

EFFECT OF CONTAINER SIZE AND LARVAL COMPETITION ON ADULT FITNESS OF *AEDES*
AEGYPTI AND *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

BY

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THESIS

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ABSTRACT

Aedes aegypti and *Aedes albopictus* (Diptera: Culicidae), co-occur in a variety of water-filled containers where they compete for limited resources. *Aedes albopictus* larvae often outcompete those of *Ae. aegypti* but variation in biotic and abiotic parameters can modify the outcome of interspecific competition between the two mosquito species. In this study, we conducted laboratory bioassays to test the hypothesis that container size alters the magnitude and direction of intra- and interspecific competition between *Ae. aegypti* and *Ae. albopictus*. Three container sizes were tested across intra- and interspecific larval competition of *Ae. aegypti* and *Ae. albopictus*, and two levels of food were used to determine if the direction of the effects of container size on the outcome of competition were consistent regardless of nutrient availability. Both *A. aegypti* and *Ae. albopictus* had higher adult emergence from small- and medium-sized containers compared to large containers. For both species, time to eclosion was longer and adult body size was smaller for larvae reared in the large containers compared to larvae reared in the small and medium containers. There was a significant difference in emergence between the two food treatments, with the lower amount of food having lower emergence rates across all treatment combinations. Whether *Ae. aegypti* fared better under intraspecific versus interspecific competition also depended on container size. The results of our experiment show that container size can affect the outcome of intra- and interspecific competition for *Ae. aegypti* and *Ae. albopictus*. Such variation in the outcomes of competition due to differences in size of the container habitat may help account for the observed patterns of competitive exclusion and coexistence seen in the field for *Ae. aegypti* and *Ae. albopictus*.

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I. EFFECT OF CONTAINER SIZE AND LARVAL COMPETITION ON ADULT FITNESS OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

Introduction

Females of container-breeding mosquitoes oviposit in a variety of natural and artificial habitats that range in size and nutrient availability (Schneider et al., 2004; Freitas et al., 2007). Mosquito larvae developing within these habitats compete for nutritional resources within the space of the container. Generally, the density of larvae within the container affects adult life history traits with negative density-dependent effects on survival to adulthood, development time, adult body size, and longevity (Braks et al., 2004; Reiskind & Lounibos, 2009; Bevins, 2008; Alto et al., 2005, 2008). The magnitude of the negative density-dependent effects has been shown to vary based upon the initial larval density and mosquito species present (Barrera, 1996; Juliano et al. 2004; Alto et al., 2005, 2008). Often interspecific competition in the larval environment is asymmetrical resulting in competitive exclusion of the inferior competitor (Lawton & Hassell, 1981; Chesson 2000; Costanzo et al., 2005a). However, environmental factors may alter the severity of interspecific competition, which may reduce asymmetry, allow for coexistence, or reverse the competitive advantage (Chesson 2000; Costanzo et al., 2005b; Murrell & Juliano, 2008; Juliano 2010). This condition-specific competition can impact species distributions and adult life history traits (Leisnham & Juliano, 2009; Costanzo et al., 2005b). Changes in adult life history traits have been shown to influence vector competence (i.e. the ability of a vector to transmit a pathogen) (Bara et al., 2015; Alto et al., 2005, 2008). However, even for the relatively well-studied mosquito species, few studies have examined the biotic and abiotic parameters that lead to condition-specific competition (Murrell & Juliano, 2008; Gardner et al., 2015; Costanzo et al., 2005b). This lack of knowledge inhibits understanding of the prevalence, pervasiveness, and mechanisms of condition-specific competition that result in either competitive exclusion or coexistence.

