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Effects of ultraviolet radiation on metabolic rate and fitness of *Aedes albopictus* and *Culex pipiens* mosquitoes

Oswaldo C Villena Corresp., [1] , **Bahram Momen** [2] , **Joseph Sullivan** [3] , **Paul T Leisnham** [2]

[1] Marine Estuarine & Environmental Sciences, University of Maryland College Park, College Park, Maryland, United States
[2] Environmental Science & Technology, University of Maryland College Park, College Park, Maryland, United States
[3] Plant Science & Landscape Architecture, University of Maryland College Park, College Park, Maryland, United States

Corresponding Author: Oswaldo C Villena
Email address: oswaldo.villena@gmail.com

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 (µwatts/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.

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Effects of ultraviolet radiation on metabolic rate and fitness of *Aedes albopictus* and *Culex pipiens* mosquitoes

Oswaldo C. Villena[1,4], Bahram Momen[2], Joseph Sullivan[3], Paul T. Leisnham[2]

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. Corresponding author: ovillena@umd.edu

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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 (µwatts/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

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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

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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.

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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

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(*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 study 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.*

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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. *Aedes 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 of both species were $F_{1\text{-}3}$ generation.

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178 ***Experiment set up***
179
180 The experimental design was a split plot-randomized complete block design (RCBD) with
181 UV-B radiation condition as the main plot, mosquito larvae cohorts (*Ae. albopictus*, *Cx. pipiens*,
182 or no larvae) as the sub-plots, and three replicate blocks. Individuals of *Ae. albopictus* and *Cx.*
183 *pipiens* were sorted into single species cohorts of 10 newly hatched individuals and added to 20
184 ml vials with 17 ml of DI water that were inoculated with 1 ml of water containing a microbial
185 community that was collected from discarded tires. A total of 45 vials were prepared. Ten newly
186 hatched *Ae. albopictus* or ten newly hatched *Cx. pipiens* were randomly added to 15 vials each. 15
187 vials only contained microbial community; no larvae were placed on these vials. Fifteen of the
188 total vials (5 of each treatment) were randomly allotted to one of three Percival reach-in
189 environmental chambers, model I-36 VL, located in the Aqua Engineering laboratory in the
190 Environmental Science and Technology Program (ENST). Each chamber was kept at 25° C, 16:8
191 (L: D), and 80- 90 % of humidity, to mimic typical summer conditions in the northeastern U.S.
192 (Day et al., 1993; Li et al., 2006). At the end, each environmental chamber had 5 vials containing
193 10 first instar larvae of *Ae. albopictus*, 5 vials containing 10 first instar larvae of *Cx. pipiens*, and
194 5 vials containing no larvae, only the microbial community. Vials represent sub-samples and each
195 experimental unit was a group of 5 vials in one of the three environmental chambers. Each vial
196 was checked daily to collect pupae and place them in individual vials with water from that vial
197 until adults emerged. I recorded the following information for each adult: date of emergence, sex,
198 species, and replicate (notebook for the experiment). On the day of emergence, the adults were
199 killed by placing them on a drying oven for further analysis such as wing length measurements.
200 The Experiment was run until all larvae had died or eclosed.

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One of three UV-B radiation conditions mimicking full-sun (10.82 umol/m^2/s), shade (6.1 umol/m^2/s), and a no-UV control group (0 umol/m^2/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 (µwatts/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 µwatts/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

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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 ampules, 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

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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 log10(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 log10(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

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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

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298 UV-B radiation comparable to that which reaches water bodies in open fields (full-sun), shaded
299 areas (shade), and no-UV radiation conditions (control group) upon the fitness (survivorship,
300 development time, and body size) and metabolic rates of two of the most broadly distributed
301 mosquito species in the world: *Ae. albopictus* and *Cx. pipiens*; and upon the microbial communities
302 on which they feed. In previously published field studies, resting metabolic rate increased in
303 mosquito larvae between emergence and day 4 to 5 (Gray and Bradley, 2003). Our results showed
304 that larval metabolic rate of both *Cx. pipiens* and *Ae. albopictus* mosquitoes significantly increased
305 in FS conditions compared to NUV conditions (Figure 1A). In addition, larval metabolic rate is
306 significantly higher at day 8 and day 15, indicating a direct effect of UV-B radiation on mosquito
307 metabolism. Furthermore, at day 15, metabolic rate was significantly higher for *Cx. pipiens*
308 compared to *Ae. albopictus* (Figure 1B).

