Article

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The Interspecific Competition between larvae of *Aedes aegypti* and Major African Malaria Vectors in a Semi-Field System in Tanzania

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Simple Summary: *Aedes* and *Anopheles* mosquitoes are major public health threats due to their ability to transmit diseases such as dengue, malaria, yellow fever, Zika, and Chikungunya to humans. Emerging studies are showing the coexistence of these vectors at larval stage particularly in urban areas. Its coexistence may lead to interspecific competition for the limited resources such as food and habitat, impacting their growth rate, development and survival. This may indirectly and negatively affect the vectorial capacity of adult's mosquitoes and reduce risks of disease transmission thereof. In addition, this phenomenon may influence cannibalistic and predatory behaviors and lead to change in species composition and abundance. This study systematically, examined the effect of interspecific competition between *Aedes aegypti* and major African malaria vectors, such as *Anopheles arabiensis*, *Anopheles gambiae* s.s., or *Anopheles funestus*, on individual fitness in semi-field settings from June to September The results showed that interspecific competition significantly affected both genera, however, *Anopheles* species were more impacted compared to *Aedes aegypti*.

Abstract: Interspecific competition between mosquito larvae may affects adult vectorial capacity, potentially reducing disease transmission. It also influences population dynamics, cannibalistic and predatory behaviors. However, knowledge of interspecific competition between *Ae. aegypti* and *Anopheles* species is limited. The study examined interspecific competition between *Ae. aegypti* larvae and either *An. arabiensis*, *An. gambiae,* or *An. funestus* on individual fitness in semi-field settings. The experiments involved density combinations of 100:100, 200:0, and 0:200 (*Ae. aegypti: Anopheles*), reared with and without food, in small habitat (8.5 cm height × 15 cm diameter) with 0.5 litre and medium habitats (15 cm height × 35 cm diameter) with 1 litre of water. The first group received Tetramin® fish food (0.02 g), while the second group was unfed to assess cannibalism and predation. While, interspecific competition affected both genera, *Anopheles* species experienced greater effect, with reduced survival and delayed development, compared to *Ae. aegypti*. The mean wing lengths of all species were significantly small in small habitats in mixed population ($p < 0.001$). The presence of food reduced cannibalism and predation compared to its absence. These interactions have implications for diseases transmission dynamics and can serve as biological indicators to signal the impacts of vector control interventions.

Keywords: Inter-specific competition; intra-specific competition; *Aedes aegypti; An. arabiensis; An. gambiae; An. funestus*; predation; cannibalism

Introduction

Mosquitoes such as *Aedes*, *Anopheles* and *Culex* species are major public health threats due to their role in transmitting vector borne disease (VBD) such as malaria, yellow fever, dengue, Zika, Chikungunya, Rift valley fever, lymphatic filariasis and West Nile fever to humans [1]. Globally, around 80% of people are at risk of being affected by at least one VBD that accounts for an estimated 17% of the global burden of infectious diseases and causes about 700,000 mortalities each year [2]. Among vectors that pose significant threats to public health such as *Aedes* and *Anopheles* species coexist at the larval stage in urban and suburban areas as documented in previous studies [3–7]. This aligns with WHO malaria report, that infrastructure development can affect the distribution and quality of breeding sites, making mosquitoes to adapt to changing environments and capable of surviving outside their natural aquatic habitats [8]. The preferred aquatic habitats for *Anopheles* species; *An. gambiae* and *An. arabiensis* species include small and temporary clean water such as puddles, hoof prints, tire tracks, and rain pools [9] and large, vegetated semi-permanent and permanent aquatic habitats such as swamps, ponds, and river streams for *An.funestus* [10,11]. On the other hand, *Aedes* species prefers man-made or natural habitats such as containers, tree holes, pitchers, flower pots, roof gutters, tires, common in urban environment [10,12,13].

When different mosquito species coexist at the larval stage, interspecific competition for limited resources such as food, space (habitat) and oxygen arise. This competitive pressure influences mosquito larval growth rate, development, survival [14] and behaviors such as cannibalism and predation [15–17], thereby affecting species composition and abundance within ecosystem [18,19]. It has been documented that interspecific competition between mosquito's larvae may indirectly and negatively influence adult life history traits such as vector competence, body size, fecundity, pathogen susceptibility, longevity, flight capability and overall vectorial capacity, potentially reducing the risks of diseases transmission [18–20]. Laboratory and field studies on interspecific competition between *Aedes* and *Culex* species reported slow larval development, low larval survival rate, small body size, reduced fecundity, and imbalance sex ratio between the test species [21]. Competitive interactions can drive cannibalistic and predatory behavior which are crucial for understanding mosquito population regulation. These behaviors drastically reduce population size below its carrying capacity contributing to self-regulation [22,23]. Understanding the effect of interspecific competition is crucial for comprehending the dynamics of mosquito populations and their implications for disease transmission. While several studies have focused within the same genus of *Aedes, Culex* and *Anopheles* [14,15,20,24–26], the knowledge of interspecific competition between *Ae. aegypti* and major African malaria vectors is still limited.

The interaction between biotic and abiotic factors collectively shape the population dynamics of adult's mosquitoes [27]. On the other hand, this interaction can alter the effects of competition between different species, potentially reducing competition, leading to the coexistence of species, or changing the advantage of one species over the other [25,28–30]. Based on environmental variations, vectorial capacity parameters vary temporally and spatially and a single environmental component might have antagonistic effects on several different vectorial capacity parameters [31,32]. High temperatures may increase vector competence, lower the extrinsic incubation period, and simultaneously shorten adult lifespan [19,33] but also yield more [34] or less competent vectors for pathogens [35]. Correspondingly, the mosquitoes larval environment (i.e. competition, larval density, nutritional status) may also influence their susceptibility to infections and disease transmission [36,37]. Understanding the consequences of interspecific competition between *Ae. aegypti* and major African malaria vectors is not just ecologically significant but also holds epidemiological importance due to their role in disease transmission [38].