The larvae of two medically important vector mosquitoes, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), co-occur in container habitats throughout portions of their invasive ranges, and provide a model system for investigating how various parameters affect condition-specific competitive outcomes (Juliano et al., 2004). *Aedes aegypti* was introduced to the Americas from sub-Saharan Africa during colonial times and is well established in the southeastern United States (Baracco et al., 2007; Jansen & Beebe, 2010; Powell & Tabahnick, 2013). Similarly, the geographic range of *Ae. albopictus*, since its introduction to Texas from Asia in the 1980s, has continued to expand throughout the eastern United States (Moore et al., 1997), resulting in increasing interspecific competition between larval *Ae. aegypti* and *Ae. albopictus* (Juliano et al., 2004; Benedict et al., 2007). Under most conditions, *Ae.*

albopictus larvae outcompete those of *Ae. aegypti*, leading to competitive exclusion of *Ae. aegypti* from larval habitats (O'Meara et al. 1995; Juliano, 2010). Yet field surveys indicate *Ae. aegypti* is able to coexist with *Ae. albopictus* in certain areas of their over-lapping ranges (Juliano et al., 2004), suggesting environmental variables may mediate the outcome of competition. Manipulations of various parameters within the container habitats can reduce or negate competitive exclusion leading to the observed coexistence at some locations (Murrell & Juliano, 2008; Braks et al., 2004; Farjana et al., 2012).

Container size is one of the major factors that likely influences the outcome of interspecific larval competition between *Ae. aegypti* and *Ae. albopictus*. The impact of the size of the larval container habitat on condition-specific competitive outcomes has received relatively scant investigation (Schneider et al., 2004). Field studies have shown that density and distribution of *Ae. aegypti* and *Ae. albopictus* larvae vary by container type and size, for containers ranging from flower plates and bottles to boat hulls and rain barrels (Morrison et al., 2004; Maciel-de-Freitas et al., 2007; Delatte et al., 2008; Bartlett-Healy et al., 2012). Few studies have measured life history traits for the adults emerging from these habitats, though Schneider et al. (2004) demonstrated that body size of female *Ae. aegypti* increases with container diameter. While the effects of intra- and interspecific competition in the larval environment on adult life history traits are well documented for *Ae. aegypti* and *Ae. albopictus* (Braks et al., 2004; Reiskind & Loubinos, 2009; Alto et al. 2005, 2008; Juliano et al, 2004), few studies have explored how biotic and abiotic conditions may account for the variation seen in the results of these competition studies (Murrell & Juliano, 2008; Juliano, 2010). Understanding how variation in container size affects adult life history traits can aid in our understanding of the underlying mechanisms that result in condition-specific competition within the larval habitat.

In this study, we examined the effect of container size and intra- and interspecific larval competition on adult emergence rates, time to adult eclosion, and adult body size for *Ae. aegypti* and *Ae. albopictus* under laboratory conditions. Three container sizes were tested across intra- and interspecific larval competition of *Ae. aegypti* and *Ae. albopictus*, and two levels of food were used to determine if the direction of the effects of container size on the outcome of competition were consistent regardless of nutrient availability. We hypothesized that A) container size would alter the outcome of both intraspecific and interspecific competition, and B) variation in emergence, time to adult eclosion, and adult body size would be correlated to these outcomes of competition determined by differences in container size.

Materials and Methods

Manipulation of the larval environment

This experiment used F₅ progeny of *Ae. aegypti* originally from Key West, Florida, and F₉ progeny of *Ae. albopictus* originally collected from Mississippi. A full factorial experimental design with three factors: food (low: 0.1 g, or high: 0.15g of 1:1:1 ratio of rabbit chow: lactalbumin : brewer's yeast), container size (small: 10.8 cm high x 6.4 cm diameter with 0.2 L water, medium: 19.1 cm high x 17.8 cm diameter with 2.0 L water, or large: 35.6 cm high x 25.4 cm diameter with 10.0 L water) and intra- and interspecific larval competition (100:0, 50:50, 0:100 *Ae. aegypti*: *Ae. albopictus*) was used in this study. Container sizes used in this experiment were within the range of containers documented to contain *Ae. aegypti* and *Ae. albopictus* larvae in the field (Delatte et al., 2008; Maciel-de-Freitas et al., 2007). Each treatment was replicated four times for a total of 72 larval containers.

