data=d;
by LeachateConc FoodRes blk;
run;
proc means data=d n mean stderr;
var eggRaft totalLarvae larvaeRaft;
by LeachateConc FoodRes blk;
output out=a mean= eggRaft totalLarvae larvaeRaft;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model eggRaft = LeachateConc|FoodRes / residual;
random blk;
lsmeans LeachateConc FoodRes LeachateConc*FoodRes / adjust=tukey;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model totalLarvae = LeachateConc|FoodRes / residual;
random blk;
lsmeans LeachateConc FoodRes LeachateConc*FoodRes / adjust=tukey;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model larvaeRaft = LeachateConc|FoodRes / residual;
random blk;
lsmeans LeachateConc FoodRes LeachateConc*FoodRes / adjust=tukey;
run;
```

References

- Afify A., and Galizia, G. 2015. Chemosensory cues for mosquito oviposition site selection. *Journal of Medical Entomology*, 52:120-130.
- Al-Doghairi, M., El-Nadi, A., Elhag E., and Al-Ayedh, H. 2004. Effect of *Solenostemma argel* on oviposition, egg hatchability and viability of *Culex pipiens L.* larvae. *Phytotherapy Research*, 18:335-338.
- Allan, S., and Kline, D. 1995. Evaluation of organic infusions and synthetic compounds mediating oviposition in *Aedes albopictus* and *Aedes aegypti*. *J. Chem. Ecol.*, 21:1847-1860.
- Allgood, D.W. 2011. Influence of detritus levels and organic pollution on interspecific resource competition, oviposition behavior, and larval survival of two tire-inhabiting mosquito species (*Diptera: Culicidae*). Master's thesis. 242. http://aquila.usm.edu/masters_theses/242/
- Alto, B.W., Lampman, R.L., Kesavaraju, B., and Muturi, E.J. 2013. Pesticide-induced release from competition among competing *Aedes aegypti* and *Aedes albopictus* (*Diptera: Culicidae*). *Journal of Medical Entomology*, 50:1240-1249.
- Barker, C.M., Brewster, C.C., and Paulson, S.L. 2003. Spatiotemporal oviposition and habitat preferences of *Ochlerotatus triseriatus* and *Aedes albopictus* in an emerging focus of La Crosse virus. *Journal of the American Mosquito Control Association*, 19:382-391.
- Bentley, M.D., and Day, J.F. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. *Annual Review of Entomology*, 34:401-421.
- Carrieri, M., Bacchi, M., Bellini, R., and Maini, S. 2003. On the competition occurring between *Aedes albopictus* and *Culex pipiens* (*Diptera: Culicidae*) in Italy. *Environmental Entomology*, 32:1313-132.
- Chadee, D.D. 1993. Oviposition response of *Aedes aegypti* (L.) to the presence of conspecifics eggs in the field in Trinidad, W.I. *J. Fla. Mosq. Control Assoc.* 64:63-66.
- Costanzo, K.S., Mormann, K., and Juliano, S.A. 2005. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (*Diptera: Culicidae*). *Journal of Medical Entomology*, 42:559-570.
- Costanzo, K.S., Muturi, E.J., Lampman, L.R., and Alto, R.W. 2011. The effects of resource type and ratio on competition with *Aedes albopictus* and *Culex pipiens* (*Diptera: Culicidae*). *Journal of Medical Entomology*, 48:29-38.
- Day, K.E., Holtze, K.E., Metcalfe-Smith, J.L., Bishop, C.T., and Dutka, B.J. 1993. Toxicity of leachate from automobile tires to aquatic biota. *Chemosphere*, 27:665-675.

- Day, J.F. 2016. Mosquito oviposition behavior and vector control. *Insects*, 7, 65; [doi:10.3390/insects7040065](https://doi.org/10.3390/insects7040065).
- Edgerly, J.S., McFarland, M., Morgan, P., and Livdahl, T. 1998. A seasonal shift in egg-laying behaviour in response to cues of future competition in a treehole mosquito. *Journal of Animal Ecology*, 67:805-818.
- Ellis, A. 2008. Incorporating density dependence into the oviposition preference-offspring performance hypothesis. *Journal of Animal Ecology*, 77:247-256.
- Freed, T.Z., and Leisnham, P.T. 2014. Roles of spatial partitioning, competition, and predation in the North American invasion of an exotic mosquito. *Oecologia*, 175:601-611.
- Himeidan, Y.E., Temu E.A., ElRayah E.A., Munga, S., and Kweka, E. 2013. Chemical cues for Malaria vectors oviposition site selection: challenges and opportunities. *Journal of insects*, 2013:1-9.
- Juliano, S.A. 2009. Species interactions among larval mosquitoes: Context dependence across habitat gradients. *Annual Review of Entomology*, 54:37-56.
- Kesavaraju, B., Leisnham, P.T., Keane, S., Delisi, N., and Pozatti, R. 2014. Interspecific competition between *Aedes albopictus* and *A. sierrensis*: Potential for competitive displacement in the Western United States. *PLoS ONE*, 9(2): eB9698. [doi:10.1371/journal.pone.0089698](https://doi.org/10.1371/journal.pone.0089698)
- Kiflawi, M., Blaustein, L., and Mangel, M. 2003. Oviposition habitat selection by the mosquito *Culiseta longiareolata* in response to risk of predation and conspecific larval density. *Ecological Entomology*, 28:168-173.
- Lounibos, L.P., and Machado-Allison C.E. 1983. Oviposition and egg brooding by the mosquito *Trichoprosopon digitatum* in cacao husks. *Ecological Entomology*, 8:475-478.
- Martinez, G., Soler, R., and Dicke, M. 2013. Chapter four – behavioral ecology of oviposition-site selection in herbivorous true bugs. *Advances in the Study of Behavior*, 45:175-207.
- Martinez, G., Finozzi, M.V., Cantero, G., Soler, R., Dicke, M., and Gonzalez, A. 2017. Oviposition preference but not adult feeding preference matches with offspring performance in the bronze bug *Thaumastocoris peregrinus*. *Entomologia experimentalis et applicata*, 163:101-111.
- Mokany, R., and Shine, R. 2003. Oviposition site selection by mosquitoes is affected by cues from conspecific larvae and anuran tadpoles. *Austral Ecology*, 28:33-37.

- Murrel, E.G., and Juliano S.A. 2008. The role of detritus type in interspecific competition and population distributions of *Aedes aegypti* and *Aedes albopictus*. *Journal of Medical Entomology*, 45:375-383.
- Navarro, D.M., De Oliveira, P.E., Potting, R.P., Brito, A.C., Fital, S.J., and Goulart Sant'Ana, A.E. 2003. The potential attractant or repellent effects of different water types on oviposition in *Aedes aegypti* L. (*Dipt.*, *Culicidae*). *Journal of Applied Entomology*, 127:46-50.
- Obenauer, P.J., Allan, S.A., and Kaufman, P.E. 2010. *Aedes albopictus* (*Diptera: Culicidae*) oviposition response to organic infusions from common flora of suburban Florida. *Journal of Vector Ecology*, 35:301-306
- Okasawa, T., Miyagi, I., Toma, T., Ramalingam, S., and Chang, M.S. 1991. Egg morphology and observations on the laboratory biology of *Armigeres (Leicesteria) digitalis* (*Diptera: Culicidae*) from Sarawak. *Journal of Medical Entomology*, 28:606-610.
- Pickett, J.A., and Woodcock. 1996. The role of mosquito olfaction in oviposition site location and in the avoidance of unsuitable host. In Bock, G.R., & Cardew, G (Eds.), *Olfaction in mosquito-host interactions* (1st edition, 109-123). New York, NY: John Wiley & Sons Ltd.
- Pintar, M.R., and Resetarits, W.J. 2017. Out with the Old, in with the New: Oviposition preference matches larval success in Cope's Gray Treefrog, *Hyla chrysoscelis*. *Journal of Herpetology*, 51: 186-189.
- Rohr, J.R., and Crumrine, P.W. 2005. Effects of an herbicide and an insecticide on pond community structure and processes. *Ecological Applications*, 15:1135-1147.
- Roitberg, B.D., Robertson, I.C., and Tyerman, J.G.A. 1999. Vive la variance: a functional oviposition theory for insect herbivores. *Entomologia Experimentalis et Applicata*, 91:187-194.
- Rubber Manufacturers Association-RMA. 2017. 2015 U.S. scrap tire management summary. Rubber Manufacturers Association, Washington, DC.
- SAS Institute Inc. 2013. Base SAS 9.4 Procedure Guide: Statistical Procedures, Second Edition. Cary, NC: SAS Institute Inc.
- Smith, C., Baldwin, A.H., Sullivan, J., and Leishnam, P.T. 2013. Effects of Elevated Atmospheric CO₂ on Competition between the Mosquitoes *Aedes albopictus* and *Ae. triseriatus* via Changes in Litter Quality and Production. *Journal of Medical Entomology*, 50:521-532.

- Spencer, M., Blaustein, L., and Cohen, J.E. 2002. Oviposition habitat selection by mosquitoes (*Culiseta longiareolata*) and consequences for population size. *Ecology*, 83:669-679.
- Stamps, J.A. 1988. Conspecific attraction and aggregation in territorial species. *American Naturalist*, 131:329-347.
- Thompson, J.N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata*, 47:3-14.
- United States Environmental Protection Agency (EPA). 1994. Method 200.7, Revision 4.4: Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry. Cincinnati, Ohio.
- United States Environmental Protection Agency (EPA). 2007. SW-846 test method 3015A: microwave assisted acid digestion of aqueous samples and extracts.
<https://www.epa.gov/sites/production/files/2015-12/documents/3015a.pdf>
- Villena, O.C., Terry, I., Iwata, K., Landa, E.R., LaDeau, S., and Leisnham, P.T. 2017. Effects of tire leachate on the invasive mosquito *Aedes albopictus* and the native congener *Aedes triseriatus*. *PeerJ*, [5:e3756](https://doi.org/10.7717/peerj.3756); DOI [10.7717/peerj.3756](https://doi.org/10.7717/peerj.3756)
- Volker, H., Rudolf, W., and Rodel, M-O. 2005. Oviposition site selection in a complex and variable environment: the role of habitat quality and conspecifics cues. *Oecologia*, 142:316-325.
- Walker, E.D., Lawson, D.L., Merritt, R.W., Morgan, W.T., and Klug, M.J. 1991. Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. *Ecology*, 72:1529-1546.
- Ward, M.P., and Schlossberg. 2004. Conspecific attraction and the conservation of territorial songbirds. *Society for Conservation Biology*, 18:519-525.
- Wik A., and Dave, G. 2009. Occurrence and effects of tire wear particles in the environment: A critical review and an initial risk assessment. *Environmental Pollution*, 157:1-11.
- Wong, J., Stoddard, S.T., Astete, H., Morrison, A.C., and Scott, T.W. 2011. Oviposition site selection by the dengue vector *Aedes Aegypti* and its implications for dengue control. *PLoS Neglected Tropical Diseases*, 5(4):
[e1015. https://doi.org/10.1371/journal.pntd.0001015](https://doi.org/10.1371/journal.pntd.0001015)
- Yoshioka, M., Couret, J., Kim, F., McMillan, J., Burkot, T.R., Dotson, E.M., Kitron, U., and Vazquez-Prokopec, G.M. 2012. Diet and density dependent competition affect larval

performance and oviposition site selection in the mosquito species *Aedes albopictus* (Diptera: Culicidae). *Parasites & Vectors*, 5:225 <https://doi.org/10.1186/1756-3305-5-225>

Zhang, H., Liu, J., Li, C., Momen, B., Kohanski, R.A., and Pick, L. 2009. Deletion of *Drosophila* insulin-like peptides causes growth defects and metabolic abnormalities. *Proceedings of the National Academy of Sciences of the United States of America*, 106:19617-19622.

Chapter 6: General Discussion