309 The increase of metabolic rate in *Cx. pipiens* larvae from day 8 to day 15 under FS
310 conditions compared to *Ae. albopictus* presumably reflects that UV-B radiation had a greater
311 negative effect on *Cx. pipiens* larvae. It has been shown in many insect species that metabolic rate
312 is strongly related to the physical and biological factors that influence metabolism (Gray and
313 Bradley, 2003). The *Culex pipiens* mosquito larvae probably increases its metabolic rate under FS
314 conditions, especially at day 15, in response to negative effects of UV-B radiation. These effects
315 could cause larvae to increase energy expenditure in order to perform physical and biological
316 activities such as getting food, growing, or competing with conspecifics, which is reflected in
317 greater metabolic rates. It has been shown previously that UV-B radiation has a greater negative
318 effect on the larval stage than the pupae stage, as the pupae stage is more resistant to damage by
319 UV radiation (MacGregor, 1932). In that study, larvae of *Cx. pipiens* exposed to UV-B radiation
320 were affected when exposed for long periods of time (more than 48 hours). Larvae lost movement

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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 (μwatts/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

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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

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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.

Conclusions

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 brigde vector for WNv (Brustolin et al., 2016).

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412 Ultraviolet radiation may have important effects on *Ae. albopictus* and *Cx. pipiens*
413 mosquitoes, especially in peridomestic areas. Understanding the factors affecting heterospecific
414 competition of the immature stages of mosquitoes is important to the understanding of their
415 distribution and of measures to control of adult populations. The overall goal of this dissertation,
416 was to test the effects of UV-B radiation on the larval ecologies of *Ae. albopictus* and *Cx. pipiens*.
417 My results showed that both *Ae. albopictus* and *Cx. pipiens* had higher metabolic rates and
418 lower survival in full-sun conditions compared to no-UV conditions, probably because they were
419 under greater physiological stress. Stress could be due to direct UV-B exposure that demand
420 greater partitioning of energy to maintaining bodily processes (e.g., feeding, growth, and
421 reproduction), which would be expressed in lower survival rates and greater development time
422 (MacGregor, 1932). Water samples exposed to full-sun conditions also showed lower microbial
423 activity than samples exposed to no-UV and shade conditions, suggesting that full-sun exposure
424 appears to decrease available microbial food resources for mosquito larvae. Thus, another form of
425 stress could be via reduced food availability that could limit energy available to maintenance, or
426 encourage larvae to forge for food for longer and incur injuries from increasing swimming, both
427 of which could lead to reduced survivorship.
428 Overall, my study suggests that UV-B radiation can have strong effects on the larval
429 ecology of both *Ae. albopictus* and *Cx. pipiens*, both through direct negative effects on metabolic
430 processes and resultant decreases in survival, and indirectly through the decrease of food
431 availability. Therefore, the effects of UV-B on larval ecology is likely to be especially important
432 in dictating the distribution and abundance of both *Ae. albopictus* and *Cx. pipiens* mosquitoes in
433 different mosquito habitats (e.g. tires) and in structuring their communities.

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The effects of UV-B radiation 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. Additional research needs to examine the effects of UV-B radiation on other community processes, such as predation, parasitism, and on vector competence across other disease systems such as chikungunya and Zika virus.

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Table 1(on next page)

Analysis of variance of the effects of UV-B radiation conditions and species on mosquito larvae metabolic rates.

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.

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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 (on next page)

Analysis of variance of the effects of UV-B conditions, species inhabiting vials, and time on the metabolic rates of microbial communities.

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 diferent times (days 1, 8, and 15) in the metabolic rate of microbial community.

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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

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Table 3(on next page)

Analysis of variance of the effects of UV-B conditions and specie on the fitness parameters of mosquitoes.

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.

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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

2

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Figure 1

Metabolic rate of mosquitoes in response to UV-B radiation conditions and time.