Larvae were reared in one of three container sizes and provisioned with larval food consisting of 1:1:1 ratio of rabbit chow : lactalbumin : brewer's yeast.. Initial food resources (0.05 g for low nutrient levels, 0.075 g for high nutrient levels) were added to the containers 24 h before first instar (< 24 h old) larvae were introduced. The remainder of the food resources (0.05 g for low nutrient levels, 0.075 g for high nutrient levels) were added to the containers five days later. Pilot studies showed that these are sufficient resources for larval development while still allowing for larval competition to manifest at both food levels (A. Parker, *unpublished data*). Larval containers were maintained at 26 ± 1°C, 70% relative humidity, and a 16:8 (L:D) photoperiod. Pupae were removed daily and placed into plastic vials with water by replicate until eclosion. The date, sex, and species of newly enclosed adults were recorded.

Measurement of adult life history traits

Adults were sorted by species, sex, date of eclosion and replicate, and their wing lengths were measured using microscopy and an image analysis system (MicroSuite Version 5.0; Olympus Soft Imaging Solutions Corp., Lakewood, CO). For both males and females, the mean proportion of adults that emerged (hereafter 'emergence'), mean time to eclosion, and mean adult body size (using the well-established proxy of wing length), were calculated for each replicate to determine the effect of container size, amount of food, and intra- and interspecific larval competition on *Ae. aegypti* and *Ae. albopictus*. Time to eclosion and adult body size are important correlates of the potential for disease transmission (i.e. vectorial capacity) in mosquitoes (Alto et al. 2005, Alto et al. 2008).

Statistical analysis

A general linear model (GLM) with a factorial treatment structure was used to test the fixed main effects of container size, amount of food, larval competition and all possible two- and three-factor interactions with adult emergence as the response. The analysis was stratified by species with separate models performed for *Ae. aegypti* and *Ae. albopictus* (males and females combined). Tukey's Means Separation Tests were used to detect significant pairwise differences between levels of each treatment. Adult emergence was calculated by dividing the total number of adults by the total number of larvae of a given species originally in the container. Separate Multivariate Analysis of Variance (MANOVA) tests were conducted to test for the fixed effects of container size, amount of food, larval competition and their interactions on time to eclosion (days) and wing length (mm) for both male and female *Ae. aegypti* and *Ae. albopictus*. Here, the models were stratified by both species and sex because male mosquitoes typically develop more rapidly and have a smaller adult body size than female mosquitoes (Murrell & Juliano, 2008, Peters & Barbosa, 1977). Standardized canonical coefficients (SCCs) were used to characterize the relative contributions of time to eclosion and wing length to significant treatment effects as well as the relationship between the three predictor variables. Shapiro-Wilk tests for normality and Brown-Forsyth tests for homogeneity of variances were used to confirm that the raw data residuals met the assumptions of the analysis for all response variables and thus no transformations were required.

Results

Emergence

Aedes aegypti emergence varied across levels of the container size and food treatments but not the competition treatment. There were significant interactions between the main effects of container size and amount of food and the main effects of container size and larval competition (Table 1). The interaction between container size and amount of food generally reflected differences in magnitude, not direction, of the effect of food across levels of container size. The lower amount of food yielded lower *Ae. aegypti* emergence rates across all container sizes, but this effect was the most pronounced in large containers (Figure 1). The interaction between container size and larval competition reflected differences in the direction as well as magnitude of the effect of competition across levels of container size (Table 1). *Aedes aegypti* larvae reared in containers exposed to interspecific competition had higher emergence in the small and medium containers compared to larvae exposed only to intraspecific competition, while the opposite was found for emergence in the large container (Figure 1).

For *Ae. albopictus*, the main effects of container size and amount of food had significant effects on emergence, but not the main effect of competition nor any interaction effects (Table 1, Figure 1). There was a significant difference in emergence for the large containers compared to both the small and medium containers, with the large containers having the lowest emergence across all treatment combinations (Table 2, Figure 1). There also was a significant difference in emergence between the two food treatments with the lower amount of food having lower emergence across all treatment combinations (Table 3, Figure 1).

