THE EFFECT OF INCREASING TEMPERATURE AND COMPETITION ON THE SIZE AND DEVELOPMENT RATE OF *AE ALBOPICTUS*

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Abstract. Mosquitoes are one of the most threatening vectors to man and transmit deadly diseases like malaria, West Nile, dengue and encephalitis viruses. Malaria infections alone are a threat to about half of the world's population and are estimated to be responsible for thousands of deaths annually. Dengue virus is responsible each year for approximately 50–100 million human cases of dengue fever and hundreds of thousands of cases of dengue hemorrhagic fever, a life-threatening form of the disease. Mosquitoes are ectothermic organisms and rely on the temperature of their environment to regulate their body temperature. *Ae albopictu*s is an invasive species of mosquito in America and is a vector for pathogens like dengue, chikungunya and West Nile viruses. *Ae albopictus* has high population growth rates in urban areas like Baltimore and thrives in temperatures around 25-30C. This species of mosquito is also known to outcompete other species of mosquitoes in container habitats. In this experiment we reared juvenile mosquitoes in 2 different species treatments (one treatment including other species and an *Ae albopictus* only treatment) in high and low densities under 2 different constant temperatures, representing ambient and future maximum July temperatures in Baltimore, Maryland (USA). We hypothesized that mostly *Ae albopictus* would emerge from the mixed treatment, that temperature would accelerate the development and increase the average size of larvae, and that larvae grown in lower densities would be bigger and have faster development rates. Our results indicated that temperature but not density impacted the survival of larvae, and neither temperature nor density affected development rate or size. However, as predicted most of the mosquitoes that emerged were *Ae albopictus.* These results mean that extreme heat due to global warming might reduce the amount of *Ae albopictus* larvae that emerge to adulthood and might suppress the population sizes of *Ae albopictus* mosquitoes in urban cities like Baltimore. The decline in the amount of *Ae albopictus* emergence due to rising temperature resulting from global warming might increase the success of other urban mosquito species.

INTRODUCTION

A vector is an organism that can transmit a disease-causing organism (pathogen) between host organisms. Vector competence is a laboratory measure of the potential for a vector (e.g. mosquitoes, ticks etc.) to become infected and subsequently to transmit a pathogen after imbibing an infectious blood meal (Alto et al. 2007). A vector ingests a pathogen while feeding on an infected host, and, in many cases, the pathogen must pass through the gut and enter the salivary glands before the vector is infectious (exceptions include *T cruzi* and *Typhus* systems in which transmission occurs via the vector's feces) (LaDeau et al 2015). Mosquitoes are one of the most concerning disease vectors to humans worldwide and they cause the deaths of thousands of humans annually. The goal of my research is to discover how rising temperature affects the size and developmental rates of mosquito larvae in high- and low-density treatments. In order to control disease risk and vector population abundance, knowledge of factors that affect physiological traits like size during the larval stage is essential.

Female mosquitoes lay their eggs in natural aquatic habitats like stagnant water bodies, tree holes and in urban habitats like car tires, trash cans and vases. After the larval stage, the larvae pupate and then emerge as full-grown adults. Mosquito larvae feed on detritus, grass, algae, and bacteria as well as other smaller organisms. Previous experiments have shown that competition for resources in the larval stage impacts the size, development rate and longevity of mosquitoes as adults. Greater competition results in significantly smaller adult female size, longer time to emergence and lower survivorship (Alto et al. 2007). Competition and other ecological interactions experienced by mosquito larvae influence morphological (e.g. size) and physiological characteristics of adult mosquitoes and may affect adult vector competence for pathogens such as arboviruses, growing evidence suggests that effects of the larval environment can continue through the adult stage because innate immunity, infection barriers, and escape barriers develop during larval stages (Buckner et al 2015). Independent investigations also show that adult longevity in *Ae aegypti* is positively correlated with body size and negatively correlated with intraspecific larval competition (Juliano et al. 2014). Environmental factors also play a role in determining which species is more successful during interspecific competition. Previous laboratory and field research show contrasting outcomes of competition between predominant *Aedes* vector species that is dependent upon resource type, where *Ae aegypti* (the yellow fever mosquito) is the superior competitor with protein-rich resources (e.g. liver powder, yeast) and *Ae albopictus* (the Asian tiger mosquito) is the superior competitor with plant detritus (e.g. leaves) (Alto et al. 2007). Likewise, density independent factors can interact with density dependent factors to affect the fitness, longevity and physiological traits of organisms in an ecosystem.