To understand the impacts of interspecific competition between mosquito's larvae, the study developed the following question; how the competitive interactions between larvae of *Ae. aegypti* versus *An. arabiensis, Ae. aegypti* versus *An. gambiae* and *Ae. aegypti* versus *An. funestus* can affect individual fitness in semi field settings.? To answer the question, the experiments were set up with intraspecific (single specie as a control) and interspecific (multiple species) competition in small and medium habitats, both with and without food.

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Materials and methods

2.Study area

Experiments were carried out between June and September 2023 in semi field system (SFS) at the Mosquito City facility of the Ifakara Health Institute (IHI), located in Kining'ina village (8.11417^0 S, 36.67484^0 E), of Kilombero district, Southern of Tanzania. As described in other studies, the SFS is a large, netted cage enclosure with vegetation and breeding habitats that mimic a natural environment [39]. The temperature and relative humidity were recorded daily using a Tiny tag® data logger placed inside the SFS.

2.Study design

The study used a full factorial experimental design to determine the effect of competition (intra and interspecific) and habitat size on mosquito fitness parameters, larval developmental time, survival to adult as well as wing length as a proxy for adult body size. Additionally, the design was used to determine the effect of food, competition and habitat size on the rate of cannibalism and predation between test species. The factorial design allows for testing all combinations of factors and their levels to assess their individual and interaction effects on the outcome of interest.

2.Larval habitats

Two habitat sizes were created from plastic basins; small (8.5 cm height × 15 cm diameter) with 0.5 liter of water and medium (15 cm height × 35 cm diameter) with 1 liter of water were used. The habitats were monitored daily and replenished as necessary to maintain the same volume. The habitats were covered with nets to prevent emerging adult mosquitoes from escaping. The temperature of the water in the habitats was measured using a thermometer placed directly in the larval habitat.

2.Experimental procedures

Three sets of species density combinations with interspecific and intraspecific at a ratio of 100:100, 200:0 and 0:200 (*Ae. aegypti: Anopheles)*, that included combinations of *Ae. aegypti* with either 1) *An. arabiensis*, 2) *An. gambiae* s.s. or 3) *An. funestus* respectively were set up. Instar two larvae from the laboratory were introduced to small and medium habitats and reared to adults, either in the presence or absence of food. This was designed specifically to assess the rate of cannibalism and predation among mosquitoes' larvae in both presence and absence of food. Those selected to receive food were fed Tetramin® fish food (0.02 g) twice per day. Each of 12 treatments was replicated six times making a total of 72 larval habitats per species combination and 216 larval habitats for three sets of species combinations (Figure 1). Experimental procedures were identical for all three experiments in both intra and interspecific competition.

2.Data collection

Larvae survival was monitored daily by recording the total number alive, dead and missing. Missing or damaged larvae were considered to have been consumed due to cannibalism or predation, while undamaged dead larvae, that were removed daily, were attributed to natural mortality [40]**.** After every 24 hours, cannibalism was recorded within same specie (intraspecific competition) and predation when *Ae. aegypti* were mixed with *Anopheles* species (interspecific competition). The pupae collected daily from each habitat were transferred to paper cups with water (50mls) and placed in a net cage (41 cm height × 35 cm length × 33 cm width) where it was monitored until all emerged to adult or died, a point that marked the end of the experiment. The emerged adults were recorded into their respective species. The number of days between introduction of larvae into the habitats to pupation and adult emergence were used to estimate developmental time and larval survival to adults respectively. Subsample of 10 mosquitoes per specie for every combination ratio were used for wing length measurement as described in a previous study (44). A single wing was removed from each female, placed on a glass microscope slide, and measured from the alular notch to the wing tip, excluding the wing fringe. Wing length was measured in millimeters using computer imaging software with a phase contrast microscope. The strong correlation between wing length and dry body weight led to its usage as a body size metric [41].