Aedes aegypti development time and adult body size

For both male and female *Ae. aegypti*, MANOVA detected significant main effects of container size and larval competition, and significant interactions between the main effects of container size and competition and the main effects of container size and amount of food (Table 4). For female *Ae. aegypti*, development time most strongly contributed to the interaction between container size and larval competition, while development time and wing length were equally strong contributors to the interaction between container size and larval competition for male *Ae. aegypti* (Table 4). Both development time and wing length of female *Ae. aegypti* contributed to the interaction between container size and amount of food but their opposite signs suggest that their relationship is inconsistent across treatment combinations (Table 4). In general, longer times to eclosion were observed in large containers compared to medium and small containers for both males and females (Figure 2). Development time showed a strong contribution to larval competition for both males and females (Table 4). For males and females, time to adult eclosion

was shorter for *Ae. aegypti* larvae reared in containers exposed to interspecific competition compared to larvae reared in containers exposed to intraspecific competition (Figure 2). For male and female *Ae. aegypti*, adult wing length was shorter for larvae reared in the lower food treatments compared to the higher food treatment (Figure 2).

Aedes albopictus development time and adult body size

For both male and female *Ae. albopictus*, MANOVA demonstrated significant main effects of container size and amount of food (Table 4). SCCs show that development time most strongly contributed to the variance observed for container size for male and female *Ae. albopictus* (Table 4). The longest time to eclosion was observed in the large containers, while the medium and small containers had a shorter time to eclosion for both males and females (Figure 2). Wing length most strongly contributed to the variance observed for the amount of food for both males and females (Table 4). In general, adult wing length was shorter for larvae reared in the lower food treatments compared to the higher food treatment for both males and females (Figure 2). For male *Ae. albopictus*, there was a significant effect of larval competition (Table 4). Wing length most strongly contributed to the variance observed for larval competition for male *Ae. albopictus* (Table 4). For male *Ae. albopictus* larvae reared in containers exposed to interspecific competition, wing length was smaller compared to male *Ae. albopictus* larvae reared in containers exposed to intraspecific competition (Figure 2). For female *Ae. albopictus*, there was a significant effect of the interactions between container size and amount of food with wing length being a strong contributor (Table 4).

Discussion

Larvae of both *Ae. aegypti* and *Ae. albopictus* can develop in a variety of container sizes (Barrera et al, 2006; Scheider et al., 2004), but no study to date has examined how co-occurrence of larvae within containers of different sizes affects the outcomes of intra- and interspecific competition. We found that variation in the container size of the larval habitat affects adult life history traits for *Ae. aegypti* and *Ae. albopictus*. These results show that container size may mediate competition between these two species with *Ae. aegypti* showing potential to outcompete *Ae. albopictus* in certain sizes of larval container habitats, which may help to account for the ongoing co-occurrence of these two species in some areas of their overlapping, introduced ranges.

Data from both emergence and adult fitness traits in this study support our hypothesis that container size affects the outcome of intra- and interspecific larval competition for *Ae. aegypti* and *Ae. albopictus*. In small- and medium-sized containers, *Ae. aegypti* larvae exposed to interspecific competition had higher adult emergence than the larvae exposed to intraspecific competition, whereas there was a higher adult emergence for *Ae. aegypti* in the larger containers for larvae exposed to intraspecific competition. In the small- and medium-sized containers, *Ae. albopictus* larvae exposed to interspecific competition had lower adult emergence than *Ae. albopictus* larvae exposed to intraspecific competition. These results suggest that *Ae. aegypti* may be able to outcompete *Ae. albopictus* within certain sizes of container habitats. Females of both species have been shown to preferentially oviposit in larger containers (Harrington et al. 2008; Reiskind & Zarrabi, 2012). Schneider et al. (2004) demonstrated that females emerging from containers with greater diameters have larger adult body sizes, but interestingly the same study found no significant relationship between container diameter and abundance of larvae and pupae. The periodic introduction of new food resources to larval habitats in the field may occur more frequently in containers with larger diameters provisioning developing larvae with additional resources and thereby accounting for the larger body size in emerging adults. In this study, we did not periodically add additional food resources to the containers, which may account for the smaller adult body sizes observed in adults emerging from the large containers.