Least squares means (± SE) for metabolic rate expressed as heat production (μW/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.

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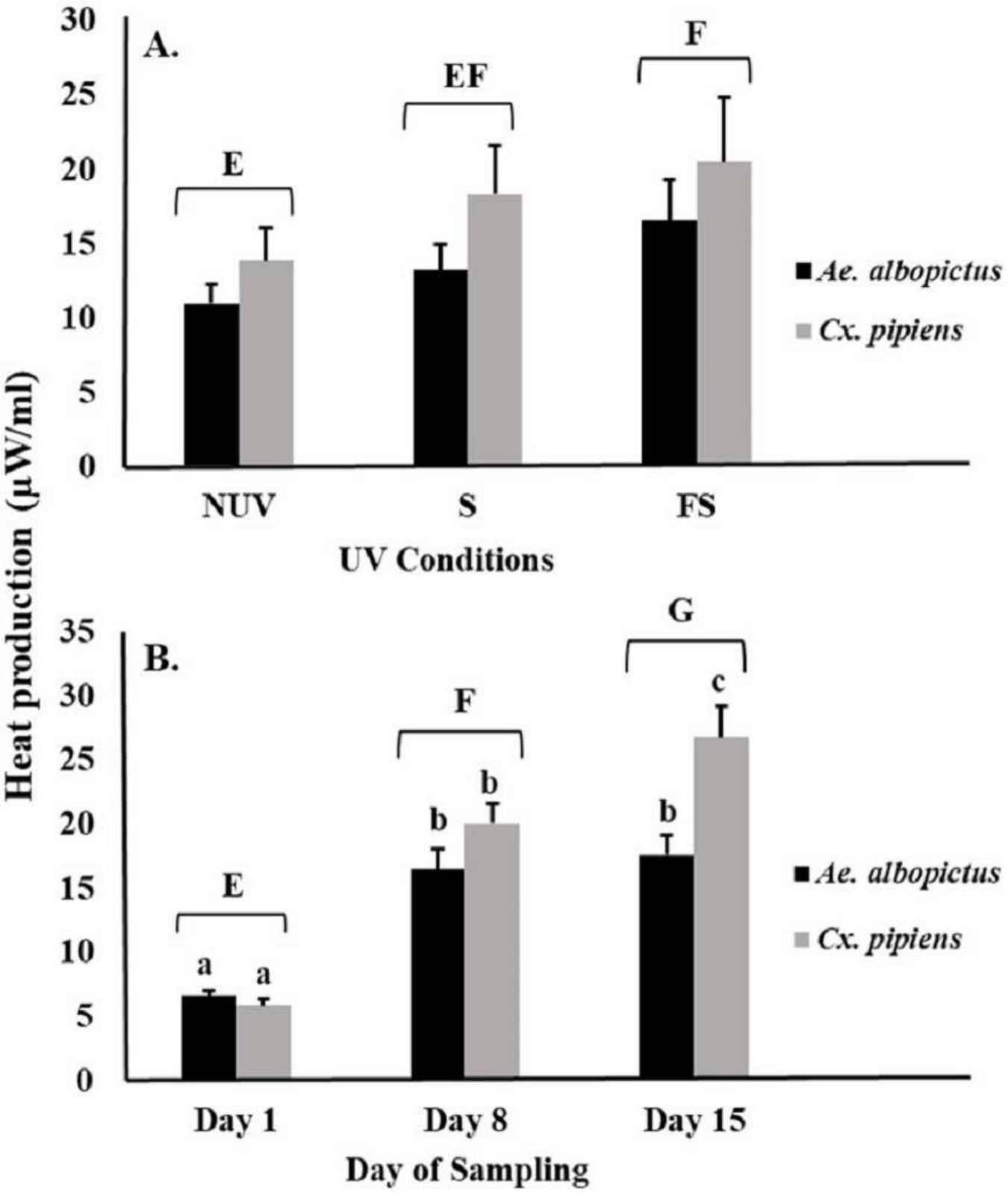

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Figure 2

Metabolic rate of microbial communities in response to container inhabitants, UV-B radiation conditions, and time.

Least squares means (± SE) for metabolic rate expressed as heat production (μW/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.