Aedes albopictus and *Culex pipiens* are two mosquito species that are highly present in urban areas of the United States (Lounibos, 2002). *Aedes albopictus* is currently present in 33 states and *Cx. pipiens* is present in 38 states (Evans et al., 2017). *Aedes albopictus* and *Cx. pipiens* mosquito populations overlap in their geographic distribution in 27 states where they coexist in spite of the fact that *Cx. pipiens* is an inferior competitor compared to *Ae. albopictus* (Carrieri et al., 2003; Costanzo et al., 2005; Costanzo et al., 2011). In the area where these mosquitoes overlap in their distribution, they could represent a threat to public health because they could promote increased human incidence of WNV, considering that *Cx. pipiens* is the main vector of WNV and that *Ae. albopictus* could act as a bridge vector for WNV (Brustolin et al., 2016).

Discarded tires are important habitats for *Ae. albopictus* and *Cx. pipiens* mosquitoes in peridomestic areas. Understanding the factors affecting heterospecific competition of the immature stages of mosquitoes is important to the understanding of their distribution and of measures to control of adult populations. The overall goal of this dissertation, was to test the effects of UV-B radiation and tire pollutants on the larval ecologies of *Ae. albopictus* and *Cx. pipiens*. Through four experiments I compared the effects of three UV-B radiation levels that mimicked conditions in the field: full-sun, shade, and no-UV on tire degradation and leaching of contaminants, the metabolism and oviposition of *Ae. albopictus* and *Cx. pipiens*, and density-dependent competition between these two species.

My results showed that both *Ae. albopictus* and *Cx. pipiens* had higher metabolic rates and lower survival in full-sun conditions compared to no-UV conditions, probably because they were under greater physiological stress. Stress could be due to direct UV-B

exposure that demand greater partitioning of energy to maintaining bodily processes (e.g., feeding, growth, and reproduction), which would be expressed in lower survival rates and greater development time (MacGregor, 1932). Water samples exposed to full-sun conditions also showed lower microbial activity than samples exposed to no-UV and shade conditions, suggesting that full-sun exposure appears to decrease available microbial food resources for mosquito larvae. Thus, another form of stress could be via reduced food availability that could limit energy available to maintenance, or encourage larvae to forage for food for longer and incur injuries from increasing swimming, both of which could lead to reduced survivorship,

Tires appeared to release higher concentrations of zinc and likely other contaminants (e.g., copper, cadmium, polycyclic aromatic hydrocarbons, benzothiazoles) when exposed to higher UV-B radiation. Released zinc and other contaminants could be suspended in the water column or attached or absorbed on the biofilm layer at the bottom of the tire casings. My findings suggested that after 150 days, zinc appeared to be ingested by mosquitoes both on fine particulate organic matter (FPOM) in the water column and in the biofilm that was on tire surfaces. Full-sun conditions promoted much greater microbial growth and zinc concentrations and overall amounts were substantially higher in biofilm. Interesting, although *Ae. albopictus* appeared to ingest much higher amounts of contaminants than *Cx. pipiens*, presumably due to its behavior of spending more time browsing biofilm than filtering in the water column, it was still the superior resource competitor, suggesting that tire leachate does not reverse the outcome of heterospecific competition. Nevertheless, although being the inferior competitor, overall *Cx. pipiens* appeared to have greater competitive effects on *Ae. albopictus* under UV-B conditions that

promote greater tire degradation than no-UV conditions, which could still help promote species coexistence and is an example of condition-specific competition. This suggests that tire leachate from tires exposed to shade and full-sun conditions could alleviate heterospecific competition, facilitating coexistence. Overall, my dissertation research suggests that UV-B can have strong effects on the larval ecology of both *Ae. albopictus* and *Cx. pipiens*, both through direct negative effects on metabolic processes and resultant decreases in survival, and indirectly through the degradation of tires and the leaching of toxic tire contaminants. Moreover, my research showed that tire leachate does not appear to alter the oviposition behavior of both *Ae. albopictus* and *Cx. pipiens*. Therefore, the effects of tire leachate on larval ecology is likely to be especially important in dictating the distribution and abundance of both *Ae. albopictus* and *Cx. pipiens* larval competition for resources in tire habitats is likely to be especially important in structuring their communities.

In addition to affecting the population dynamics of both species, my dissertation indicates a number of important implications to public health. *Aedes albopictus* adults have 14 times higher bodily zinc concentrations compared to *Cx. pipiens* when emerging from tires exposed to full-sun conditions. Two previous studies have demonstrated high and greater tolerances to tire leachate of *Ae. albopictus* than other mosquito species, which could lead to the development of insecticide resistance. Suwanchaichinda and Brattsten (2002) showed that *Ae. albopictus* exposed to benzothiazoles from tire leachate showed resistance to carbaryl, rotenone, and temephos, and the study of Nkya et al. (2013) showed that *Ae. albopictus* exposed to tire leachate developed resistance to pyrethroid insecticides. In both cases, *Ae. albopictus* was able to detoxify tire contaminants through the increased

induction of the cytochrome P450s monooxygenases. Insecticide resistance in *Ae. albopictus* exposed to tire leachate should be further studied because it would have a direct impact on mosquito distribution and the increase in the risk of virus transmission to humans and animals.

An additional finding of my dissertation is that although *Cx. pipiens* and *Ae. albopictus* experienced lower survival from tires exposed to shade and full-sun than no-UV conditions, the adults that did emerge were larger. Juliano et al. (2014) has shown that although dengue virus disseminates through the mid-gut to the salivary glands more slowly in larger *Ae. aegypti* females, larger individuals are overall superior disease vectors because they have a higher rate of biting, longer life expectancy, greater flying range, and production of higher number of eggs compared to smaller mosquitoes. Further studies should assess if lower numbers of larger mosquitoes from tires with higher tire leachate concentration have greater negative effect on virus transmission compared to higher numbers of smaller mosquitoes from tires with lower tire leachate concentration.

The effects of UV-B radiation and tire leachate are likely to be complex and may be manifest in both the immature (larval) and adult life stages, as well as in the microbial communities on which mosquito larvae feed. The main goal of my work was to test if tire leachate from UV-B degradation could alter the competitive superiority of *Ae. albopictus* over *Cx. pipiens*; it showed evidence of increased competitiveness of *Cx. pipiens* over *Ae. albopictus* in tire habitats exposed to shade and full-sun conditions. Additional research needs to examine the effects of tire leachate on other community processes, such as predation, parasitism, and on vector competence across other disease systems such as chikungunya and Zika virus. Also, more research is needed in a multigenerational study on

the development of insecticide resistance by mosquitoes previously exposed to tire leachate. It is obvious that the elimination of stockpiles of scrap tires will eliminate a breeding mosquito habitat along with the associated risk of virus transmission. It also clear that the spread of *Ae. albopictus* since its discovery in 1985 in Houston, Texas has been favored by interstate shipments of scrap tires. If elimination of stockpiles of scrap tires is not feasible, mosquito control programs may be required to suppress mosquito populations at tire piles, which it would be probably more problematic and costly. Reduction or elimination of stockpiles of discarded tires should be a priority for state recycling programs and tire disposal and recycling should be addressed at the federal level.

References

- Brustolin, M., Talavera, S., Santamaria, C., Rivas, R., Pujol, N., Aranda, C., Marques, E., Valle, M., Verdun, M., Pages, N., and Busquets, N. 2016. *Culex pipiens* and *Stegomyia albopicta* (= *Aedes albopictus*) populations as vectors for lineage 1 and 2 West Nile virus in Europe. *Medical and Veterinary Entomology*, 30:166-173.
- Carrieri, M., Bacchi, M., Bellini, R., and Maini, S. 2003. On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. *Environmental Entomology*, 32:1313-132.
- Costanzo, K.S., Mormann, K., and Juliano, S.A. 2005. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology*, 42:559-570.
- Costanzo, K.S., Muturi, E.J., Lampman, L.R., and Alto, R.W. 2011. The effects of resource type and ratio on competition with *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology*, 48:29-38.
- Evans, M.V., Dallas, T.A., Han, B.A., Murdock, C.C., and Drake, J.M. 2017. Data-driven identification of potential Zika virus vectors. *Computational and Systems Biology, Ecology*, DOI: [10.7554/eLife.22053](https://doi.org/10.7554/eLife.22053)
- Juliano, S.A., Ribeiro, G.S., Maciel-de-Freitas, R., Castro, M.G., Codeco, C., Lourenco-de-Oliveira, R., and Lounibos, L.P. 2014. She's femme fatale: low-density larval development produces good disease vectors. *Mem Inst Oswaldo Cruz*, 109:96-103.
- Lounibos, L.P. 2002a. Invasions by insect vectors of human disease. *Annual Review of Entomology*, 47:233-266.
- MacGregor, M.E. 1932. Certain pathological effects of ultra-violet radiation on mosquito larvae and pupae. *Proceedings of the Royal Society of London*, 112:27-38.
- Nkya, T.E., Akhouayri, I., Kisinza, W., and David, J.P. 2013. Impact of environment on mosquito response to pyrethroid insecticides: Facts, evidences and prospects. *Insect Biochemistry and Molecular Biology*, 43:407-416.
- Suwanchaida, C., and Brattsten, L.B. 2002. Induction of microsomal cytochrome P450s by tire-leachate compounds, habitat components of *Aedes albopictus* mosquito larvae. *Archives of Insect Biochemistry and Physiology*, 49:71-79.

Appendices

Appendix 1: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) and species (*Ae. albopictus* and *Cx. pipiens*) at three different times (days 1, 8, and 15) on the larvae metabolic rate of *Ae. albopictus* and *Cx. pipiens* mosquitoes