Temperature is an important abiotic factor affecting the biological processes and physiological functions, including locomotion, growth and reproduction in ectotherms. Mosquitoes reared at higher temperatures may develop more rapidly compared to lower temperatures (Ezeakacha and Yee 2019). Rising average temperature is a concern due to global warming, deforestation and urbanization. Global average temperature has increased by about 1.8°F (1C) from 1901 to 2016, largely due to human activities, especially emissions of greenhouse or heat-trapping gases (Doherty et al. 2019). With significant reductions in emissions, global temperature increase could be limited to 3.6°F (2°C) or less compared to preindustrial temperatures. Without significant reductions, annual average global temperatures could increase by 9°F (5°C) or more before the end of this century compared to preindustrial temperatures (Doherty et al. 2019). The term Urban Heat Island (UHI) effect refers to the phenomenon that ambient air and surface temperatures in urban areas are several degrees higher than surrounding rural areas (Huang et al 2011). This happens due to urbanization and the lack of enough surrounding vegetation in some urban areas. The combined effect of UHIs and global warming will affect conditions experienced by juvenile mosquitoes.

The size of an adult mosquito is set at the end of the larval stage. Size is an important determinant for vector competence because it is correlated with longevity. The size of a female mosquito, determined by its genetic background and larval environment, has long been suspected to be an important contributing factor to vector competence (Barreaux et al 2018), although there is a debate over the magnitude and even sign of the effect. Positive relationships have been observed between size and vector longevity, which enhances vector competence (Alto et al 2008). In past experiments larger *Ae albopictus* females have been found to be more fecund than their smaller counterparts (Buckner et al 2015). Density also affects growth and development rates, resulting in smaller adults with lower fecundity that would be the case for uncrowded individuals (Cochrane 1972, Hawley 1985a, b, Léonard & Juliano 1995, Frankino & Juliano 1999, Lounibos et al. 2002) or with delayed reproduction (Frogner 1980), and either of these nonlethal effects may regulate population size. Effects of small adult size, induced by food shortage, may also include reduced adult longevity (Haramis 1985, Juliano 2007). The frequency at which female mosquitoes feed determines the transmission rate of pathogens. The bigger mosquitoes will therefore have more opportunities to transmit pathogens than smaller females. Experiments done in the field on *Ae aegypti* indicate that the frequency of dengue infection increases with female size (Juliano et al. 2014). Another factor that affects vector competence is the development rate of mosquito larvae. Larvae that develop faster will be able to escape harsh conditions like the presence of predators or habitats drying out.

The Asian tiger mosquito, *Ae albopictus* is a competent vector for at least 22 arboviruses, including Zika, dengue fever and chikungunya and the rapid expansion of *Ae albopictus* around the globe is a current problem (Nejati et al. 2017). Despite being incapable of flying a distance greater than 800 m, this species has been able to spread from native tropical and subtropical areas of Southeast Asia to America, Europe and Africa as well as to Indo-Pacific and Australian regions in a matter of decades (Nejati et al. 2017). *Ae aegypti* and *Ae albopictus* have become established in many urbanized areas of the Americas, including the United States (Metzger et al 2016). In temperate cities, *Ae albopictus* is commonly found in container habitats, often cohabitating with invasive *Ae japonicus* and resident *Culex* species. Research on interspecific competition between *Ae albopictus* and *Ae aegypti* in the southern United States shows that the introduction and spread of *Ae albopictus* in the 1980s and 1990s was associated with declines in range and abundance of resident *Ae aegypti* (Alto et al. 2007). Intraspecific competition among *Ae albopictus* larvae is also an important factor limiting this species growth and emergence. In addition, to temperature, rate of growth of mosquito larva is affected by density-dependent factors, such as quantity of food and larval density (Hwa-Jen Teng, Apperson 2000). An experiment conducted to determine the effect of intraspecific competition on *Ae albopictus* competition for food and overcrowding of larvae resulted in high mortality, smaller adult size and smaller proportion of surviving females (Macchioni et al. 2016). The presence of co-occurring species (e.g., *Culex* species) may favor greater larval survival if intraspecific competition is stronger than competition between species.