We varied food levels to ensure the observed results were not solely driven by amount of food available within the container habitats, but instead to see if the observed results were due to container size, and to explore for any interactions between food availability and container size. For both *Ae. aegypti* and *Ae. albopictus*, differences between food treatments in the effects of competition on emergence and body size were generally differences of magnitude and not direction. Low levels of larval nutritional resources can cause increased levels of larval competition. This results in lower adult emergence rates and

lower adult fitness across mosquito species when compared to similar larval habitats with higher levels of food resources (Merritt et al., 1992; Reiskind et al., 2004; Araújo & Gil, 2012; Braks et al., 2004), consistent with the results of this experiment. *Aedes albopictus* outcompetes *Ae. aegypti* in container habitats where the dominant resource is leaf detritus while *Ae. aegypti* outcompetes *Ae. albopictus* in container habitats where the dominant resource is animal carcasses, which are high in protein (Barrera 1996). The artificial diet used in this study has a high protein content from the inclusion of lactalbumin (Schlaeger, 1996). Thus it is possible that the reduced intraspecific competition experienced by larval *Ae. aegypti* within interspecific competition treatments conferred a development advantage to *Ae. aegypti*. In order to examine this hypothesis, lower levels of intraspecific competition (e.g., fifty total larvae of an individual species per container), which were not tested in this study, would need to be included. Thus it is possible that the results seen in this study may have differed if food resources better mimicked those found when leaf detritus is the primary resource (Murrell & Julinao, 2008; Daugherty et al., 2000).

One potential explanation for the difference in emergence between the small and medium containers compared to the large containers is the energy expended by larvae when foraging for food. *Aedes aegypti* and *Ae. albopictus* larvae are considered collector-gathers, removing food particles loosely deposited on submerged substrate, but can also filter feed in the water column on suspended food particles (Merritt et al., 1992; Skiff & Yee, 2014). Larvae in large containers may have to expend more energy to forage along the bottom of the container, which was at a greater depth compared to the small and medium containers, in order to locate food particles. Along with increased difficulty finding food in the large container, increased separation between food particles at the bottom of the container and access to air at the surface of the large containers may have led to the lowest foraging efficiency and the highest expenditure of energy per food resources acquired. This may also explain the higher emergence of larvae reared in the high food treatments where more food particles likely are available allowing the larvae to forage at a higher efficiency. The results of this study suggest that larval *Ae. aegypti* may be better foragers than *Ae. albopictus* in small- and medium-sized containers when limited, protein-rich resources are available. This may indicate another mechanism for the co-existence of *Ae. aegypti* and *Ae. albopictus* within certain areas of their over-lapping geographic ranges.

Understanding how larval container size may affect the distribution and abundance of *Ae. aegypti* and *Ae. albopictus* is not only of fundamental ecological interest, but may also have medical implications. Both species occur globally in tropical and subtropical regions (Kraemer et al., 2015) and are vectors of pathogens such as dengue, chikungunya, and Zika viruses (Furuya-Kanamori et al., 2016; Wong et al., 2013). Studies have shown that eliminating the most productive larval container habitats can reduce the

density of adult mosquitoes (Maciel-de-Freitas & Lourenço-de-Oliveira, 2011) thereby reducing human-vector contact. Implementation of proactive vector control measures, such as removal of the most productive larval habitats, not only reduces the density of adult mosquitoes, but can also reduce the occurrence of human cases of mosquito-borne diseases (Eisen et al., 2009).