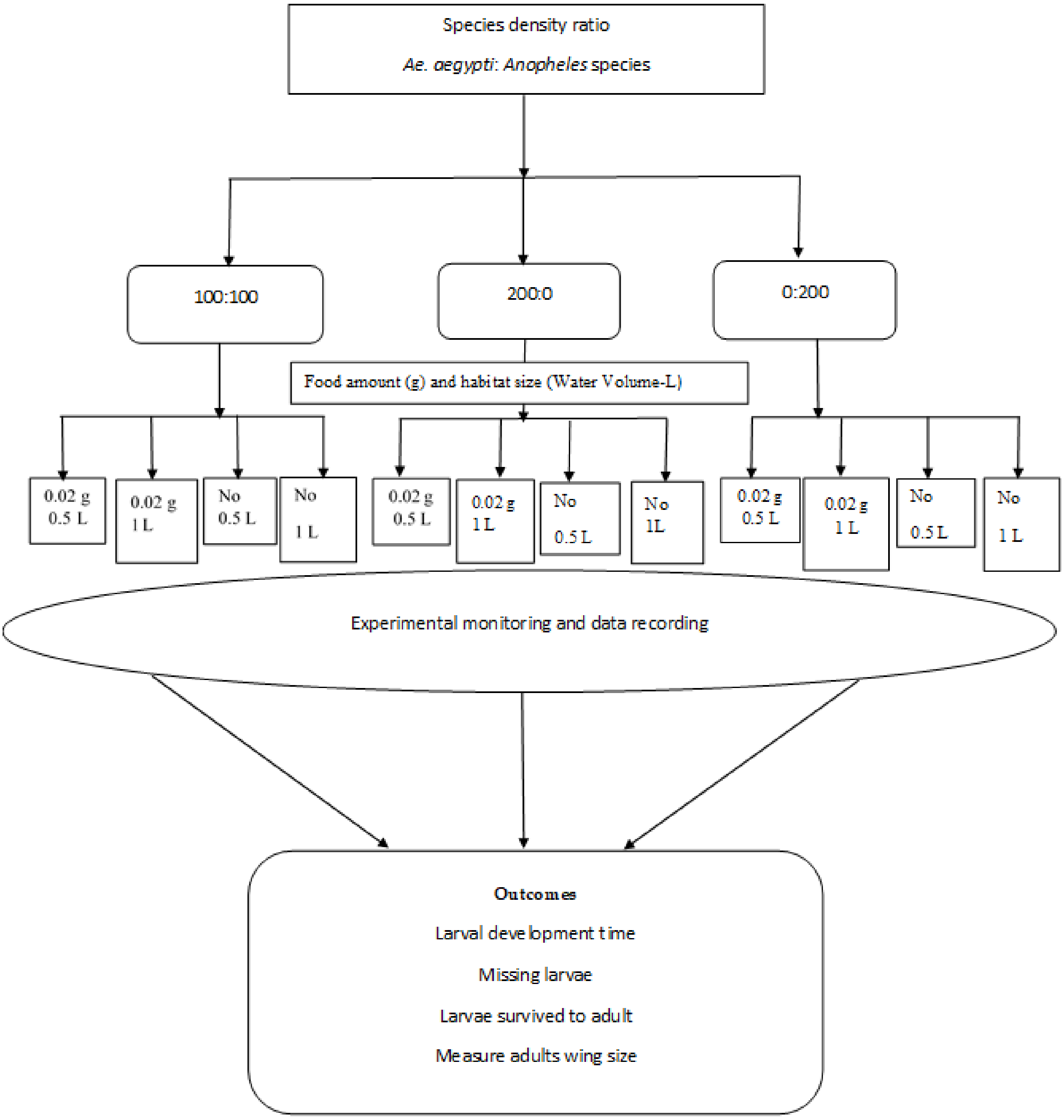

Figure Schematic presentation of the experimental setup and procedures for inter and intraspecific competition between *Aedes aegypti* and either *Anopheles arabiensis*, *Anopheles gambiae* and *Anopheles funestus* in small and medium habitat sizes.

2.Data management and statistical analysis.

The data were analyzed using STATA 18 software (Stata Corp LLC, USA). Shapiro Wilk test used for testing data normality and statistical significance was set as a $p<0.05$. Descriptive statistics, mean and 95% confidence intervals (CI) of developmental time, larvae survival to adulthood, wing length and missing larvae for each *Ae. aegypti* and *Anopheles* species experiment were calculated.

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The percentage larvae survival to adulthood was obtained as the total number of emerged adults divided by the initial number of larvae introduced in the habitat per species multiplied by hundred. Also, the number of missing larvae was obtained by adding the total number of dead larvae, the total number of live pupae, and the number of dead pupae found in the larval habitat, then subtracting this sum from the total number of larvae that remained in the basin from the previous day. Its percentage was obtained by taking the total number of missing larvae divide by the initial number of larvae introduced in the habitat multiplied by hundred.

A generalized linear mixed model (GLMM) was used; negative binomial regression was used to examine the fixed effects of competition, habitat and their interactions on the number of larvae survived to adulthood, whereby the effect of food was also included in the analysis to account for missing larvae. The experimental day was fitted as a random variable in the model. Additionally, binomial regression was used to test the fixed effects of competition (intra or interspecific competition), habitat (small or medium) and their interactions on wing size.

Results

The daily recorded temperature and relative humidity inside the semi-field system (SFS) from June to September averaged 27.21 ± 0.05ºC and 74.74 ± 0.16%, respectively. Additionally, the average water temperature in the habitats was also 27.21 ± 0.05ºC.

3.Larval developmental time

In populations of single species, *An. arabiensis* had a mean pupation time of 9.7 (9.07, 10.28) days in small and 9.5 (8.80, 10.24) days in medium habitats. When reared with *Ae. aegypti*, this increased to 12 (11.35, 12.65) in small and 12.3 (11.79, 12.90) days in medium habitats, significantly prolonging the time to pupation. For *Ae. aegypti*, the mean time to pupation in single populations was 9.5 (8.84, 10.22) and 9.5 (8.88, 10.12) days in small and medium habitats respectively. In mixed populations with *An. arabiensis*, the time to pupation decreased to 8.5 (7.81, 9.10) and 8.7 (8.14, 9.23) days in small and medium habitats respectively.

Anopheles gambiae exhibited a mean pupation time of 7.8 (7.01, 8.51) and 8.17 (7.36, 8.97) days in small and medium habitats in populations of single species that increased to 9 (8.75, 9.78) and 8.9 (8.99, 9.68) days in small and medium habitats respectively, when reared with *Ae. aegypti*. Conversely, *Ae. aegypti* showed a reduced pupation time of 6.7 (6.03, 7.37) and 7.14 (6.38, 7.90) days in small and medium habitats respectively, compared to 8.7 (7.91, 9.43) and 8.7 (7.99, 9.31) days in small and medium habitat sizes when reared alone.