```
PROC IMPORT OUT= WORK.LmetrateRM
    DATAFILE= "C:\Documents\Data\MRLrepcon.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
proc print data=LmetrateRM;
run;
proc sort data=LmetrateRM;
key trt sp blk/ascending;
run;
data b;
set LmetrateRM;
mr1=log10(mr1);
mr8=log10(mr8);
mr15=log10(mr15);
run;
proc print data=b;
run;
data l;
set b;
day= 1; mr=mr1; output;
day= 8; mr=mr8; output;
day= 15; mr=mr15; output;
drop mr1 mr8 mr15;
proc print data=l;
run;
proc mixed data=l covtest;
class vial trt sp blk day;
model mr = trt|sp|day / residual ddfm=kr;
repeated day/ subject= trt*sp*blk type=cs;
random blk;
lsmeans trt sp day trt*day sp*day / pdiff adjust=tukey;
run;
```

Appendix 2: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) and species (*Ae. albopictus* and *Cx. pipiens*) at three different times (days 1, 8, and 15) on the metabolic rate of the microbial communities.

```
PROC IMPORT OUT= WORK.MbMetrate
    DATAFILE= "C:\Documents\Data\MRMbrepcon.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
Proc print data=MbMetrate;
run;
proc sort data=Mbmetrate;
key trt sp blk/ascending;
run;
data e;
set MbMetrate;
mr1=log10(mr1);
mr8=log10(mr8);
mr15=log10(mr15);
run;
proc print data=e;
run;
data k;
set e;
day= 1; mr=mr1; output;
day= 8; mr=mr8; output;
day= 15; mr=mr15; output;
drop mr1 mr8 mr15;
proc print data=k;
run;
proc mixed data=k covtest;
class vial trt sp blk day;
model mr = trt|sp|day / residual ddfm=kr;
repeated day/ subject=trt * sp * blk type=cs;
random blk;
lsmeans trt sp day trt*day / pdiff adjust=tukey;
run;
```


Appendix 3: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) and species (*Ae. albopictus* and *Cx. pipiens*) on the fitness parameters (survival, development time, and body size) of *Ae. albopictus* and *Cx. pipiens* mosquitoes

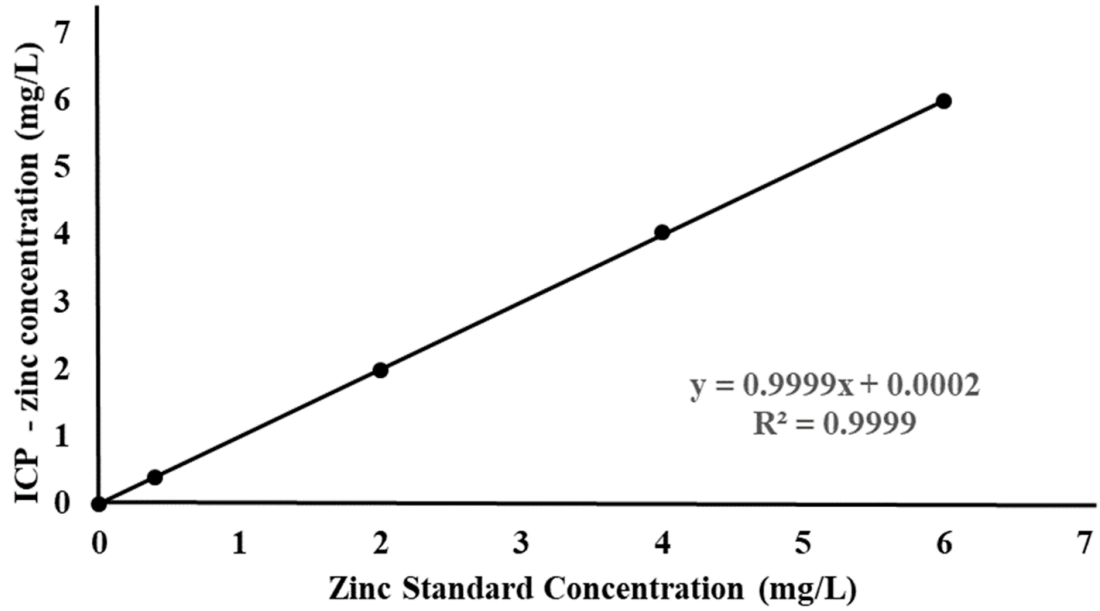
```
PROC IMPORT OUT= WORK.UpSurvLarva
    DATAFILE= "C:\Documents\Data\SurLarvae.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
Proc print data=UpSurvLarva;
run;
proc sort data=UpSurvlarva;
key sp trt blk rep/ascending;
run;
proc means data=UpSurvlarva n mean stderr stddev;
by sp trt blk;
var eclo;
output out=sural n=count nmiss=nmiss;
run;
proc print data=sural;
run;
data surA2;
set sural;
ss=log10(count+1);
run;
Proc print data=surA2;
run;
*survival;
proc mixed data=surA2;
class trt sp;
model ss = trt|sp /residual ddfm=sat;
random blk;
LSMEANS trt sp trt*sp/pdiff adjust=tukey;
run;
*development time;
proc means data=Survlarvae n mean stderr stddev;
by trt sp blk;
var eclo;
output out=devtime1 mean=mean;
run;
data devtime2;
set devtime1;
dt=log10(mean+1);
run;
proc mixed data=devtime2;
```

```

class trt sp blk;
model dt = trt|sp /residual ddfm=kr;
random blk;
lsmeans trt sp trt*sp /adjust=tukey;
run;
*wing length;
proc means data=Survlarvae n mean stderr;
by trt sp;
var wingL;
output out=wing1 mean=mean stderr=stderr;
run;
proc print data=wing1;
run;
data wing3;
set Survlarvae;
ss=log10(wingL+1);
run;
proc means data=wing3 n mean stderr;
by trt sp blk;
var wingL;
output out=wing1 mean=mean stderr=stderr;
run;
proc mixed data=wing1;
class trt sp blk;
model mean = trt|sp/ residual ddfm=sat;
random blk;
lsmeans trt sp trt*sp /adjust=tukey;
run;

```

Appendix 4: Calibration of the Inductively coupled plasma atomic emission spectroscopy (ICP-AES), using the following zinc concentrations: 0, 0.4, 2, 4, 6 mg/L.



Appendix 5: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) on the dissolved and total zinc concentration in discarded tires.

```
PROC IMPORT OUT= WORK.zinc
    DATAFILE= "C:\Documents\Data\Zinc\waterZnCov.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
PROC PRINT DATA=zinc;
RUN;
proc sort data=zinc;
key trt day /ascending;
run;
proc means data=zinc;
var ivdzn ivtzn DZn TZn;
by trt day block;
output out=a mean=ivdzn ivtzn DZn TZn;
run;
proc print data=a;
run;
proc sort data=a;
by day trt block;
run;
proc mixed data=a;
class block day trt;
model DZn= ivdzn trt/residual ddfm=sat;
random block;
lsmeans trt/ adjust=tukey;
by day;
run;
proc sort data=a;
by day trt block;
run;
proc mixed data=a;
class block day trt;
model TZn= ivtzn trt/residual ddfm=sat;
random block;
lsmeans trt/ adjust=tukey;
by day;
run;
```

Appendix 6: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) on the total recoverable zinc concentration in biofilm from discarded tires.

```
PROC IMPORT OUT= WORK.Znbio2
  DATAFILE= "C:\Documents\Data\Zinc biofilm2.csv"
  DBMS=CSV REPLACE;
  GETNAMES=YES;
  DATAROW=2;
run;
Proc print data=Znbio2;
run;
proc sort data=Znbio2;
  by trt blk;
  run;
*Amount of biofilm per trt**;;
proc means data=Znbio2 n mean median stderr;
  by trt blk;
  var Drybiofilm;
  output out=biofilm1 n=count mean=meanwbio median=medianwbio stderr=stderrwbio
  nmiss=nmiss;
  run;
*Amount of Zn per trt mg/g**;;
proc means data=Znbio2 n mean median stderr;
  by trt blk;
  var TZnGram;
  output out=zn1 n=count mean=meanzn median=medianzn stderr=stderrzn nmiss=nmiss;
  run;
*data transformation**;;
data b;
set Znbio2;
TZnGram=log10(TZnGram);
DryBiofilm=log10(DryBiofilm);
run;
proc mixed data=b;
class trt Tire blk;
model TZnGram = trt /ddfm=sat residual;
random blk;
LSMEANS trt/pdiff adjust=tukey;
run;
proc mixed data=b;
class trt blk;
model DryBiofilm = trt /ddfm=sat residual;
random blk;
LSMEANS trt/pdiff adjust=tukey;
run;
```

Appendix 7: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) and competition treatments (*Ae. albopictus*: *Cx. pipiens*, 100:0, 50:0, 50:50, 0:100, 0:50) on the per capita rate of population change (λ') and fitness parameters (survivorship, development time, and body size) of *Ae. albopictus*

```

PROC IMPORT OUT= WORK.SurvAlbo
      DATAFILE= "C:\Documents\Data\SurAlbo.csv"
      DBMS=CSV REPLACE;
      GETNAMES=YES;
      DATAROW=2;
RUN;
proc print data=SurvAlbo;
run;
proc sort data=SurvAlbo;
  by sex trt combAC A C blk;
run;

***survival analyses***;
proc sort data=SurvAlbo;
  by trt combAC A C blk;
run;
proc means data=SurvAlbo;
  by trt combAC A C blk;
  var eclosion;
  output out=sura1 n=count nmiss=nmiss;
run;
*Calculating proportion survival for each cohort in each tire;
data surA2;
  set sura1;
  s=count/A;
run;
data surA2;
  set surA2;
  ss = arsin(sqrt(count/A));
proc print data=surA2;
run;
proc mixed data=surA2;
  class trt combAC A C blk;
  model ss = trt|combAC /ddfm=kenwardroger residual;
  random blk;
  LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;

*survival by sex;
proc sort data=SurvAlbo;
  by sex trt combAC A C blk;

```

```

run;
proc means data=SurvAlbo;
  by sex trt combAC A C blk;
  var eclosion;
  output out=sura1 n=count nmiss=nmiss;
run;
*Calculating proportion survival for each cohort in each tire;
data surA2;
  set sura1;
  s=count/(A/2);
  if s>1 then s=1;
run;
data surA2;
  set surA2;
  ss = arsin(sqrt(s));
proc print data=surA2;
run;
proc mixed data=surA2;
  by sex;
  class sex trt combAC A C blk;
  model ss = trt|combAC /ddfm=kenwardroger residual;
  random blk;
  LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;

***Development time analyses***;
proc sort data=SurvAlbo;
  by sex trt combAC A C blk;
run;
proc means data=SurvAlbo;
  by sex trt combAC A C blk;
  var eclosion;
  output out=ecl2 n=count mean=meandays median=mediandays;
run;
proc mixed data=ecl2;
  by sex;
  class sex trt combAC A C blk;
  model mediandays = trt|combAC /outp=b2;
  random blk;
  LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;
  options ps=45;
proc plot data=b2;
  plot resid*pred = trt;
run;

```

```

***Body Size***;
proc means data=SurvAlbo;
  by trt combAC A C blk;
  var wingL;
  output out=wingl2 n=count mean=meanwingL median=medianwingL;
run;
proc mixed data=wingl2;
  by sex;
  class trt combAC A C blk;
  model medianwingL = trt|combAC /outp=b2;
  random blk;
  LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;
  options ps=45;
proc plot data=b2;
  plot resid*pred = trt;
run;
data lambda;
  set SurvAlbo;
  if sex='M' then delete;
run;
proc sort data=lambda;
  by trt combAC A C blk eclosion;
run;

proc means data=lambda;
  by trt combAC A C blk eclosion;
  var wingL;
  output out=lambda1 mean=meanwingL median=medianwingL n=count
  nmiss=nmiss;
run;
proc print data=lambda1;
run;
/*calculations of lifetable stats*/;
data lambda2;
  set lambda1;
  ax=count+nmiss;
  fwx= 0.5*(-121.240+78.02*medianwingL);
  lxxmx=ax*fwx;
  xlxxmx=eclosion*ax*fwx;
run;
/*gets sums*/;
proc means data=lambda2 noprint;
  by trt combAC A C blk;
  var lxxmx xlxxmx;
  output out=lambda3 sum=sumlxxmx sumxlxxmx;

```