The main objective of this study is to evaluate how temperature affects the size and development rate of mosquito larvae in high and low levels of competition. I hypothesized that rising temperature will accelerate the development rates of mosquitoes as well as increase the average size of mosquitoes, with more of an impact in high density treatments. I further expected *Ae albopictus* reared in mixed species colonies to grow bigger and faster compared to those in *Ae albopictus* only colonies.

METHODS

In this experiment high-density populations had 50 larvae per 125mL, and low-density treatments had 25 larvae per 125mL. Two groups of eggs were collected. One group was collected from the field in standardized ovitraps while the other group was obtained from an *Ae albopictus* colony from the University of Maryland-College Park. Eggs that were collected from the field were a mix of species (*Ae albopictus, Ae aegypti, Ae japonicus* and *Culex* mosquitoes). The 2 species treatments (Mixed vs *Ae albopictus* only) were exposed to 4 temperature/density treatments 1(High temperature/high density, high temperature/low density, low temperature/high density, low temperature/low density). Larvae were grown at constant temperature in incubators for 19 days. Jars were used as mesocosms, food came from the water collected in the standardized ovitraps from the field egg collections and was added once in equal amounts to each mesocosm at the beginning of the experiment. The ambient temperature (hereafter, low temperature) was chosen to reflect current mean high temperature for July in Baltimore, MD (31.6ºC). The second temperature (high temperature) reflects predicted increases in temperature in the next decade (36.6ºC).

- 1st Jar - *Ae albopictus* larvae in low density (25 larvae/125mL) (All *Ae albopictus* larvae).
- 2nd Jar - *Ae albopictus* larvae in high density (50 larvae/125mL) (All *Ae albopictus* larvae).
- 3rd Jar - Other species mixed with *Ae albopictus* larvae in low density (25larvae/125mL).
- 4th Jar - Other species mixed with *Ae albopictus* larvae in high density (50 larvae/125L).

A total of 4 repetitions were done for each species treatment and each temperature, giving a total of 32 jars. The number of larvae alive, number of pupae and number of adults that emerged were recorded for each jar each day until the end of the experiment. After larvae pupated, they were transferred to separate jars and the days until pupation were recorded. Once mosquitoes emerged their wing sizes were measured to the nearest millimeter.

RESULTS

Mixed-species treatment: At the end of the 19-day period significantly more larvae survived until the end of the experiment in the low temperature relative to the high temperature treatment (0 alive in high temperature vs 11 alive in low temperature) (Figure 1; t=-5.65, df=9.45, p=0.0003). Larvae alive were still in the larval stage, adults were not included in this count.

Density

There was no significant difference in the proportion of the larvae remaining in low (mean 0.055 ±.034) versus high density (mean 0.048±0.018) treatments reared at low temperatures (Figure 2; t=0.37, df=4.31, p=0.73).

Ae albopictus only treatment: At the end of the 19-day period only 16 out of 600 initial larvae were alive in *the Ae albopictus* treatment.

Temperature

Significantly more larvae from the *Ae. albopictus*-only group survived the experiment in the low temperature (all 16 surviving larvae) relative to the high temperature treatment (Figure 3; t=-3.73, df=7, p=0.007).

Density

There was no significant difference in the proportion of the larvae remaining in low (mean 0.045 ±.030) versus high density (mean 0.060±0.052) treatments reared at low temperatures (Figure 4; t=-0.50, df=4.82, p=0.64).

Adults Emerged

Mixed treatment: A total of 26 adult mosquitoes emerged from the mixed treatment under low temperature, 24 of the adults were *Ae albopictus*, 1 was *Ae aegypti* and 1 was *Culex quinquefasciatus*. Significantly more larvae from the mixed species group emerged as adults from the low temperature (26 adults) relative to the high temperature (1 adult) treatment (Figure 5; t=-2.87, df=8.43, p=0.02). The single adult that emerged from a high temperature treatment was reared in a low density mesocosm. When reared in the low temperature treatment, there was no significant effect of density treatment on adult emergence in the mixed species group.

Ae albopictus treatment: Only nine adults emerged from the *Ae albopictus* treatment. One adult emerged from the high temperature low density treatment, four adults emerged from the low temperature low density treatment and four adults emerged from the low temperature high density treatment. There were no statistical differences across treatments (Figure 6).