Anopheles funestus had a mean pupation time of 14.4 (13.73, 14.99) and 14.4 (13.71, 14.99) days in both small and medium habitats in single populations, increasing to 15.5 (15.02, 16.0) and 15.2 (15.10, 15.82) days in the same habitats when reared with *Ae. aegypti*. By contrast, *Ae. aegypti* took longer to pupate in single populations, with mean times of 10.6 (9.97, 11.29) days in small and 11 (10.40, 11.67) days in medium habitats, compared to 7 (6.49, 7.59) and 7.7 (7.14, 8.30) days in the same habitats, in mixed populations.

3.Effects of competition on mosquito larvae survived to adults

Ae. aegypti consistently exhibited higher survival rates in interspecific competition compared to *An. arabiensis*, *An. gambiae* and *An. funestus,* with both genera showing higher larval survival to adults in single species populations than mixed populations (Figure 2A, 2B, 2C; and Table 1).

Table Generalized linear mixed model for the effects of competition and habitats on larvae survived to adult for *Aedes aegypti, Anopheles arabiensis, Anopheles gambiae* and *Anopheles funestus***.**

Population	**Species**	**Effects**		**RR (95% CI)**	**P-value**
Ae.aegypti & An.arabiensis	*Ae.aegypti*	Competition	Intraspecific	1	
			Interspecific	0.40 (0.30, 0.55)	<0.001
		Habitat	Small	1	
			Medium	0.88 (0.66, 1.16)	0.359

	An.arabiensis	Competition	Intraspecific	1	
			Interspecific	0.23 (0.15, 0 .35)	<0.001
		Habitat	Small	1	
			Medium	0.84 (0.56, 1.29)	0.441
Ae.aegypti & An.gambiae	*Ae.aegypti*	Competition	Intraspecific	1	
			Interspecific	0.50 (0.34, 0.74)	0.001
		Habitat	Small	1	
			Medium	0.89 (0.63, 1.27)	0.55
	An.gambiae	Competition	Intraspecific	1	
			Interspecific	0.43 (0.26, 0.71)	0.001
		Habitat	Small	1	
			Medium	0.82 (0.52, 1.28)	0.393
Ae.aegypti & An.funestus	*Ae.aegypti*	Competition	Intraspecific	1	
			Interspecific	0.26 (0.17, 0.39)	<0.001
		Habitat	Small	1	
			Medium	0.65 (0.45, 0.94)	0.901
	An.funestus	Competition	Intraspecific	1	
			Interspecific	0.19 (0.13, 0.28)	<0.001
		Habitat	Small	1	
			Medium	1.02 (0.69, 1.52)	0.024