```

run;
data lambda4;
  set lambda3;
  lambda=exp((log((2/A)*sumlxx))/(14+(sumlxx/sumlxx)));
  if sumlxx=. then lambda=0;
  if sumlxx<1 then lambda=0;
  r=log(lambda);
run;
proc print data=lambda4;
run;
proc means data=lambda4 n mean stderr;
  by trt combAC;
  var lambda;
run;
data lambda4;
  set lambda4;
  log10lambda=log10(lambda+1);
run;
proc print data=lambda4;
run;
proc mixed data=lambda4;
  class trt combAC A C blk;
  model lambda = trt|combAC /outp=b2;
  random blk;
  LSMEANS trt|combAC /pdiff adjust=sidak;
run;
  options ps=45;
proc plot data=b2;
  plot resid*pred = trt;
run;

```

Appendix 8: SAS code to assess the effects of UV-B conditions (FS, S, and NUV) and competition treatments (*Ae. albopictus*: *Cx. pipiens*, 100:0, 50:0, 50:50, 0:100, 0:50) on the per capita rate of population change (λ') and fitness parameters (survivorship, development time, and body size) of *Cx. pipiens* mosquitoes

```
PROC IMPORT OUT= WORK.SurvCxPip
    DATAFILE= "C:\Documents\Data\SurCulex.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
data survcu;
set SurvCxPip;
run;
proc print data=survcu;
run;
proc sort data=survcu;
    by sex trt combAC A C blk;
run;

***survival analyses***;
proc sort data=survcu;
    by trt combAC A C blk;
run;
proc means data=survcu;
    by trt combAC A C blk;
    var eclosion;
    output out=surc1 n=count nmiss=nmiss;
run;
data surC2;
set surc1;
s=count/C;
run;
data surC2;
set surC2;
ss = arsin(sqrt(count/C));
proc print data=surC2;
run;
proc mixed data=surC2;
class trt combAC A C blk;
model ss = trt|combAC /ddfm=kenwardroger residual;
random blk;
LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;
```

```

*survival by sex;
proc sort data=survcu;
  by sex trt combAC A C blk;
run;
proc means data=survcu;
  by sex trt combAC A C blk;
  var eclosion;
  output out=surc1 n=count nmiss=nmiss;
run;
data surC2;
  set surc1;
  s=count/(C/2);
  if s>1 then s=1;
run;
data surC2;
  set surC2;
  ss = arsin(sqrt(s));
proc print data=surC2;
run;
proc mixed data=surC2;
  by sex;
  class sex trt combAC A C blk;
  model ss = trt|combAC /ddfm=kenwardroger residual;
  random blk;
  LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;

***Development time analyses***;
proc sort data=survcu;
  by sex trt combAC A C blk;
run;
proc means data=survcu;
  by sex trt combAC A C blk;
  var eclosion;
  output out=ecl2 n=count mean=meandays median=mediandays;
run;
proc mixed data=ecl2;
  by sex;
  class sex trt combAC A C blk;
  model mediandays = trt|combAC /out=b2;
  random blk;
  LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;
  options ps=45;
proc plot data=b2;
  plot resid*pred = trt;

```

```

run;
***Body Size***;
proc means data=survcu;
  by sex trt combAC A C blk;
  var wingL;
  output out=wingl2 n=count mean=meanwingL median=medianwingL;
run;
proc mixed data=wingl2;
by sex;
class sex trt combAC A C blk;
model medianwingL = trt|combAC /outp=b2;
random blk;
LSMEANS trt combAC trt*combAC /pdiff adjust=tukey;
run;
options ps=45;
proc plot data=b2;
plot resid*pred = trt;
run;

data lambda;
  set survcu;
  if sex='M' then delete;
run;
proc sort data=lambda;
  by trt combAC A C blk eclosion;
run;
/*determines mean wing lengths for each day and counts individuals*/;
proc means data=lambda noprint;
  by trt combAC A C blk eclosion;
  var wingL;
  output out=lambda1 mean=meanwingL median=medianwingL n=count
nmiss=nmiss;
run;
proc print data=lambda1;
run;
/*calculations of lifetable stats*/;
data lambda2;
  set lambda1;
  ax=count+nmiss;
  fwx= 0.5*(-383.82+148.05*medianwingL);
  lxxmx=ax*fwx;
  xlxmx=eclosion*ax*fwx;
run;
proc means data=lambda2 noprint;
  by trt combAC A C blk;
  var lxxmx xlxmx;

```

```

        output out=lambda3 sum=sumlxxmx sumxlxxmx;
    run;
data lambda4;
    set lambda3;
    lambda=exp((log((2/C)*sumlxxmx))/(10+(sumxlxxmx/sumlxxmx)));
    if sumxlxxmx=. then lambda=0;
    if sumlxxmx<1 then lambda=0;
    r=log(lambda);
    run;
proc print data=lambda4;
    run;
proc means data=lambda4 n mean stderr;
    by trt combAC;
    var lambda;
    run;
data lambda4;
    set lambda4;
    log10lambda=log(lambda+1);
    run;

```

*model using randomization - because data violated parametric assumptions;

```

%macro rand_gen(
indata=lambda4,
outdata=outrand,
depvar=lambda,
numreps=1000,
seed=0);
proc sql noprint;
select count(*) into :numrecs from
&INDATA;
quit;
data __temp_1;
retain seed &SEED ; drop seed;
set &INDATA;
do replicate = 1 to &NUMREPS;
call ranuni(seed,rand_dep);
output;
end;
run;
proc sort data=__temp_1;
by replicate rand_dep;
run;
data &OUTDATA ;
array deplist{ &NUMRECS } _temporary_ ;
set &INDATA(in=in_orig)

```

```

__temp_1(drop=rand_dep);
if in_orig then do;
replicate=0;
deplist{ _n_ } = &DEPVAR ;
end;
else &DEPVAR =
deplist{ 1+ mod(_n_,&NUMRECS) };
run;
%mend rand_gen;
%macro rand_anl(
randdata=outrand,
where=,
testprob=probf,
testlabel=F test,);
data _null_;
retain pvalue numsig numtot 0;
set &RANDDDATA end=endofile;
%if "&WHERE" ne ""
%then where &WHERE %str(;) ;
if Replicate=0 then pvalue = &TESTPROB ;
else do;
numtot+1;
numsig + ( &TESTPROB < pvalue );
end;
if endofile then do;
ratio = numsig/numtot;
put "Randomization test for &TESTLABEL "
%if "&WHERE" ne "" %then "where &WHERE";
" has significance level of "
ratio 6.4 ;
end;
run;
%mend rand_anl;
%rand_gen(indata=lambda4,outdata=outrand,
depvar=lambda,numreps=1000,seed=1230568)
ods output OverallANOVA=overall;
/* change model */
proc glm data=outrand outstat=outstat1;
by replicate;
class trt combAC A C blk;
model lambda = blk trt combAC trt*combAC ;
run;
%rand_anl(randdata=outstat1,
where=_source_='trt' and _type_='SS3',
testprob=prob,testlabel=main effect test)
ods output close;

```

```

%rand_ani(randdata=outstat1,
where=_source_='combAC' and _type_='SS3',
testprob=prob,testlabel=main effect test)
ods output close;
%rand_ani(randdata=outstat1,
where=_source_='blk' and _type_='SS3',
testprob=prob,testlabel=main effect test)
ods output close;
%rand_ani(randdata=outstat1,
where=_source_='trt*combAC' and _type_='SS3',
testprob=prob,testlabel=interaction test)
%rand_ani(randdata=outstat1,
run;

***proc mixed random***;
%macro rand_gen(
indata=lambda4,
outdata=outrand,
depvar=lambda,
numreps=1000,
seed=0);
proc sql noprint;
select count(*) into :numrecs from
&INDATA;
quit;
data __temp_1;
retain seed &SEED; drop seed;
set &INDATA;
do replicate = 1 to &NUMREPS;
call ranuni(seed,rand_dep);
output;
end;
run;
proc sort data=__temp_1;
by replicate rand_dep;
run;
data &OUTDATA ;
array deplist{ &NUMRECS } _temporary_ ;
set &INDATA(in=in_orig)
__temp_1(drop=rand_dep);
if in_orig then do;
replicate=0;
deplist{ _n_ } = &DEPVAR ;
end;
else &DEPVAR =
deplist{ 1+ mod(_n_,&NUMRECS) };

```

```

run;
%mend rand_gen;
%macro rand_anl(
randdata=outrand,
where=,
testprob=probf,
testlabel=F test,);
data _null_;
retain pvalue numsig numtot 0;
set &RANDDDATA end=endofile;
%if "&WHERE" ne ""
%then where &WHERE %str(;) ;
if Replicate=0 then pvalue = &TESTPROB ;
else do;
numtot+1;
numsig + ( &TESTPROB < pvalue );
end;
if endofile then do;
ratio = numsig/numtot;
put "Randomization test for &TESTLABEL "
%if "&WHERE" ne "" %then "where &WHERE";
" has significance level of "
ratio 6.4 ;
end;
run;
%mend rand_anl;
%rand_gen(indata=lambda4,outdata=outrand,
depvar=lambda,numreps=1000,seed=1230568)
ods output OverallANOVA=overall;
/* change model */
proc mixed data=outrand outstat=outstat1;
by replicate;
class trt combAC A C blk;
model lambda = trt combAC trt*combAC;
random blk;
run;
%rand_anl(randdata=outstat1,
where=_source_='trt' and _type_='SS3',
testprob=prob,testlabel=main effect test)
ods output close;
%rand_anl(randdata=outstat1,
where=_source_='combAC' and _type_='SS3',
testprob=prob,testlabel=main effect test)
ods output close;
%rand_anl(randdata=outstat1,
where=_source_='blk' and _type_='SS3',

```



```
testprob=prob,testlabel=main effect test)
ods output close;
%rand_anl(randdata=outstat1,
where=_source_='trt*combAC' and _type_='SS3',
testprob=prob,testlabel=interaction test)
%rand_anl(randdata=outstat1,
run
```

Appendix 9: SAS code to assess the amount levels of food resources (filter and unfiltered) in tire leachate with high and low zinc concentrations.

```
PROC IMPORT OUT= WORK.metrate
    DATAFILE= "C:\Documents\Data\metab rate.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
proc print data=metrate;
run;
data b;
set metrate;
metrate=log10(metrate+1);
run;
proc print data=b;
run;
proc sort data=b;
key blk LeachateConc FoodRes/ascending;
run;
proc print data=b;
run;
proc means data=b;
var metrate;
by blk LeachateConc FoodRes;
output out=a mean=metrate;
run;
proc print data=a;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model metrate= LeachateConc|FoodRes/residuals ddfm=sat;
random blk;
run;
```

Appendix 10: SAS code to assess the effects of zinc concentration (high and low) and amount of food resources (filter and unfiltered) on the oviposition behavior of *Ae. albopictus* mosquitoes

```
PROC IMPORT OUT= WORK.albovip
    DATAFILE= "C:\Documents\Data\AlboOvip.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
proc print data=albovip;
run;
data e;
set albovip;
NumEggs=log10(NumberEggs+1);
totalLarvae=log10(HatchedLarvae+1);
PercHatch=log10(PercHatching+1);
run;
proc sort data=e;
key LeachateConc FoodRes blk/ascending;
run;
proc means data=e n mean stderr;
var NumEggs totalLarvae PercHatch;
by LeachateConc FoodRes blk;
output out=a mean= NumEggs totalLarvae PercHatch;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model NumEggs = LeachateConc|FoodRes / residual ddfm=kr;
random blk;
lsmeans LeachateConc FoodRes LeachateConc*FoodRes / adjust=tukey;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model totalLarvae = LeachateConc|FoodRes / residual ddfm=kr;
random blk;
lsmeans LeachateConc FoodRes LeachateConc*FoodRes / adjust=tukey;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model PercHatch = LeachateConc|FoodRes / residual ddfm=kr;
random blk;
lsmeans LeachateConc FoodRes LeachateConc*FoodRes / adjust=tukey;
run;
```

Appendix 11: SAS code to assess the effects of zinc concentration (high and low) and amount of food resources (filter and unfiltered) on the oviposition behavior of *Cx. pipiens* mosquitoes