The results of this laboratory experiment provides the first evidence that the size of the container can mediate the outcomes of intra- and interspecific competition for *Ae. albopictus* and *Ae. aegypti*. In order to use container size as an indicator of condition-specific competition, field studies need to survey the types and sizes of containers found in areas where the ranges of these two species overlap. Future studies need to examine how changing various parameters of the container size and shape (e.g. manipulating the surface area while holding volume constant) affect competition between *Ae. aegypti* and *Ae. albopictus*, which will help define the mechanistic relationship between container shape and size on the outcome of condition-specific competition.

Figures

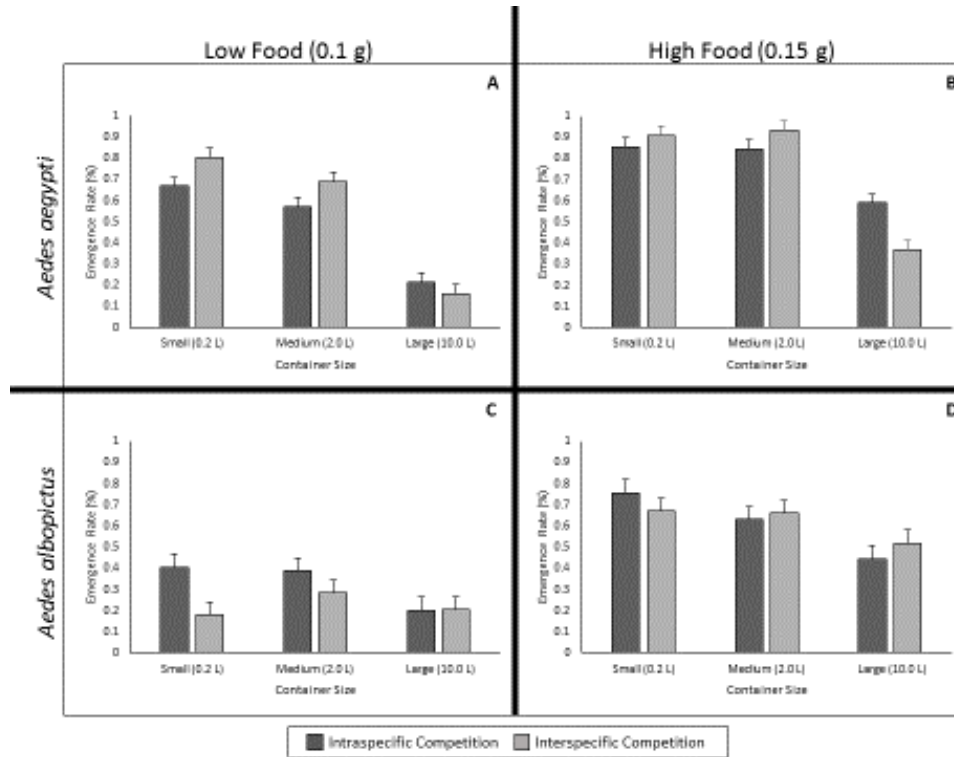


Figure 1. Mean ($\pm$ s.e.) for emergence rates across intra- and interspecific competition by amount of food and container size for A.) *Ae. aegypti* developing in the low food treatment, B.) *Ae. aegypti* developing in the high food treatment, C.) *Ae. albopictus* developing in the low food treatment, and D.) *Ae. albopictus* in developing the high food treatment.