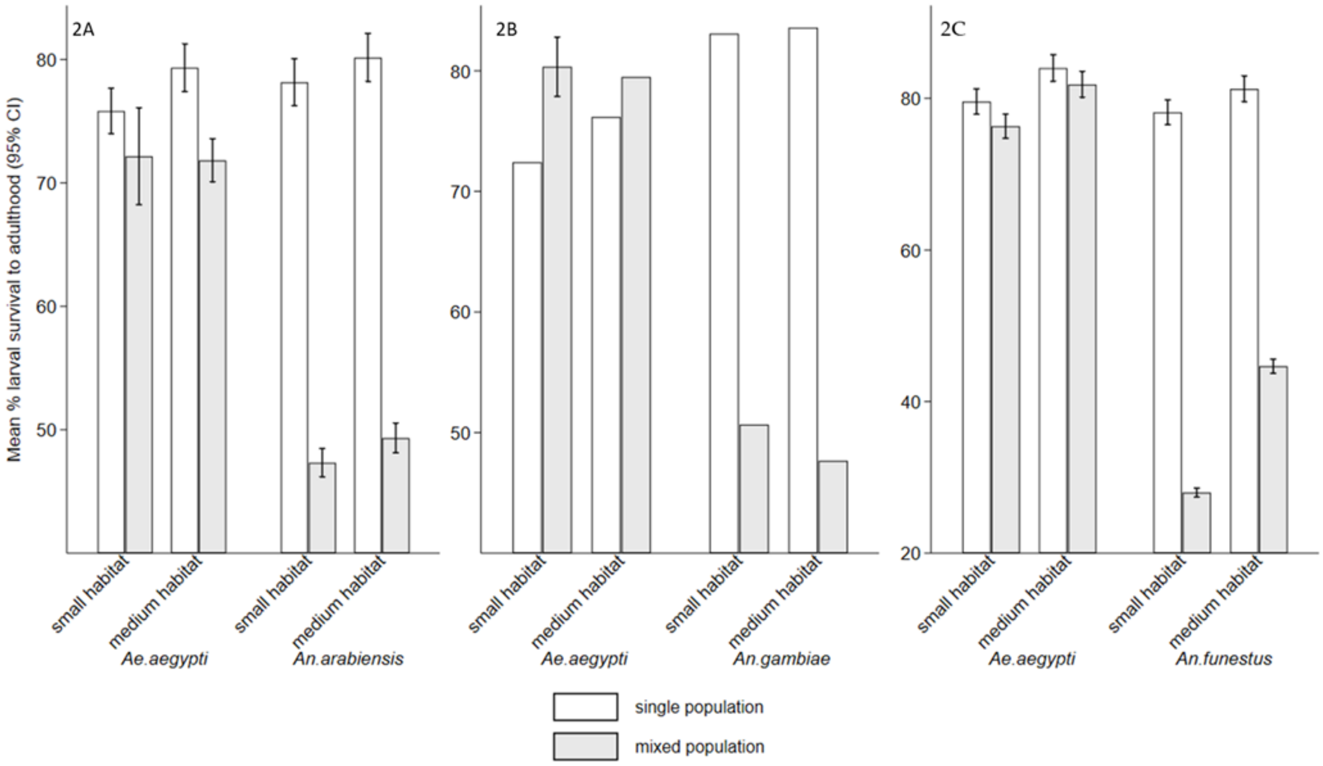

Figure 2. Mean percent (95% CI) of larvae survived to adulthood for a) *Aedes aegypti* and *Anopheles arabiensis*, b) *Aedes aegypti* and *Anopheles gambiae*, c) *Aedes aegypti* and *Anopheles funestus* exposed in single and mixed population across small and medium habitats.

3.Adults body size via wing length (mm)

Mosquito body sizes of all test species were significantly affected by competition, habitat size, and their interaction (p<0.001, Table 2). Overall, the means wing length of all test species were significantly small for mosquitoes emerging from small habitat compared to those emerging from medium habitats in interspecific competition (Figure 3A, 3B, 3C). In comparison, the mean wing lengths of mosquitoes emerging from small and medium habitats were significantly large in intraspecific competition compared to those in interspecific competition for both genera.

Table 2. Generalized linear model of the effects of competition, habitat and their interactions on the adults wing length (mm) for *Aedes aegypti* mixed with either *Anopheles arabiensis*, *Anopheles gambiae* or *Anopheles funestus*.

Population	**Species**	**Effects**		**RR (95% CI)**	**P-value**
Ae.aegypti & An.arabiensis	*Ae.aegypti*	Competition	Alone	1	
			Mixed	0.76 (0.72, 0 .80)	<0.001
		Habitat	Small	1	
			Medium	1.16 (1.09, 1.22)	<0.001
		Competition× Habitat	Mixed ×Medium	1.21 (1.11, 1.31)	<0.001
	An.arabiensis	Competition	Alone	1	
			Mixed	0.47 (0.44, 0.50)	<0.001
		Habitat	Small	1	
			Medium	1.13 (1.08, 1.19)	<0.001
		Competition ×Habitat	Mixed ×Medium	1.61 (1.48, 1.74)	<0.001
Ae.aegypti & An.gambiae	*Ae.aegypti*	Competition	Alone	1	
			Mixed	0.80 (0.76, 0.84)	<0.001
		Habitat	Small	1	
			Medium	0.20 (1.15, 1.26)	<0.001
		Competition× Habitat	Mixed ×Medium	1.04 (0.98, 1.12)	0.196
	An.gambiae	Competition	Alone	1	
			Mixed	0.56 (0.54, 0.58)	<0.001
		Habitat	Small	1	
			Medium	1.09 (1.04, 1.14)	<0.001
		Competition× Habitat	Mixed ×Medium	0.29 (0.21, 1.37)	<0.001
Ae.aegypti & An.funestus	*Ae.aegypti*	Competition	Alone	1	
			Mixed	0.77 (0.73, 0.82)	<0.001
		Habitat	Small	1	
			Medium	1.22 (1.17, 1.29)	<0.001
		Competition× Habitat	Mixed ×Medium	1.13 (1.05, 1.22)	0.001
	An.funestus	Competition	Alone	1	
			Mixed	0.58 (0.56, 0.60)	<0.001
		Habitat	Small	1	
			Medium	1.18 (1.14, 1.22)	<0.001
		Competition× Habitat	Mixed ×Medium	0.28 (1.21, 1.35)	<0.001