```
PROC IMPORT OUT= WORK.culexovip
    DATAFILE= "C:\Documents\Data\CulexOvip.csv"
    DBMS=CSV REPLACE;
    GETNAMES=YES;
    DATAROW=2;
RUN;
proc print data=culexovip;
run;
data d;
set culexovip;
eggRaft=log10(eggrafts+1);
totalLarvae=log10(total_larvae+1);
larvaeRaft=log10(larvae_per_raft+1);
run;
proc sort data=d;
by LeachateConc FoodRes blk;
run;
proc means data=d n mean stderr;
var eggRaft totalLarvae larvaeRaft;
by LeachateConc FoodRes blk;
output out=a mean= eggRaft totalLarvae larvaeRaft;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model eggRaft = LeachateConc|FoodRes / residual;
random blk;
lsmeans LeachateConc FoodRes LeachateConc*FoodRes / adjust=tukey;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model totalLarvae = LeachateConc|FoodRes / residual;
random blk;
lsmeans LeachateConc FoodRes LeachateConc*FoodRes / adjust=tukey;
run;
proc mixed data=a;
class LeachateConc FoodRes blk;
model larvaeRaft = LeachateConc|FoodRes / residual;
random blk;
lsmeans LeachateConc FoodRes LeachateConc*FoodRes / adjust=tukey;
run;
```

References

- Abernethy, S.G., Montemayor, B.P., and Penders, J.W. 1996. The Aquatic toxicity of scrap automobile tires. Ontario Ministry of Environment and Energy. pp 1-112.
- Afify A., and Galizia, G. 2015. Chemosensory cues for mosquito oviposition site selection. *Journal of Medical Entomology*, 52:120-130.
- Al-Doghairi, M., El-Nadi, A., Elhag E., and Al-Ayedh, H. 2004. Effect of *Solenostemma argel* on oviposition, egg hatchability and viability of *Culex pipiens L.* larvae. *Phytotherapy Research*, 18:335-338.
- Allan, S., and Kline, D. 1995. Evaluation of organic infusions and synthetic compounds mediating oviposition in *Aedes albopictus* and *Aedes aegypti*. *J. Chem. Ecol.*, 21:1847-1860.
- Allgood, D.W. 2011. Influence of detritus levels and organic pollution on interspecific resource competition, oviposition behavior, and larval survival of two tire-inhabiting mosquito species (*Diptera: Culicidae*). Master's thesis. 242. http://aquila.usm.edu/masters_theses/242/
- Alto, B.W., Lampman, R.L., Kesavaraju, B., and Muturi, E.J. 2013. Pesticide-induced release from competition among competing *Aedes aegypti* and *Aedes albopictus* (*Diptera: Culicidae*). *Journal of Medical Entomology*, 50:1240-1249.
- Andrady, A.L., Hamid, H.S., Hu, X., and Torikai, A. 1998. Effects of increased solar ultraviolet radiation on materials. *Journal of Photochemistry and Photobiology*, 46:96-103.
- Andrady, A.L., Hamid, H.S., and Torikai, A. 2003. Effects of climate change and UV-B on materials. *Journal of Photochemistry and Photobiology*, 2:68-72.
- Ballare, C.L., Caldwell, M.M., Flint, S.D., Robinson, S.A., and Bornman, J.F. 2011. Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. *Photochemical and Photobiological Sciences*, 10, 226. [doi: 10.1039/c0pp90035d](https://doi.org/10.1039/c0pp90035d)
- Barker, C.M., Brewster, C.C., and Paulson, S.L. 2003. Spatiotemporal oviposition and habitat preferences of *Ochlerotatus triseriatus* and *Aedes albopictus* in an emerging focus of La Crosse virus. *Journal of the American Mosquito Control Association*, 19:382-391.
- Becker, N., Petric, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., and Kaiser, A. 2010. *Mosquitoes and Their Control*. Chapter 2: Biology of Mosquitoes. Springer, 2nd ed. Heidelberg, Germany.

- Benedict, M.Q., Levine, R.S., Hawley, W.A., and Lounibos, L.P. 2007. Spread of the Tiger: Global risk of invasion by the mosquito *Aedes albopictus*. *Vector Borne Zoonotic Diseases*, 7:76-85.
- Bentley, M.D., and Day, J.F. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. *Annual Review of Entomology*, 34:401-421.
- Blaustein, L. and Chase, J.M. 2007. Interactions between mosquito larvae and species that share the same trophic level. *Annual Review of Entomology*, 52:489-507.
- Bradshaw, W.E., and Holzapfel, C.M. 1986. Habitat segregation among tree-hole mosquitoes. *National Geographic Research*, 2:167-178.
- Bradshaw, W.E., and Holzapfel, C.M. 1992. Reproductive consequences of density-dependent size variation in the Pitcherplant mosquito, *Wyeomyia smithii* (Diptera: Culicidae). *Annual Entomological Society of America*, 85:274-281.
- Bradshaw, W.E., Holzapfel, C.M., and O'Neill T. 1993. Egg size and reproductive allocation in the Pitcherplant mosquito, *Wyeomyia smithii* (Diptera: Culicidae). *Journal of Medical Entomology*, 30:384-390.
- Brustolin, M., Talavera, S., Santamaria, C., Rivas, R., Pujol, N., Aranda, C., Marques, E., Valle, M., Verdun, M., Pages, N., and Busquets, N. 2016. *Culex pipiens* and *Stegomyia albopicta* (= *Aedes albopictus*) populations as vectors for lineage 1 and 2 West Nile virus in Europe. *Medical and Veterinary Entomology*, 30:166-173.
- Burke, W.F., McDonald, K.O. and Davidson, E.W. 1983. Effect of UV light on spore viability and mosquito larvicidal activity of *Bacillus sphaericus* 1593. *Applied and Environmental Microbiology*, 46:954-956.
- Caldwell, M.M., Ballare, C.L., Bornman, J.F., Flint, S.D., Bjorn, L.O., Teramura A.H., Teramura, A.H., Kulandaivelu, G., and Tevini, M. 2003. Terrestrial ecosystems, increased solar ultraviolet radiation and interactions with other climatic change factors. *Photochemical and Photobiological Sciences*, 2:29-38.
- Camponelli, K.M., Casey, R.E., Snodgrass, J.W., Lev, S.M., and Landa, E.R. 2009. Impacts of weathered tire debris on the development of *Rana sylvatica* larvae. *Chemosphere*, 74:717-722.
- Cancrini, G., Scaramozzino, P., Gabrielli, S., Di Paolo, M., and Romi, R. 2007. *Aedes albopictus* and *Cx. pipiens* implicated as natural vectors of *Dirofilaria repens* in Central Italy. *Journal of Medical Entomology*, 44:1064-1066.
- Carrieri, M., Bacchi, M., Bellini, R., and Maini, S. 2003. On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. *Environmental Entomology*, 32:1313-132.

- Cassell, D.L. 2011. A randomization-test wrapper for SAS PROCs. *Statistics and Data Analysis*, Paper 251-27.
- Center for Disease Control – CDC. 2016. Estimated range of *Aedes albopictus* and *Aedes aegypti* in the United States, 2016. U.S. Department of Health and Human Services, Atlanta, U.S.
- Chadee, D.D. 1993. Oviposition response of *Aedes aegypti* (L.) to the presence of conspecifics eggs in the field in Trinidad, W.I. *J. Fla. Mosq. Control Assoc.* 64:63-66.
- Chase, J.M., and Leibold, M.A. 2003. *Ecological niches: linking classical and contemporary approaches*. University of Chicago Press, Chicago, Illinois
- Chesson, P., and Huntly, N. 1997. The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *American Naturalist*, 150:519-553.
- Chesson, P. 2000. Mechanism of maintenance of species diversity. *Annual Review of Ecology and Systematics*, 31:343-366.
- Clements, A.N. 2000. *The biology of mosquitoes. Development, nutrition and reproduction*. Volume 1. CABI publishing, New York, NY, USA.
- Costanzo, K.S., Mormann, K., and Juliano, S.A. 2005a. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology*, 42:559-570.
- Costanzo, K.S., Kesavaraju, B., and Juliano, S.A., 2005b. Condition-specific competition in container mosquitoes: the role of noncompeting life-history stages. *Ecology*, 86:3289-3295.
- Costanzo, K.S., Muturi, E.J., Lampman, L.R., and Alto, R.W. 2011. The effects of resource type and ratio on competition with *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). *Journal of Medical Entomology*, 48:29-38.
- Crampton, M., Ryan, A., Eckert, C., Baker, K.H., and Herson, D.S. 2014. Effects of leachate from crumb rubber and zinc in green roofs on the survival, growth, and resistance characteristics of *Salmonella enterica* subsp. *Enterica* Serovar *Typhimurium*, *Applied and Environmental Microbiology*, 80:2804-2810.
- Cuong, D.T., Karuppiah, S., Obbard, J.P. 2008. Distribution of heavy metals in the dissolved and suspended phase of the sea-surface microlayer, seawater column, and in sediments of Singapore's coastal environment. *Environmental Monitoring and Assessment*, 138:255-272.

- Darsie, R.F., and Ward, A.R. 2005. Identification and geographical distribution of the mosquitos of North America, North of Mexico. Gainesville, University Press of Florida.
- Davidson, A., and Belbin, L. 2002. Exposure of natural Antarctic marine microbial assemblages to ambient UV radiation: effects on the marine microbial community. *Aquatic Microbial Ecology*, 27:159:174.
- Davis, M.A. 2009. *Invasion Biology*. Oxford University Press, UK. 241 pp.
- Day, K.E., Holtze, K.E., Metcalfe-Smith, J.L., Bishop, and C.T., Dutka, B.J. 1993. Toxicity of leachate from automobile tires to aquatic biota. *Chemosphere*, 27:665-675.
- Day, J.F. 2016. Mosquito oviposition behavior and vector control. *Insects*, 7, 65; [doi:10.3390/insects7040065](https://doi.org/10.3390/insects7040065).
- Dehghan, H., Sadraei, J., Moosa-Kasemi, S.H. 2010. The morphological variations of *Culex pipiens* larvae (*Diptera: Culicidae*) in Yazd Province, Central Iran. *Iran Journal Arthropod-Borne Disease*, 4:42-49.
- De Lange, H.J., Morris, D.P., and Williamson, C.E. 2003. Solar ultraviolet photodegradation of DOC may stimulate freshwater food webs. *Journal of Plankton Research*, 25:111-117.
- De Hoop, L., De Troch, M., Hendriks, A.J., and De Laender. 2013. Modeling toxic stress by atrazine in a marine consumer-resource system. *Environmental Toxicology Chemistry*, 32:1082-1095.
- Dunson, W.A., and Travis, J. 1991. The role of abiotic factors in community organization. *American Naturalist*, 138:1067-1091.
- Dyer, A.G. 2001. Ocular filtering of ultraviolet radiation and the spectral spacing of photoreceptors benefit von Kries colour constancy. *The Journal of Experimental Biology*, 204:2391-2399.
- Edgerly, J.S., McFarland, M., Morgan, P., and Livdahl, T. 1998. A seasonal shift in egg-laying behaviour in response to cues of future competition in a treehole mosquito. *Journal of Animal Ecology*, 67:805-818.
- Ellis, A. 2008. Incorporating density dependence into the oviposition preference-offspring performance hypothesis. *Journal of Animal Ecology*, 77:247-256.