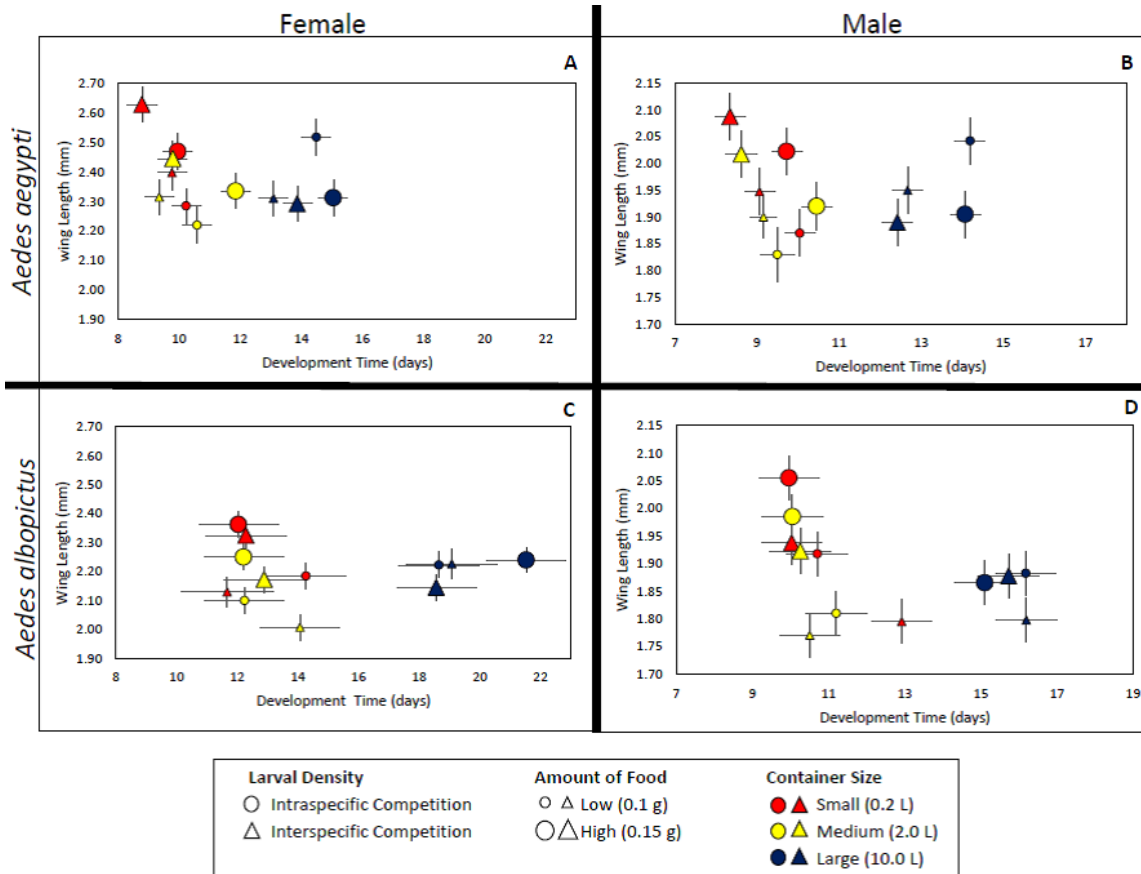


Figure 2. Bivariate plot of least square (LS) means ($\pm$ s.e.) for time to eclosion and wing length across intra- and interspecific competition by amount of food and container size for A.) *Ae. aegypti* females, B.) *Ae. aegypti* males, C.) *Ae. albopictus* females, and D.) *Ae. albopictus* males.

Species	Variable	df	SS	MS	F	P
<i>Ae. aegypti</i>	Container size	2	2.1910	1.0955	156.72	<.001
	Food	1	0.6487	0.6487	92.80	<.001
	Container size x Food	2	0.0469	0.0235	3.36	0.046
	Competition	1	0.0052	0.0052	0.75	0.3928
	Container size x Competition	2	0.1505	0.0753	10.77	<0.0010. 0002
	Food x Competition	1	0.0252	0.0252	3.61	0.066
	Container Size x Food x Competition	2	0.0096	0.0048	0.69	0.511
<i>Ae. albopictus</i>	Container size	2	0.2534	0.1267	8.37	0.001
	Food	1	1.3568	1.3568	89.58	<.0001
	Container size x Food	2	0.0452	0.0226	1.49	0.239
	Competition	1	0.0315	0.0315	2.08	0.158
	Container size x Competition	2	0.0774	0.0387	2.56	0.092
	Food x Competition	1	0.0391	0.0391	2.58	0.117
	Container Size x Food x Competition	2	0.0026	0.0013	0.09	0.917

Table 1. Analysis of Variance (ANOVA) for the effect of container size, amount of food, and intra- and interspecific competition on adult emergence for *Ae. aegypti* and *Ae. albopictus*.