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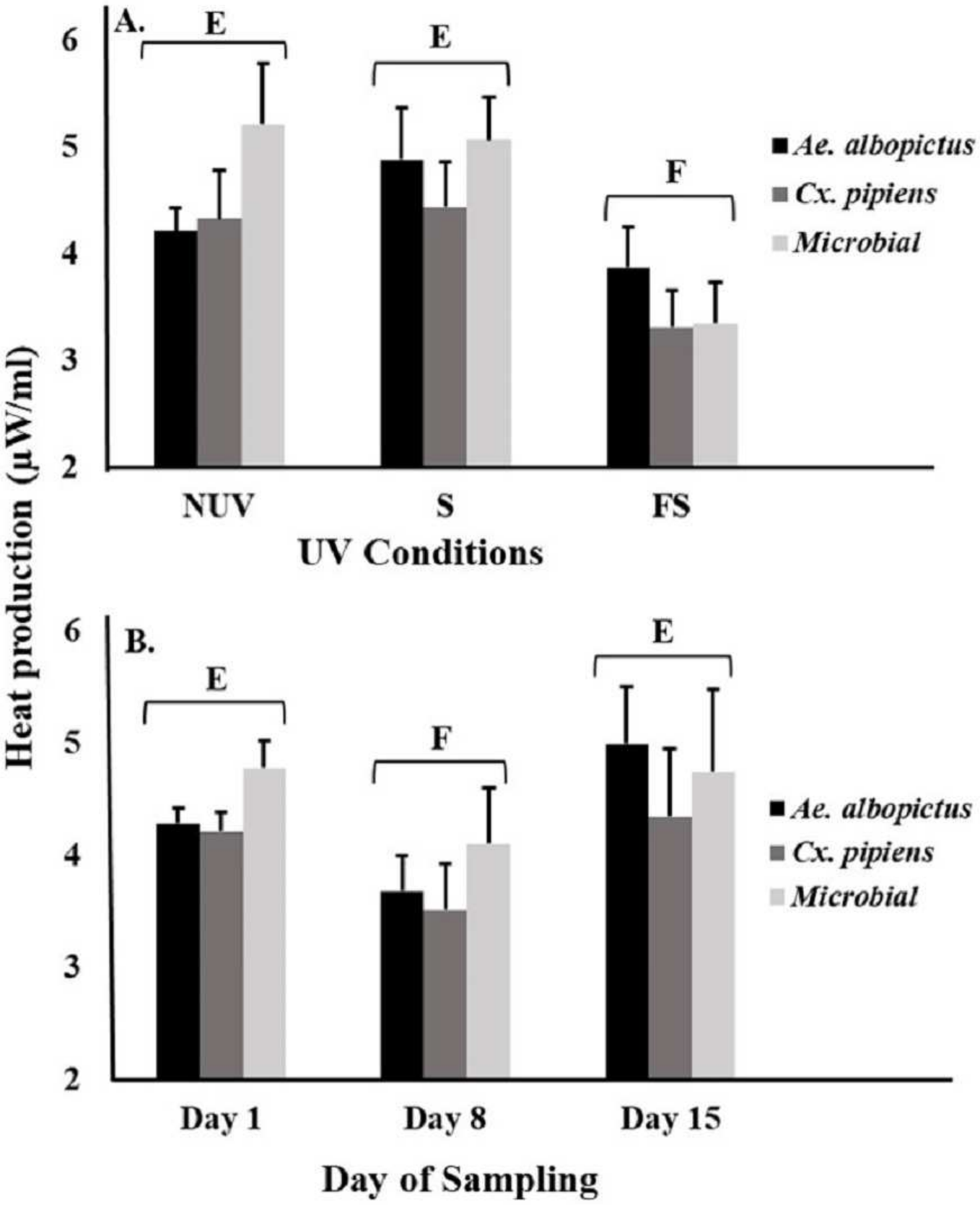

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Figure 3

Mosquito fitness parameters in response to UV-B radiation conditions.

Least squares means (± SE) for fitness parameters of *Ae. albopictus* and *Cx. pipiens* mosquitoes. (a) survival percentage of *Ae. albopictus* (b) survavil 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.

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