- Estrada-Franco, J.G., Craig, G.B. 1995. Biology, disease relationships and control of *Aedes albopictus*. Technical paper No. 42, Pan American Health Organization, Washington, D.C.
- Evans, J.J. 1997. Rubber tire leachates in the aquatic environment. *Reviews in Environmental Contamination and Toxicology*, 151:67-115.

- Evans, A., and Evans, R. 2006. UK waste tyre management best practice: Handling of post-consumer tyres – collection & storage. Tyres report, The Waste & Resources Action Programme.
- Evans, M.V., Dallas, T.A., Han, B.A., Murdock, C.C., and Drake, J.M. 2017. Data-driven identification of potential Zika virus vectors. *Computational and Systems Biology, Ecology*, DOI: [10.7554/eLife.22053](https://doi.org/10.7554/eLife.22053)
- Farooqui, T. 2012. Review of octopamine in insect nervous system. *Open Access Insect Physiology*, 4:1-17.
- Fausser, P., Tjell, J.C., Mosbaek, H., Pileegard, K. 1999. Quantification of tire-tread particles using extractable organic zinc as tracer. *Rubber Chemistry and Technology*, 72:969-977.
- Fish, D..1985. An analysis of adult size variation within natural mosquito populations. In L.P. Lounibos, J.R. Rey, and J.H. Frank, (eds.) *Ecology of mosquitoes: Proceedings of Florida Medical Entomology Workshop*, pg, 419-30.
- Fisher, I.J., Bradshaw, W.E., and Kammeyer, C. 1990. Fitness and its correlates assessed by intra- and interspecific interactions among tree-hole mosquitoes. *Journal of Animal Ecology*, 59:819-829.
- Fonseca, D., M., Keyghobadi, N., Malcolm, C.A., Mehmet, C, Schaffner, F., Mogi, M., Fleischer, R.C., and Wilkerson R.C. 2004. Emerging vectors in the *Culex pipiens* complex. *Science*, 303: 1535-1538.
- Freed, T.Z., and Leisnham, P.T. 2014. Roles of spatial partitioning, competition, and predation in the North American invasion of an exotic mosquito. *Oecologia*, 175:601-611.
- Gao, K., Li, P., Watanabe, T., and Helbling, E.W. 2008. Combined effects of ultraviolet radiation and temperature on morphology, photosynthesis, and DNA of *Arthrospira* (Spirulina) *Platensis* (Cyanophyta). *Journal of Phycology*, 44:777-786.
- Gerhardt, R., Gottfried, K., Apperson, C., Davis B., Erwin, P., Smith A., Panella, N., Powell E., and Nasci, R. 2001. First isolation of LaCrosse virus from naturally infected *Aedes albopictus*. *Emerging Infectious Diseases*, 7:807-811.
- Grant, R.H., Apostol, K.G., and Schmitz, H.F. 2010. Physiological impacts of short-term UV irradiance exposures on cultivars of *Glycine max*. Book *UV radiation in global climate change*, pp 458-487.
- Gray, E.M., and Bradley, T.J. 2003. Metabolic rate in female *Culex tarsalis* (Diptera: Culicidae): Age, Size, Activity, and Feeding effects. *Journal of Medical Entomology*, 40:903-911.

- Gray, E.M., 2013. Thermal acclimation in a complex life cycle: The effects of larval and adult thermal conditions on metabolic rate and heat resistance in *Culex pipiens* (Diptera: Culicidae). *Journal of Insect Physiology*, 59:1001-1007.
- Gualtieri, M., Andrioletti, M., Vismara, C., Milani, M. and Camatini, M. 2005a. Toxicity of tire debris leachates. *Environment International*, 31:723-730.
- Gualtieri, M., Andrioletti, M., Mantecca, P., Vismara, C., and Camatini, M. 2005b. Impact of tire debris on in vitro and in vivo systems. *Particle and Fibre Toxicology*, 2:1-14.
- Hadapad, A.B., Vijayalakshmi, N., Hire, R.S., Dongre, Tk. 2008. Effect of ultraviolet radiation on spore viability and mosquitocidal activity of an indigenous ISPC-8 *Bacillus sphaericus* Neide strain. *Acta Tropica*, 107:113-116.
- Hard, J.J., and Bradshaw. 1993. Reproductive allocation in the western tree-whole mosquito, *Aedes sierrensis*. *OIKOS journal*. 66:55-65.
- Hader, D.P., Kumar, H.D., Smith, R.C., Worrest, R.C. 2007. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical & Photobiological Sciences*, 6:267-285.
- Hartwell, S.I., Jordahl, D.M., and Dawson, C.E. 2000. The effect of salinity on Tire Leachate Toxicity. *Water, Air, and Soil Pollution*, 121:119-131.
- Hedberg, C.W., Washburn, J.W., and Sjogren, R.D. 1985. The association of artificial containers and LaCrosse encephalitis cases in Minnesota 1979. *Journal of the American Mosquito Control Association*, 1:89-90.
- Helbling, E.W., and Zagarese, H. 2003. UV effects in aquatic organisms and ecosystems. *Journal of Plankton Research*, 26:255-256.
- Hemphill, N. 1991. Disturbance and variation in competition between two stream insects. *Ecology*, 72:864-872.
- Himeidan, Y.E., Temu E.A., ElRayah E.A., Munga, S., and Kweka, E. 2013. Chemical cues for Malaria vectors oviposition site selection: challenges and opportunities. *Journal of insects*, 2013:1-9.
- Hori, M., Shibuya, K., Sato, M., and Saito Y. 2014. Lethal effects of short-wavelength visible light on insects. *Scientific Reports*, 4:7383, [doi: 10.1038/srep07383](https://doi.org/10.1038/srep07383)
- Horner, J.M. 1996. Environmental health implications of heavy metal pollution from car tires. *Reviews on Environmental Health*, 11:175-178.
- Huang, J., Ge, X., and Wang, D. 2012. Distribution of heavy metals in the water column, suspended particulate matters and the sediment under hydrodynamic conditions using an annular flume. *Journal of Environmental Science (China)*, 24:2051-2059.

- Johansen, N.S., Vanninen, I., Pinto, D.M., Nissinen, A.I., and Shipp, L. 2011. In the light of new greenhouses technologies: 2. Direct effects of artificial lighting on arthropods and integrated pest management in greenhouse crops. *Annals of Applied Biology*, 159:1-27.
- Joy, J.E., Hanna, A.A., and Kenned, B.A. 2003. Spatial and Temporal Variation in the Mosquitoes (*Diptera: Culicidae*) inhabiting waste tires in Nicholas County, West Virginia. *Journal of Medical Entomology*, 40:73-77.
- Joy, J.E. 2004. Larval mosquitoes in abandoned tire pile sites from West Virginia. *Journal of the American Mosquito Control Association*, 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., O'Meara, G.F., Morrill, J.R., and Cutwa, M.M. 2002. Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. *Oecologia*, 130: 458-469.
- Juliano, S.A., and Lounibos, L.P. 2004. A field test for competitive effects of *Aedes albopictus* on *Ae. aegypti* in South Florida: differences between sites of coexistence and exclusion? *Oecologia*, 139:583-593.
- Juliano, S.A., and Lounibos, L.P. 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. *Ecology letters*, 8:558-574.
- Juliano, S.A., Ribeiro, G.S., Maciel-de-Freitas, R., Castro, M.G., Codeco, C., Lourenco-de-Oliveira, R., and Lounibos, L.P. 2014. She's femme fatale: low-density larval development produces good disease vectors. *Mem Inst Oswaldo Cruz*, 109:96-103.
- Juliano, S.A. 2009. Species interactions among larval mosquitoes: context dependence across habitat gradients. *Annual Review of Entomology*, 54:37-56.
- Kellough, R.M. 1991. The effects of scrap automobile tires in water. Waste Management Branch, Ontario Ministry of the Environment, Ontario, pp 54.
- Kesavaraju, B., Leisnham, P.T., Keane, S., Delisi, N., Pozatti, R. 2014. Interspecific competition between *Aedes albopictus* and *Ae. sierrensis*: Potential for competitive displacement in the Western United States. *PLoS ONE*, 9(2): [e89698](https://doi.org/10.1371/journal.pone.0089698). [doi:10.1371/journal.pone.0089698](https://doi.org/10.1371/journal.pone.0089698)
- Kiflawi, M., Blaustein, L., and Mangel, M. 2003. Oviposition habitat selection by the mosquito *Culiseta longiareolata* in response to risk of predation and conspecific larval density. *Ecological Entomology*, 28:168-173.

- Kilpatrick, A.M., Meola, M.A., Moudy, R. M., and Kramer, L.D. 2008. Temperature, viral genetics, and the transmission of West Nile Virus by *Culex pipiens*. PLoS Pathogens, 4:1-7.
- Kim, H. C., Wilkerson, R., Pecor, J., Lee, W., Lee, J., O'Guinns, M., and Klein, T. 2005. New records and reference collection of mosquitoes (*Diptera: Culicidae*) on Jeju Island, Republic of Korea. Entomological Research, 35:55-56.
- Klowden, M.J. 2007. Physiological Systems in Insects. Academic press Elsevier, London, UK.
- Kreider, M.L., Panko, J.M., McAtee, B.L., Sweet, and Finley, B.L. 2010. Physical and chemical characterization of tire-related particles: Comparison of particles generated using different methodologies. Science of the Total Environment, 408:652-659.
- Kumata, H., Takada, H., and Ogura, N. 1997. 2-(4-Morpholinyl) benzothiazole as an indicator of tire-wear particles and road dust in the urban environment. In: Molecular markers in environmental geochemistry. American Chemical Society Symposium Series, vol. 671, Washington, DC, pp. 291-305.
- Kumata, H., Yamada, J., Masuda, K., Takada, H., Sato, Y., Sakurai, T., and Fujiwara, K. 2002. Benzothiazolamines as tire-derived molecular markers: sorptive behavior in street runoff and application to source apportioning. Environmental Science and Technology, 36:702-708.
- Kwon, E., and Castaldi, M.J. 2009. Fundamental understanding of the thermal degradation mechanisms of waste tires and their air pollutant generation in a N₂ atmosphere. Environmental Science and Technology, 43:5996-6002.
- Lambrechts, L., Scott, T.W., and Gubler, D.J. 2010. Consequences of the expanding global distribution of *Aedes albopictus* for Dengue Virus Transmission. PLoS ONE, 4(5):e646. [doi:10.1371/journal.pntd.0000646](https://doi.org/10.1371/journal.pntd.0000646)
- Lane, S.J., and Mahony, M.J. 2002. Larval anurans with synchronous and asynchronous development periods: contrasting responses to water reduction and predator presence. Journal of Animal Ecology, 71:780-792.
- Lawton, J.H., and Hassell, M.P. 1981. Asymmetrical competition in insects. Nature, 289:793-795.
- Leisnham, P.T., and Juliano, S.A. 2010. Interpopulation differences in competitive effect and response of the mosquito *Aedes aegypti* and resistance to invasion by a superior competitor. Oecologia, 164:221-230.

- Leisnham, P.T., LaDeau, S.L., and Juliano, S.A. 2014. Spatial and temporal habitat segregation of mosquitoes in urban Florida. *PLoS ONE*, 9(3): e91655. doi:10.1371/journal.pone.0091655