Effect	Levels	Emergence LSMean	Significance Letters
Containers	Small (0.2 L)	0.8094	A
	Medium (2.0 L)	0.7606	A
	Large (10.0 L)	0.3338	B
Amount of food	0.1 g	0.5183	B
	0.15 g	0.7508	A

Table 2. Tukey comparison lines for least square means for adult emergence for *Ae. aegypti* for the effect of container size and amount of food. Different letters reflect significant pairwise differences between treatments.

Effect	Levels	Emergence LSMean	Significance Letters
Container Size	Small (0.2 L)	0.5031	A
	Medium (2.0 L)	0.4906	A
	Large (10.0 L)	0.3431	B
Amount of food	0.1 g	0.2775	B
	0.15 g	0.6138	A

Table 3. Tukey comparison lines for least square means for adult emergence for *Ae. albopictus* for the effect of container size and amount of food. Significance letters that are different reflect significant pairwise differences between treatments.

Species	Sex	Variable	df	Pillai's trace	P	SCCs	
						Development Time	Wing Length
<i>Ae. aegypti</i>	Male	Container size	4	1.0248	<0.001	3.2722	0.6087
		Food	2	0.0996	0.160	0.2038	1.2537
		Container size x Food	4	0.4331	0.001	1.4910	1.3634
		Competition	2	0.5220	<0.001	3.1974	0.3398
		Container size x Competition	4	0.2460	0.048	2.2805	1.3005
		Food x Competition	2	0.0669	0.298	3.0280	0.1054
		Container Size x Food x Competition	4	0.0872	0.516	3.1902	0.8684
	Female	Container size	4	0.9742	<0.001	2.3365	0.0617
		Food	2	0.1458	0.064	1.3844	1.1402
		Container size x Food	4	0.3430	0.008	-0.7794	1.2119
		Competition	2	0.2632	0.005	2.2734	-0.1959
		Container size x Competition	4	0.3229	0.012	0.2963	1.3318
		Food x Competition	2	0.0805	0.230	1.7410	-0.7979
		Container Size x Food x Competition	4	0.1026	0.428	2.3257	0.2576
<i>Ae. albopictus</i>	Male	Container size	4	0.8713	<0.001	1.9136	0.2344
		Food	2	0.4031	<0.001	-0.3746	1.2388
		Container size x Food	4	0.1859	0.130	0.4358	1.4128
		Competition	2	0.1996	0.020	0.0197	1.3533
		Container size x Competition	4	0.0871	0.517	-0.7934	1.0510
		Food x Competition	2	0.0093	0.850	-0.2013	1.2961
		Container Size x Food x Competition	4	0.1064	0.408	1.7973	0.9053
	Female	Container size	4	0.9647	<0.001	1.6026	-0.3225
		Food	2	0.3306	0.001	-0.4300	1.4236
		Container size x Food	4	0.3430	0.011	-0.8017	1.3702
		Competition	2	0.1284	0.104	-0.0280	1.3864
		Container size x Competition	4	0.0958	0.495	1.4862	-0.8410
		Food x Competition	2	0.0074	0.885	0.8666	0.9725
		Container Size x Food x Competition	4	0.0911	0.522	1.3832	0.3962

Table 4. Multivariate Analysis of Variance (MANOVA) for the effect of container size, amount of food, and intra- and interspecific competition on time to eclosion (days) and wing length (mm) for both male and female *Ae. aegypti* and *Ae. albopictus*. Standardized canonical coefficients (SCCs) show the relative contribution to each life history trait to the multivariate effect.

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