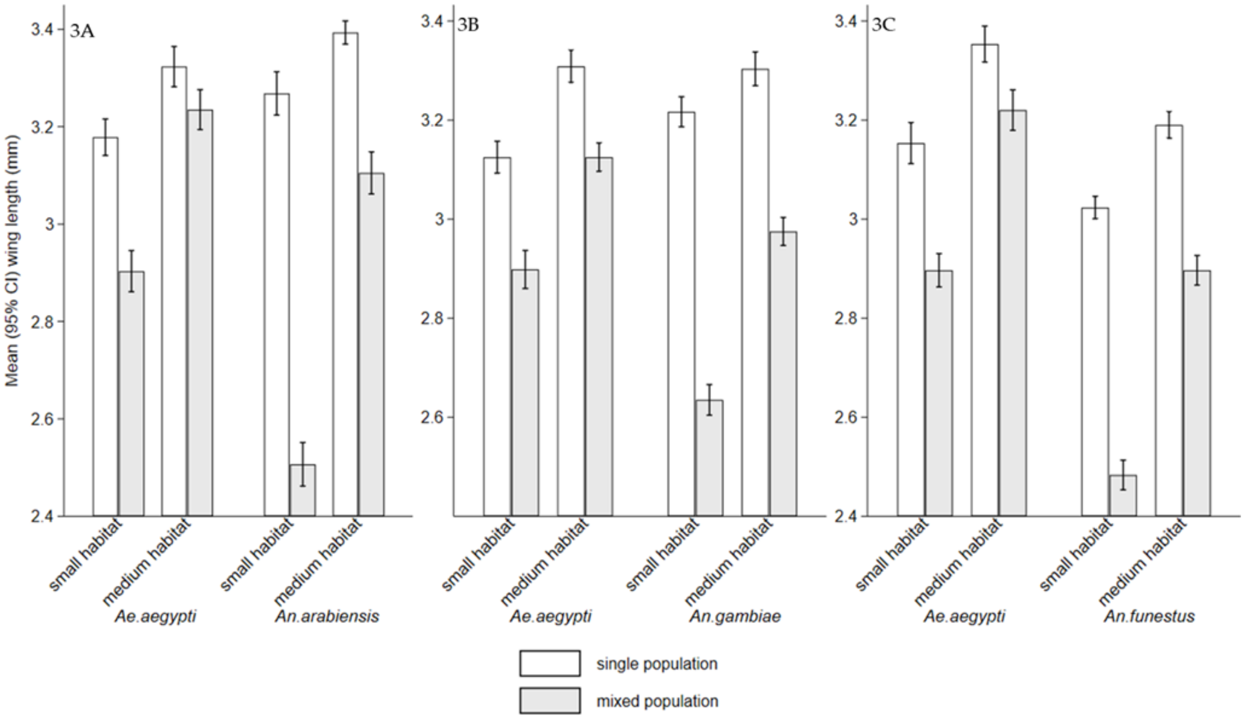

Figure 3. Mean (95% CI) wing length of female a) *Aedes aegypti* with *Anopheles arabiensis, b) Aedes aegypti* with *Anopheles gambiae* and c) *Aedes aegypti* with *Anopheles funestus* in single and mixed population across different habitat size.

3.Cannibalism and predation effects

The study observed notable variation in the rate of missing larvae in presence or absence food; and during competition of Ae. aegypti with either An. arabiensis, An. gambiae or An. funestus (Figure 4). With either presence or absence of food, An. arabiensis, An. gambiae, and An. funestus experienced higher rate of missing larvae in interspecific competition than Ae. aegypti (Figure 5A, 5B,5C, 5D, 5E, 5F). This indicates that Anopheles species encounter greater survival challenges when competing with Ae. aegypti than when they are alone. The presence of food decreased the rate of missing larvae in all mosquito species (Figure 5A, 5B,5C, 5D, 5E, 5F, Table 3).

Figure 4. Showing *Ae. aegypti* larvae feeding on the larvae of *Anopheles* species sharing the same habitat.

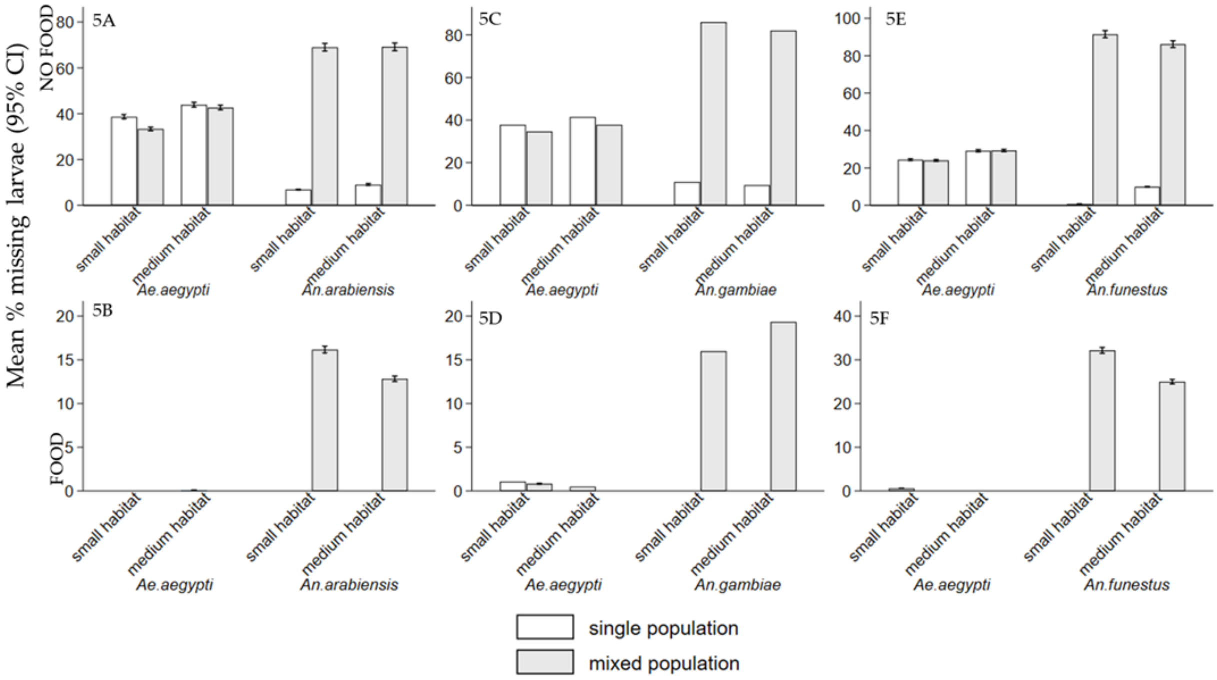

Figure 5. Mean percent (95% CI) of missing larvae for a) *Aedes aegypti* and *Anopheles arabiensis*, b) *Aedes aegypti* and *Anopheles gambiae*, c) *Aedes aegypti* and *Anopheles funestus* exposed in single and mixed population across small and medium habitats.

Table 3. Generalized linear mixed model for the effects of competition, food and habitats on cannibalistic and predacious behavior for Aedes aegypti, Anopheles arabiensis, Anopheles gambiae and Anopheles funestus.