Wing sizes of adults

All adults but one emerged from the low temperature mixed treatment since the high temperature mixed treatment and *Ae albopictus* treatment yielded few or no adults. Sample size was only enough to test for density effects on wing sizes when reared in the low temperature treatment. There was no significant difference in the sizes of wings in male mosquitoes or female mosquitoes reared in high density (mean male size 1.66mm ± 0.21 (p=0.65), mean female size 1.820 ± 0.22 (p=0.27)) versus low density (mean male size 1.80 ± 0.17, mean female size 1.875 ± 0.1612624) treatments (Figure 7).

Development rate

We did not find that density had any influence on the development rate of larvae. Across all low temperature treatments, larvae took an average of (5.63±1.8 days) for female adult *Ae albopictus* and (8.00±4.73 days) for males to emerge as adults. There was no significant difference in the average development rate between males and females.

DISCUSSION

My results contradict some results from some previous experiments, where higher temperatures have been associated with faster development and smaller adult mosquito sizes. However, the high temperatures used in this experiment reflect a much hotter future and may have been above the thermal tolerance of this species. We did observe that algae grew in the high temperature treatment on day 9 and in the low temperature treatment on day 14 of the experiment. The algae might have been a bad food source for larvae compared to the food in the mesocosms in the beginning of the experiment and its earlier appearance may have limited growth in the high temperature treatments.

We also expected to see density and species mixing to reflect different levels of competition in the larval environment. Since *Ae albopictus* is known to be a superior competitor in temperatures lower than 30°C, (Lounibos et al. 2002) but to be sensitive to intraspecific competition, "competition for food and overcrowding of *Ae albopictus* larvae resulted in high mortality, smaller adult size and smaller proportion of surviving females" (Macchioni et al. 2016), it was expected that *Ae albopictus* would do better in mixed treatment than in the single treatment. *Ae albopictus* survival was low in both temperature treatments but the species did do better in the mixed treatment in terms of the total number of adults that emerged compared to the single species treatment. This result suggests that intraspecific competition may have been a factor in the poor performance of larvae in the single species treatment.

I conclude that temperature not density was the key determinant of larval survival and emergence to adulthood, but neither factor influenced development rate or size of *Ae albopictus* in my experiment. *Ae albopictus* did significantly better in mixed treatment than in single treatment which indicates that *Ae albopictus* is a better competitor in interspecific competition than when in intraspecific competition.

My results mean that extreme heat due to global warming might reduce the amount of *Ae albopictus* larvae that emerge to adulthood and might suppress the population sizes of *Ae albopictus* mosquitoes in urban cities like Baltimore. This might have a ripple effect and increase the population sizes of other mosquito species due to the absence of *Ae albopictus*. It could also increase the presence of more heat tolerant mosquito species. My experiment also suggests that the presence of other species in competition with *Ae albopictus* larvae might ensure a greater emergence rate of this species.

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APPENDIX

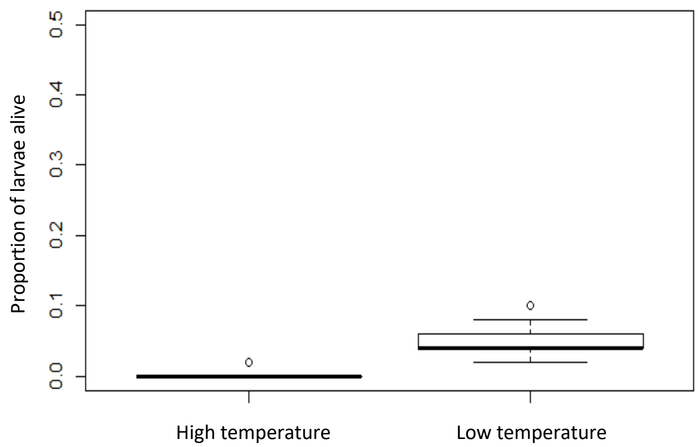

FIGURE 1. A boxplot comparing the proportion of larvae alive (number alive at the end divided by total number alive at the beginning of the experiment) (High temperature vs Low temperature) in mixed treatment at the end of the 19-day period (t=-5.65, df=9.45, p=0.0003).