- Li, W., Seifert, M., Xu, Y., and Hock, B. 2006. Assessment of estrogenic activity of leachate from automobiles tires with two in vitro bioassays. *Fresenius Environmental Bulletin*, 15:74-79.
- Lockwood, J.L., Blackburn, T.M., and Cassey, P. 2009. The more you introduce the more you get: the role of colonization pressure and propagule pressure in invasion ecology. *Diversity and Distributions*, 15:904-910.
- Lounibos, L.P., and Machado-Allison C.E. 1983. Oviposition and egg brooding by the mosquito *Trichoprosopon digitatum* in cacao husks. *Ecological Entomology*, 8:475-478.
- Lounibos, L.P. 2002a. Invasions by insect vectors of human disease. *Annual Review of Entomology*, 47:233-266.
- Lounibos, L.P., Suarez, S., Menendez, Z., Nishimura, N., Escher, R.L., O'Connell, S.M., and Rey, J.R. 2002b. Does temperature affect the outcome of larval competition between *Aedes aegypti* and *Aedes albopictus*? *Journal Vector Ecology*, 27:86-95.
- Lounibos, L.P., Escher, R.L., and Nishimura, N. 2011. Retention and adaptiveness of photoperiodic egg diapause in Florida populations of invasive *Aedes albopictus*. *Journal of American Mosquito Control Association*, 27:433-436.
- Lounibos, L.P., Bargielowski, I., Carrasquilla, M.C., and Nishimura, N. 2016. Coexistence of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in peninsular Florida two decades after competitive displacements. *Journal of Medical Entomology*, 53:1385-1390.
- Macia, A. 2006. Differences in performance of *Aedes aegypti* larvae raised at different densities in tires and ovitraps under field conditions in Argentina. *Journal of Vector Ecology*, 37:371-377.
- MacGregor, M.E. 1932. Certain pathological effects of ultra-violet radiation on mosquito larvae and pupae. *Proceedings of the Royal Society of London*, 112:27-38.
- Marini, G., Guzzetta, G., Baldacchino, F., Arnoldi, D., Montarsi, F., Capelli, G., Rizzoli, A., Merler, S., and Rosa, R. 2017. The effect of interspecific competition on the temporal dynamics of *Aedes albopictus* and *Culex pipiens*. *Parasites & Vectors*, 10:102 [DOI 10.1186/s13071-017-2041-8](https://doi.org/10.1186/s13071-017-2041-8)
- Markowski, D. 2015. Mosquito of the month: *Culex pipiens* – Northern House Mosquito. *Vector Disease Control International. Mosquito of the month series*. www.vdci.net/blog/topic/mosquito-of-the-month-series

Martinez, G., Soler, R., and Dicke, M. 2013. Chapter four – behavioral ecology of oviposition-site selection in herbivorous true bugs. *Advances in the Study of Behavior*, 45:175-207.

Martinez, G., Finozzi, M.V., Cantero, G., Soler, R., Dicke, M., and Gonzalez, A. 2017. Oviposition preference but not adult feeding preference matches with offspring performance in the bronze bug *Thaumastocoris peregrinus*. *Entomologia experimentalis et applicata*, 163:101-111.

Marwood, C., McAtee, B., Kreider, M., Scott Ogle, R., Finley, B., Sweet, L., and Panko, J. 2011. Acute aquatic toxicity of tire and road wear particles to alga, daphnid, and fish. *Ecotoxicology*, 20:2079-209.

Mazza, C.A., Izaguirre, M.M., Curiales, J., and Ballare, C.L. 2010. A look into the invisible: ultraviolet-B sensitivity in an insect (*Caliothrips phaseoli*) revealed through a behavioural action spectrum. *Proceedings Biological Science*, 277:367-373.

McCann, S., Day, J.F., Allan, S., and Lord, C.C. 2010. Age modifies the effect of body size on fecundity in *Culex quinquefasciatus* Say (*Diptera: Culicidae*). *Journal of Vector Ecology*, 34:174-181.

McKenzie, R.L., Bjorn, L.O., Bais, A., Ilyasd, M. 2003. Changes in biologically active ultraviolet radiation reaching the Earth surface. *Photochemical and Photobiological Sciences*, 2:5-15.

McMahon, T.J., Galloway, T.D., and Anderson, R.A. 2008. Tires as larval habitats for mosquitoes (*Diptera: Culicidae*) in southern Manitoba, Canada. *Journal of Vector Ecology*, 33:198-204.

McMichael, A.J., Lucas, R., Ponsonby, A.L., and Edwards, S.J. 2003. Stratospheric ozone depletion, ultraviolet radiation and health. In: McMichael et al. (Ed.), *Climate change and human health, risk and responses*. WMO library cataloguing, Geneva, pp. 159-178.

Merritt, R.W., Dadd, R.H., Walker, E.D. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annual Review of Entomology*, 37:349-376.

Miller, K.L. 2014. Waste tire recycling programs. Office of Legislative Research. Research Report 2014-R-0276, Harford, CT, U.S.

Mitchell, C.J., Haramis, L.D., Karabatsos, N., Smith, G.C., and Starwalt, V.J. 1998. Isolation of La Crosse, Cache Valley, and Potosi viruses from *Aedes* mosquitoes (*Diptera: Culicidae*) collected at used tire-sites in Illinois during 1994-1995. *Journal Medical Entomology*, 35:573-577.

Mokany, R., and Shine, R. 2003. Oviposition site selection by mosquitoes is affected by cues from conspecific larvae and anuran tadpoles. *Austral Ecology*, 28:33-37.

- Molaei, G., Andreadis, T.G., Armstrong, P.M., Anderson, J.F., and Vossbrinck, C.R. 2006. Host feeding patterns of *Culex* mosquitoes and West Nile virus transmission, northeastern United States. *Emerg. Infect. Dis.*, 12:468-474.
- Moller-Jacobs, L.L., Murdock, C.C., and Thomas, M.B. 2014. Capacity of mosquitoes to transmit malaria depends on larval environment. *Parasites & Vectors*, 7:593.
<http://www.parasitesandvectors.com/content/7/1/593>
- Moore, G.C., and Mitchell, C.J. 1997. *Aedes albopictus* in the United States: Ten year presence and public health implications. *Emerging Infectious Diseases*, 3:39-334.
- Murrel, E.G., and Juliano S.A. 2008. The role of detritus type in interspecific competition and population distributions of *Aedes aegypti* and *Aedes albopictus*. *Journal of Medical Entomology*, 45:375-383.
- Navarro, D.M., De Oliveira, P.E., Potting, R.P., Brito, A.C., Fital, S.J., and Goulart Sant'Ana, A.E. 2003. The potential attractant or repellent effects of different water types on oviposition in *Aedes aegypti* L. (*Dipt.*, *Culicidae*). *Journal of Applied Entomology*, 127:46-50.
- Nelson, S.M., Mueller, G., and Hemphill, D.C. 1994. Identification of tire leachate toxicants and a risk assessment of water quality effects using tire reefs in canals. *Bulletin of Environmental Contamination and Toxicology*. 52:574-581.
- Newman, P.A., Oman, L.D., Douglas, A.R., Fleming, E.L., Frith, S.M., Hurwitz, M.M., Kawa, S.R., Jackman, C.H., Krotkov, E.R., Nash, E.R., Nielsen, J.E., Pawson, S., Stolarski, R.S., and Velders, J.M. 2009. What would have happened to the ozone layer if chlorofluorocarbons (CFCs) had not been regulated? *Journal of Atmospheric Chemistry and Physics*. 9:2113-2128.
- Ngoagouni, C., Kamgang, B., Nakoune, E., Paupy, C., and Kasanji, M. 2015. Invasion of *Aedes albopictus* (*Diptera: Culicidae*) into central Africa: what consequences for emerging diseases? *Parasites & Vectors*, 8:1-7.
- Nkya, T.E., Akhouayri, I., Kisinza, W., and David, J.P. 2013. Impact of environment on mosquito response to pyrethroid insecticides: Facts, evidences and prospects. *Insect Biochemistry and Molecular Biology*, 43:407-416.
- Obenauer, P.J., Allan, S.A., and Kaufman, P.E. 2010. *Aedes albopictus* (*Diptera: Culicidae*) oviposition response to organic infusions from common flora of suburban Florida. *Journal of Vector Ecology*, 35:301-306
- Okasawa, T., Miyagi, I., Toma, T., Ramalingam, S., and Chang, M.S. 1991. Egg morphology and observations on the laboratory biology of *Armigeres* (*Leicesteria*) *digitalus* (*Diptera: Culicidae*) from Sarawak. *Journal of Medical Entomology*, 28:606-610.

- Oli, M.K., and Dobson, F.S. 2003. The relative importance of life-history variables to population growth rate in mammals: Cole's prediction revisited. *American Naturalist*, 161:422-440.
- Pancotto, V.A., Sala, O.E., Cabello, M., Lopez, N.I., Robson, T.M., Ballare, C.L., Caldwell, M.M., and Scopel, A.L. 2003. Solar UV-B decrease decomposition in herbaceous plant litter in Tierra del Fuego, Argentina: potential role of an altered decomposer community. *Global Change Biology*, 9: 1465-1474.
- Pelz-Stelinski, K., Kaufman, M.G., and Walker, E.D. 2011. Beetle (*Coleoptera: Scirtidae*) facilitation of larval mosquito growth in tree hole habitats is linked to multitrophic microbial interactions. *Microbial Ecology*, 3:690-703.
- Peric-Mataruga, V., Nenadovic, V., and Ivanovic, J. 2006. Neurohormones in insect stress: A review. *Arch. Biol. Sci.*, 58:1-12.
- Peterson, J.C., Clark, D.R., and Sleevi, P.S. 1986. Tire fire oil: Monitoring a new environmental pollutant. *Analytical Chemistry*, 58:70-75.
- Pickett, J.A., and Woodcock. 1996. The role of mosquito olfaction in oviposition site location and in the avoidance of unsuitable host. In Bock, G.R., & Cardew, G (Eds.), *Olfaction in mosquito-host interactions* (1st edition, 109-123). New York, NY: John Wiley & Sons Ltd.
- Pintar, M.R., and Resetarits, W.J. 2017. Out with the Old, in with the New: Oviposition preference matches larval success in Cope's Gray Treefrog, *Hyla chrysoscelis*. *Journal of Herpetology*, 51: 186-189.
- Rhodes, E.P., Zhiyong, R., and Mays, D.C. 2012. Zinc leaching from tire crumb rubber. *Environmental Science & Technology*, 46:12856-12863.
- Rizzoli, A., Bolzoni, L., Chadwick, E.A., Capelli, G., Montarsi, F., Grisenti, M., Martinez de la Puente, J., Muñoz, J., Figuerola, J., Soriguer, R., Anfora, G., Di Luca, M., and Rosa, R. 2015. Understanding West Nile virus ecology in Europe: *Culex pipiens* host feeding preference in a hotspot of virus emergence. *Parasites and Vectors*, 8:213. [DOI 10.1186/s13071-015-0831-4](https://doi.org/10.1186/s13071-015-0831-4)
- Rohr J.R., and Crumrine, P.W. 2005. Effects of an herbicide and an insecticide on pond community structure and processes. *Ecological Applications*, 15:1135-1147.
- Roitberg, B.D., Robertson, I.C., and Tyerman, J.G.A. 1999. Vive la variance: a functional oviposition theory for insect herbivores. *Entomologia Experimentalis et Applicata*, 91:187-194.

- Roux O., Vantaux, A., Roche, B., Yameogo, K.B., Dabire, K.R., Diabate, A., Simard, F., and Lefevre, T. 2015. Evidence for Carry-over effects of predator exposure on pathogen transmission potential. *Proceedings of Biological Science*, 282: 20152430. <http://dx.doi.org/10.1098/rspb.2015.2430>