Population	**Species**	**Effects**		**RR (95% CI)**	**P-value**
Ae.aegypti & An.arabiensis	*Ae.aegypti*	Competition	Alone	1	
			Mixed	0.54 (0.38, 0 .79)	0.001
		Habitat	Small	1	
			Medium	1.15 (0.82, 1.62)	0.423
		Food	No	1	
			Yes	0.001 (0.0001, 0 .005)	<0.001
	An.arabiensis	Competition	Alone	1	
			Mixed	8.24 (4.91, 13.83)	<0.001
		Habitat	Small	1	
			Medium	1.28 (0.79, 2.06)	0.303
		Food	No	1	
			Yes	0.13 (0.07, 0 .21)	<0.001
Ae.aegypti & An.gambiae	*Ae.aegypti*	Competition	Alone	1	
			Mixed	0.49 (0.36, 0.66)	<0.001
		Habitat	Small	1	
			Medium	0.86 (0.64, 1.16)	0.326
		Food	No	1	
			Yes	0.02 (0.01, 0 .03)	<0.001
	An.gambiae	Competition	Alone	1	
			Mixed	6.35 (4.34, 9.29)	<0.001
		Habitat	Small	1	
			Medium	1.16 (0.83, 1.63)	0.386
		Food	No	1	

			Yes	0.16 (0.11, 0.24)	<0.001
Ae.aegypti & *An.funestus*	*Ae.aegypti*	Competition	Alone	1	
			Mixed	0.71 (0.49, 1.03)	0.07
		Habitat	Small	1	
			Medium	0.98 (0.67, 1.45)	0.942
		Food	No	1	
			Yes	0.01 (0.003, 0.013)	<0.001
	An.funestus	Competition	Alone	1	
			Mixed	14.09 (8.55, 23.22)	<0.001
		Habitat	Small	1	
			Medium	1.99 (1.27, 3.11)	0.002
		Food	No	1	
			Yes	0.26 (0.16, 0.42)	<0.001

Discussion

This is the first study documenting the effect of competition of cohabiting *Ae. aegypti* and *Anopheles* species at larval stages on mosquito fitness. Overall, The coexistence of *Ae. aegypti* with *An. arabiensis*, *An. gambiae*, or *An. funestus* led to competition, resulting in decreased larval survival, delayed pupation, reduced body size, and an increased rate of missing larvae of those species. When *An. arabiensis, An. gambiae,* and *An. funestus* were reared individually higher larval survival rate, larger adult body sizes, short pupation time and reduced rate of missing larvae were observed compared to when they were reared with *Ae. Aegypti.* These findings are consistent with previous studies conducted under laboratory and semi-field settings that recorded delayed developmental time, reduced larvae survival rate to adult, and reduced adult body size of cohabiting *Aedes* and *Culex* species [21,25,42,43].

This study documented interrupted developmental time from larvae to adults as a mosquito fitness cost caused by competition from cohabitation. This could be attributed to intense competition for limited food resources, which reduces food intake per larvae, slowing their growth and delaying pupation. While both species shared the same habitats and resources, this overlap increased closely contact within the same ecological niche that could also be a probable cause of the delayed development. A prior study of *Aedes cantans* under field conditions indicated that frequent contact between larvae could interfere with their feeding, resulting in prolonged developmental time similar to those caused by food scarcity [44]. In addition, growth-inhibiting cues released by *Ae. aegypti* larvae that make it grow fast, may prolong the development of *An. arabiensis*, *An. gambiae*, or *An. funestus* [45,46]. Moreover, because some of the food particles given to the larvae tends to settle at the bottom of the larval habitat, *Ae. aegypti* larvae might have an advantage to access these food particles owing to its diving ability compared to coexisting *An. arabiensis*, *An. gambiae* and *An. funestus*. Also, the differences in the mouth brushes of Culicine mosquitoes and the frequency of strokes might result in varying amounts of food intake per unit of time, which it turn might favor shorter developmental compared to *Anopheles* species in the same habitats [47,48]. Subsequently, fast growth rate benefits larval survival by reducing their exposure to vulnerable larvae stages such as cannibalism, predation and environmental factors i.e. rainfall leading to the flushing of breeding sites or drought periods causing desiccation of larval habitats [15,49].

These findings clearly indicated the effect of food accessibility on larval survival in a mixed population and its emergence to adult mosquitoes. *Ae. aegypti* larvae are often more aggressive and efficient in resource acquisition, outcompeting *Anopheles* larvae. In a mixed population, *Ae. aegypti* exhibited a higher adult emergence rate than *An. arabiensis, An. gambiae,* and *An. funestus*. These results align with previous laboratory studies that examined the effect of interspecific competition within *Aedes* species and between *Ae.aegypti* and *An.stephensi* on survival [50,51]. A separate study has indicated that species capable of sustaining positive population growth tend to hold a competitive advantage over their counterparts [52]. For that case, *Ae. aegypti* outcompeted *An. arabiensis, An. gambiae,* and *An. funestus* due to its superior survival rates. The rationale behind this competitive advantage is likely attributed to the enhanced food intake [51,52]. Another study observed a similar trend of *Ae. aegypti* and *Ae. polynesiensis*, whereby *Ae. polynesiensis* showed a competitive advantage over *Ae. aegypti* under field conditions [53]. Similarly, when considering *Ae. albopictus* and *Ae. aegypti*, *Ae. albopictus* maintained a positive population growth over *Ae. aegypti* [54].