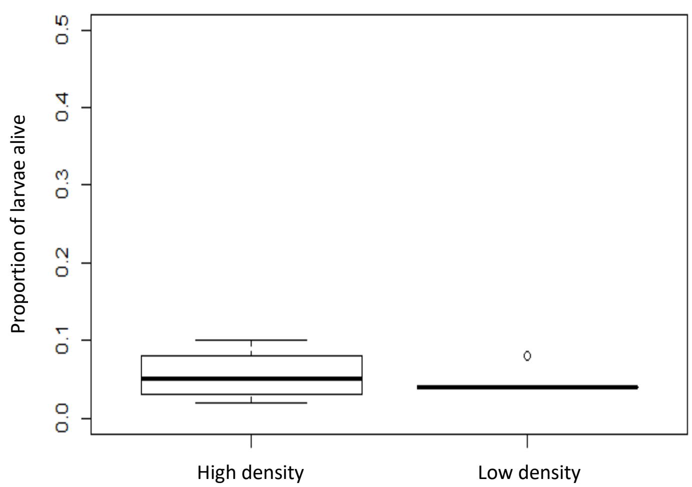

FIGURE 2. Boxplot comparing the proportion of larvae alive (number alive at the end divided by total number alive at the beginning of the experiment) (High density vs Low density) in low temperature mixed mesocosms at the end of the 19-day period (t=0.37, df=4.31, p=0.73).

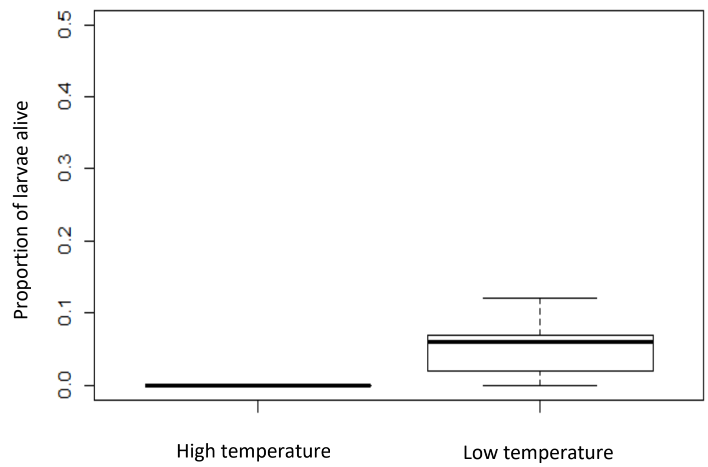

FIGURE 3. A boxplot comparing the proportion of larvae alive (number alive at the end divided by total number alive at the beginning of the experiment) (High temperature vs Low temperature) in the *Ae albopictus* only treatment at the end of the 19-day period in the Ae albopictus only treatment (t=-3.73, df=7, p=0.007).

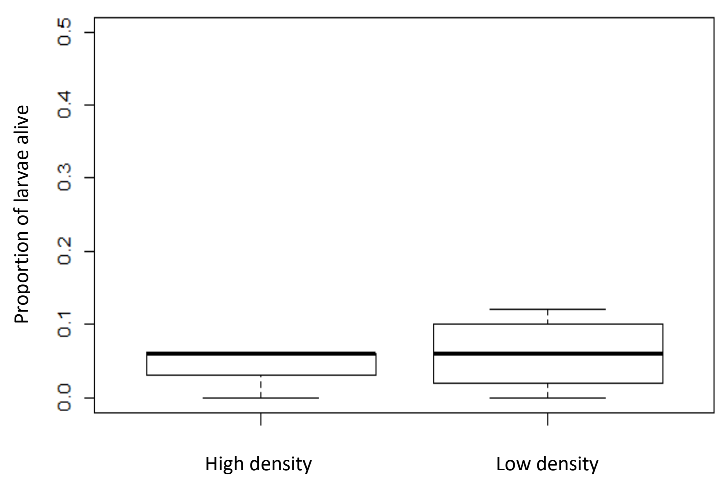

FIGURE 4. Boxplot comparing the proportion of larvae alive (number alive at the end divided by total number alive at the beginning of the experiment) (High density vs Low density) in low temperature *Ae albopictus* only mesocosms under low temperature alive at the end of the 19-day period (t=-0.50, df=4.82, p=0.64).