- Rubber Manufacturers Association-RMA. 2017. 2015 U.S. scrap tire management summary. Rubber Manufacturers Association, Washington, DC.
- Sanchez-Bayo, F., Van Den Brink, P.J., and Mann, R.M. 2012. Ecological impacts of toxic chemicals. Benthan Science Publisher, Sidney, Australia. 250 pp.
- Sang, W., Yu, L., He, L., Ma, W.H., Zhu, Z.H., Zhu, F., Wang, X.P., and Lei, C.L. 2016. UVB radiation delays *Tribolium castaneum* metamorphosis by influencing ecdysteroid metabolism. *PLoS ONE*, 11(3): [e0151831](https://doi.org/10.1371/journal.pone.0151831)
- SAS institute Inc. 2013. Base SAS 9.4 Procedures Guide: Statistical Procedures, Second Edition. Cary, NC: SAS Institute Inc.
- Savage, H. and Miller, B. 1995. House mosquitos of the U.S.A., *Culex pipiens*. *Wing Beats*, 6:8-9.
- Sawabe, K., Isawa, H., Hoshino, K., Sasaki, T., Roychoudhury, S., Higa, Y., Kasai, S., Tsuda, Y., Nishiumi, I., Hisai, N., Hamao, S., and Kobayashi, M. 2010. Host-feeding habits of *Culex pipiens* and *Aedes albopictus* (*Diptera: Culisidae*) collected at the urban and suburban residential areas of Japan. *Journal of Medical Entomology*, 47:442-450.
- Scott, J.G. 1999. Cytochromes P450 and insecticide resistance. *Insect Biochemistry and Molecular Biology*, 29:757-777.
- Scotto, J., Cotton, G., Urbach, F., Berger, D., and Fears, T. 1988. Biologically effective ultraviolet radiation: surface measurements in the United States, 1974 to 1985. *Science*, 239:762-764.
- Selbes, M., Yilmaz, O., Khan, A.A., and Karanfil, T. 2015. Leaching of DOC, DN, and inorganic constituents from scrap tires. *Chemosphere*, 139:617-623.
- Shimoda, M., and Honda, K-I. 2013. Insects reaction to light and its application to pest management. *Applied Entomology and Zoology*. 48:413-421.
- Shuman, E.K. 2011. Global Climate Change and Infectious Diseases. *International Journal of Occupational and Environmental Medicine*, 2(1). www.theijoem.com
- Sliney, D.H., Gilbert II, D.W., and Lyon, T. 2016. Ultraviolet safety assessment on insect light traps. *Journal Occupational Environmental Hygiene*, 13:413-424.

- Smith, C., Baldwin, A.H., Sullivan, J., and Leisnham, P.T. 2013. Effects of Elevated Atmospheric CO₂ on Competition between the Mosquitoes *Aedes albopictus* and *Ae. triseriatus* via Changes in Litter Quality and Production. *Journal of Medical Entomology*, 50:521-532.
- Spencer, M., Blaustein, L., and Cohen, J.E. 2002. Oviposition habitat selection by mosquitoes (*Culiseta longiareolata*) and consequences for population size. *Ecology*, 83:669-679.
- Spies, R.B., Anderson, B.D., and Rice, D.W. 1987. Benzothiazoles in estuarine sediments as indicators of street runoff. *Nature*, 327:697-699.
- Sprenger, D. and Wuithiranyagool, T. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. *Journal of the American Mosquito Control Association*, 2:217-219.
- Stamps, J.A. 1988. Conspecific attraction and aggregation in territorial species. *American Naturalist*, 131:329-347.
- Stephensen, E., Adolfsson-Erisi, M., Celander, M., Hulander, M., Parkkonen, J., Hegelund, T., Sturve, J., Hasselberg, L., Bengtsson, M., and Forlin, L. 2003. Biomarker responses and chemical analysis in fish indicate leakage of polycyclic aromatic hydrocarbons and other compounds from car tire rubber. *Environmental Toxicology*, 22:2926-2931.
- Sullivan, J.H., Pope, L.C., Sutherland, B.M., Bennett, P.V., Blum, J.E., Stapleton, A.E., and Gitz, D.C. 2010. Assessment of DNA damage as a tool to measure UV-B tolerance in soybean lines differing in flavonoid composition. *Book UV Radiation in global change*, pp 437-457.
- Suwanchaichinda, C., and Brattsten, L.B. 2002. Induction of microsomal cytochrome P450s by tire-leachate compounds, habitat components of *Aedes albopictus* mosquito larvae. *Archives of Insect Biochemistry and Physiology*, 49:71-79.
- Terblanche, J.S., Sinclair, B.J., Klok, C.J., McFarlane, M.L., and Chown, S.L. 2005. The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica chalconota* (Coleoptera: Chrysomelidae). *Journal of Insect Physiology*, 51:1013-1023.
- Terblanche, J.S., and Chown, S.L. 2007. Physiological diversity in insects: ecological and evolutionary contexts. *Advances in Insect Physiology*, 33:50-152.
- Terblanche, J.S., Clusella-Trullas, S., Deere, J.A., Van Vuuren, B.J., and Chown, S.L. 2009. Directional evolution of the slope of metabolic rate-temperature relationship is correlate with climate. *Physiological and Biochemical Zoology*, 82:495-503.

- Thompson, J.N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis et Applicata*, 47:3-14.
- Tiawsirisup, S., Platt, K.B., Evans, R.B., and Rowley, W.A. 2005. A comparison of West Nile Virus transmission by *Ochlerotatus trivittatus* (COQ.), *Culex pipiens* (L.), and *Aedes albopictus* (Skuse). *Vector Borne Zoonotic Disease*, 5:40-47.
- Tilman, D. 1982. Resource competition and community structure. Princeton University Press, Princeton, New Jersey, USA.
- Turner, J. 2011. Ultraviolet radiation reflection from building materials: characterization, quantification and the resulting effects. Thesis for a PhD degree, University of Southern Queensland, Australia, pp. 282.
- United States Environmental Protection Agency (U.S. EPA). 1993. Scrap Tire Handbook. U.S Environmental Protection Agency Region 5. 77 West Jackson Blvd, Chicago, IL.
- United States Environmental Protection Agency (U.S. EPA). 1994. Method 200.7, Revision 4.4: Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry. Cincinnati, Ohio.
- United States Environmental Protection Agency (U.S. EPA). 2007. SW-846 test method 3015A: microwave assisted acid digestion of aqueous samples and extracts. <https://www.epa.gov/sites/production/files/2015-12/documents/3015a.pdf>
- Villena, O., Terry, I, Kayoko, I., Landa, E., LaDeau, S., and Leisnham, P. 2017. Effect of tire leachate on the invasive mosquito *Aedes albopictus* and the native congener *Aedes triseriatus*. *PeerJ Preprints* 5:e3065v1 <https://doi.org/10.7287/peerj.preprints.3065v1>
- Vinogradova, E.B. 2000. *Culex pipiens* mosquitoes; taxonomy, distribution, ecology, physiology, genetics, applied importance and control. Pensoft, Sofia, Bulgaria.
- Volker, H., Rudolf, W., and Rodel, M-O. 2005. Oviposition site selection in a complex and variable environment: the role of habitat quality and conspecifics cues. *Oecologia*, 142:316-325.
- Walker, E.D., and Merrit, R.W. 1988. The significance of leaf detritus to mosquito (*Diptera, Culicidae*) productivity from treeholes. *Environmental Entomology*, 17:199-206.
- Walker, E.D., Lawson, D.L., Merrit, R.W., Morgan, W.T., and Klug, M.J. 1991. Nutrient dynamics, bacterial populations, and mosquito productivity in tree hole ecosystems and microcosms. *Ecology*, 72:1529-1546.
- Ward, M.P., and Schlossberg. 2004. Conspecific attraction and the conservation of territorial songbirds. *Society for Conservation Biology*, 18:519-525.

- Wik, A., and Dave, G. 2005. Environmental labeling of car tires - toxicity to *Daphnia magna* can be used as a screening method. *Chemosphere*, 58:645-651.
- Wik, A., and Dave, G. 2006. Acute toxicity of leachates of tire material to *Daphnia magna* – Variability and toxic components. *Chemosphere*, 64:1777-1784.
- Wik A., and Dave, G. 2009. Occurrence and effects of tire wear particles in the environment – A critical review and an initial risk assessment. *Environmental Pollution*, 157:1-11.
- Wong, J., Stoddard, S.T., Astete, H., Morrison, A.C., and Scott, T.W. 2011. Oviposition site selection by the dengue vector *Aedes Aegypti* and its implications for dengue control. *PLoS Neglected Tropical Diseases*, 5(4):
[e1015. https://doi.org/10.1371/journal.pntd.0001015](https://doi.org/10.1371/journal.pntd.0001015)
- Wong, P-SJ., Li, M.I., Chong, C-S., Ng L-C., Tan, C-H., 2013. *Aedes* (*Stegomyia*) *albopictus* (Skuse): A potential vector of Zika Virus in Singapore. *PLoS Neglected Tropical Diseases*, 7(8): e2348. [doi:10.1371/journal.pntd.0002348](https://doi.org/10.1371/journal.pntd.0002348)
- World Health Organization-WHO. 2003. Guidelines for dengue surveillance and mosquito control. 2nd edition. WHO Regional office for the Western Pacific, Manila, Philippines.
- Wu, H., Gao, K., Villafañe, V.E., Watanabe, T., Helbling, E.W. 2005. Effects of solar radiation on morphology and photosynthesis of filamentous cyanobacterium *Arthrospira platensis*. *Applied Environmental Microbiology*, 71:5004-5013.
- Wu, H., Gao, K., and Wu, H. 2009. Responses of a marine red tide alga *Skeletonema costatum* (*Bacillariophyceae*) to long-term UV radiation exposures. *Journal of Photochemistry and Photobiology*, 94:82-86.
- Xue, R., Barnard, D.R., and Schreck, C.E. 1995. Effect of body size, parity, and age of *Aedes albopictus* on human host attack rates and the repellency of deet. *Journal of American Mosquito Control Association*, 11:50-53.
- Yee, D.A. 2008a. Tires as habitats for mosquitoes: A review of studies within the eastern United States. *Journal of Medical entomology*, 45:581-593.
- Yee, D.A., Kesavaraju, B., and Juliano, S.A. 2008b. Larval feeding behavior of three co-occurring species of container mosquitoes. *Journal of Vector Ecology*, 29:315-322.
- Yee, D.A., Kneitel, J.M., and Juliano, S.A. 2010. Environmental correlates of abundances of mosquito species and stages in discarded vehicle tires. *Journal of Medical Entomology*, 47: 53-62.

Yoshioka, M., Couret, J., Kim, F., McMillan, J., Burkot, T.R., Dotson, E.M., Kitron, U., and Vazquez-Prokopec, G.M. 2012. Diet and density dependent competition affect larval performance and oviposition site selection in the mosquito species *Aedes albopictus* (Diptera: Culicidae). *Parasites & Vectors*, 5:225 <https://doi.org/10.1186/1756-3305-5-225>

Zhang, H., Liu, J., Li, C., Momen, B., Kohanski, R.A., and Pick, L. 2009. Deletion of *Drosophila* insulin-like peptides causes growth defects and metabolic abnormalities. *Proceedings of the National Academy of Sciences of the United States of America*, 106:19617-19622.