Interspecific competition in mosquitoes sharing the habitats is recognized as a key factor influencing species distribution and population structure [55,56]. This has been reported in the Southeastern United States, where the reduction in *Ae. aegypti* abundance resulted from its competition with *Ae. albopictus* [57]. The coexistence of *Ae. aegypti* and *Anopheles* species have been observed in natural environments, particularly in urban and suburban areas of Benin, Gezira Sudan, Nigeria and Kinshasa Congo [4–7].

During these experiments, variations in foraging behaviors between test mosquito species were observed. *Ae. aegypti* were observed to predominantly spent more time at the bottom and walls of the larval habitat, whereas *Anopheles* species spent more time at the surface. These behavioral differences could result in differential resource utilization, potentially reducing or avoiding interspecific competition [58]. Similar foraging patterns has been recorded in the coexisting *Ae. albopictus* and *Ae. aegypti*, where *Ae. albopictus* foraged at detritus surfaces, while *Ae. aegypti* occupied the column and bottom of the larval habitat [59]. In addition, *Cx. quinquefasciatus* demonstrated a feeding preference on the lower surfaces microlayer, whereas *An. gambiae* predominantly fed on the upper surfaces microlayer [60,61].

In this study, wing length estimates, revealed that interspecific competition had an effect on body size of all test mosquito species [62–64]. Mixed populations in small larval habitats had relatively small body sizes compared to those emerging from medium larval habitats. Considering the notable difference in body size of the two mosquito species influenced by habitat size, it is reasonable to infer that limited space serves as a variable in the larval environment, prompting competition that affects the adult mosquito and its associated host seeking, mating, fecundity, and vector competence [63,65,66].

The current study demonstrated that cannibalism and predation occurred in both *Ae. aegypti* and *Anopheles* species. Cannibalism and predation were established from missing larvae / unrecovered dead larvae. These behaviors between and within *Ae. aegypti* and *Anopheles* species were observed from third day of monitoring at larvae stage three. As *Ae.aegypti* larvae grew faster than *Anopheles* species, exhibited these behaviors, consistent with earlier findings that suggested these behaviors involve older and relatively larger individual larvae [15,67]. *Anopheles* larvae may be physically less capable of defending themselves against aggressive *Ae. aegypti*, making them easier targets for predation. It was observed that, *Ae. aegypti* were predating on *Anopheles* probably due to their physical differencies, but also to some extent cannibalizing themselves. Because the two species shared the same habitats and resources, this overlap increased the chances of predation as both species interacted closely within the same ecological niche. Previous research reported that cannibalism or predation among mosquito larvae may result from the circular currents created by the mouth brushes of older larvae during filtering [68], or through active attacks by conspecifics or heterospecifics and when species are in close proximity [69]. On the other hand, this study suggests that the amount of food given did not affect cannibalism and predation, because few *Anopheles* larvae were missing in the presence of *Ae. aegypti*. This imply that, these behaviors are facultative processes and are not dependent on food availability [17].

While the study objectives were achieved, several limitations may have influenced the observed outcomes. The study was conducted in a controlled environment in a semi-field setting designed to mimic realistic conditions. While exposed to fluctuating microclimatic conditions, the environment allowed for control over specific factors, such as predators and varying food availability that could have influenced the outcome. Additionally, laboratory-reared mosquitoes at the second instar stage were used and transferred to the semi-field system, potentially slowing their development as they adapted to the new environmental conditions. Furthermore, the study did not attempt to confirm cannibalism and predation behaviors through methods such as polymerase chain reaction (PCR) analysis of prey DNA, examination of larvae feces, or midguts content analysis. Instead, the study relied on the missing larvae to infer cannibalism and predation, which may have affected the accuracy of these observations.

This study focused on a specific time frame, further studies should investigate the underlying mechanisms driving these competitive interactions, allowing for more comprehensive understanding of mosquito population dynamics. Also, further studies should focus on exploring the variables that impact competitive interactions and evaluating the prevalence of such interactions in natural settings. Also, future studies should focus on other fitness parameters such as fecundity, longevity, host seeking behavior and flight capabilities of adult mosquitoes resulting from interspecific competition.

Conclusions

These findings demonstrate superior competitiveness of *Ae. aegypti* over major African malaria vectors. This study has implications for diseases transmission dynamics, due to environmental changes such as urbanizations, climate changes or human interventions like water management practices in urban areas can lead to new scenarios where these species do overlap more frequently. By understanding competitive interactions between *Ae. aegypti* and major African malaria vectors is vital for predicting changes in the population dynamics of these species, which are both important diseases vectors. For instance, a decline in the *Anopheles* population due to competition can lead to an increase in *Aedes* population which inadvertently may increase the risks of *Aedes* borne diseases. Additionally, this unique and antagonist interaction between these species can be used as biological indicator to signal the impact of vector control intervention on mosquito ecology, particularly the biotechnology approaches such as gene derive technology.

Conflicts of Interest" The authors affirm that no conflicts of interest to declare.

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Abbreviations

VBD: Vector - borne diseases, WHO: World Health Organization, IHI: Ifakara Health Institute, SFS: Semi field system, GLMM: Generalized linear mixed model. CI: Confidence interval, PCR: Polymerase chain reaction and DNA: Deoxyribonucleic acid.

Authors' contributions: SCL and DWL conceived, designed and implemented the study. SCL executed the experiment. SCL, JKS, YAM and LLM carried out data analysis and interpretation of the results. SCL wrote the manuscript. JKS, YAM, LLM, ASM, NKN, AM, HJK, AM, SM, MFM and DWL revised the manuscript. All authors participated in reviewing and approving of the final version of the manuscript for submission.

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