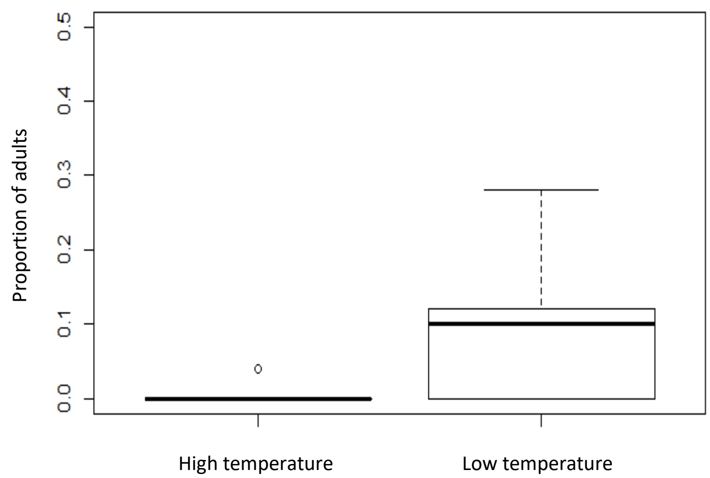

FIGURE 5. Boxplot comparing the proportion of adults that emerged (number of adults that emerged by the end divided by total number alive at the beginning of the experiment) (High temperature vs Low temperature) in mixed treatment at the end of the 19-day period (t=-2.87, df=8.43, p=0.02).

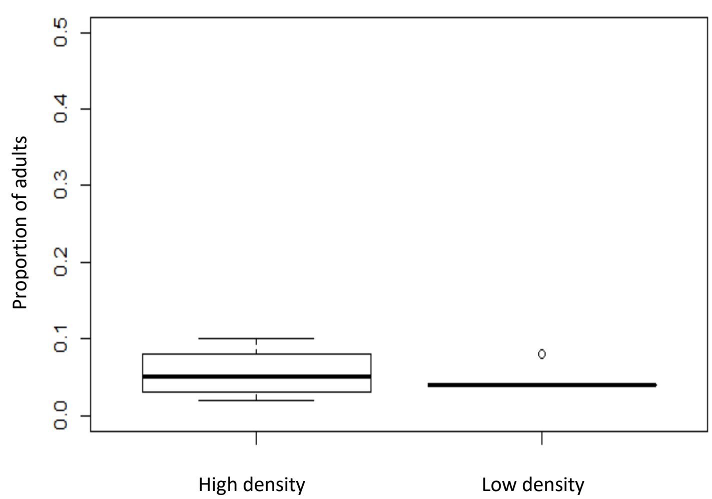

FIGURE 6. Boxplot comparing the proportion of adults that emerged (number of adults that emerged by the end divided by total number of larvae alive at the beginning of the experiment) (High density vs Low density) in low temperature *Ae albopictus* only mesocosms under low temperature at the end of the 19-day period.

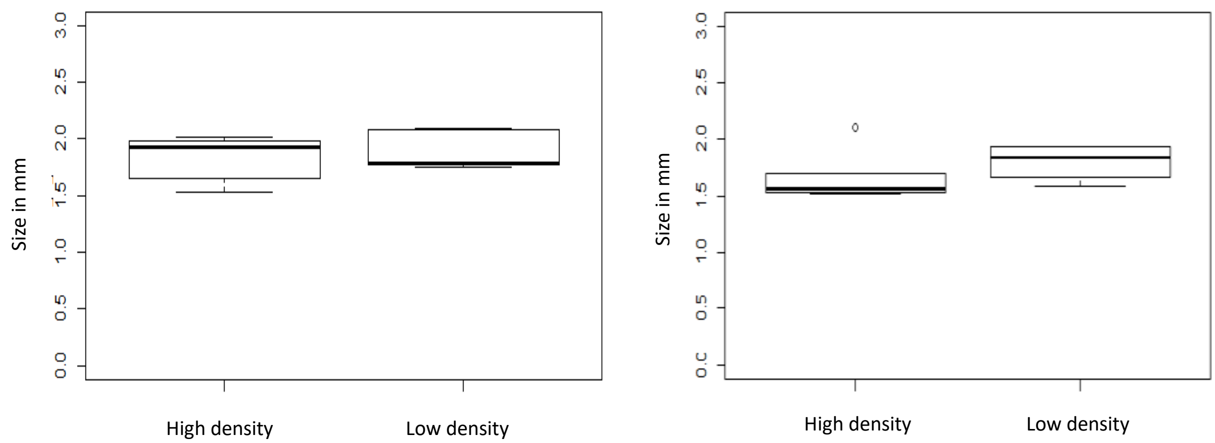

FIGURE 7. Boxplots comparing the wing sizes (mm) of the two density treatments under low temperature. Left- Female wing sizes, right – male wing sizes.