Impacts of biotic and abiotic parameters on immature populations of *Aedes aegypti*

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Abstract

In recent centuries, the mosquito *Aedes aegypti* has spread into most urban areas throughout the tropics. This species is considered the main vector of the chikungunya, dengue, yellow fever and Zika viruses and causes major public health issues. The aim of this study is to investigate the relative influence of biotic and abiotic parameters on immature populations of *Ae. aegypti*. During a one-year-long field experiment, we monitored 108 macroinvertebrate aquatic communities inhabiting four types of water containers across three different urbanized sites in a Neotropical city. A multimodel inference approach revealed that, in addition to abiotic parameters, biotic interactions with aquatic organisms had an important influence on the abundance of *Ae. aegypti* and that the urbanized site considered influences the outcomes of the interactions. Controphic species other than mosquitoes aided *Ae. aegypti* development, suggesting a mechanism of facilitation through a chain of processes. However, the abundance of *Ae. aegypti* was lowered by competition with native mosquito species in the slightly urbanized area and by predation in more urbanized areas. Competitive displacement and reduction, as well as predation by native aquatic organisms, can be considered a form of ecosystem service. The conservation and/or augmentation of natural enemies should improve the short- and long-term success of incompatible and/or sterile insect techniques, thus opening up perspectives for the future of mosquito management.

Keywords: Biocontrol agents; Competition; Ecosystem services; Mosquito control; Predation; Mosquito management

Key message

- In recent centuries, the mosquito vector *Aedes aegypti* has spread throughout the tropics and caused public health issues.
- The relative influence of biotic and abiotic parameters on the abundance of *Ae. aegypti* immatures was investigated during a one-year-long field experiment.
- Both biotic and abiotic parameters impacted *Ae. aegypti*, and biotic interactions resulted in antagonistic outcomes depending on the urbanized site considered.

- This opens up perspectives for mosquito management, including by combining biological insect techniques with the reinforcement of natural enemies.

Introduction

The prevention of arthropod-borne diseases (e.g., arboviral diseases, filariasis and malaria) is contingent on controlling vector populations due to the lack of effective cures. Thus, understanding vector ecology in relation to microclimatic conditions and land use is essential and can better direct the use of incompatible (e.g., *Wolbachia*-infected) and/or sterile (e.g., radiation-based) insect technique control (Dufourd and Dumont [13]; Aliota et al. [2]; Zheng et al. [52]). For example, under similar precipitation regimes, urban zones are warmer and drier than their rural surroundings (i.e., the urban heat island effect) as a consequence of less vegetation cover related to greater imperviousness increasing the re-emission of solar radiation (Mohajerani et al. [39]). These urban conditions have been associated with the lower survival, dispersal and fecundity rates of adult mosquitoes and egg viability, but, by reducing the development time of immatures (i.e., larvae and pupae), they result in the production of more offspring and more generations per year (Honório et al. [24], [23]; Delatte et al. [12]).

Immature mosquitoes develop within aquatic communities organized into complex food webs implying multiple trophic interactions (Blaustein and Chase [5]; Juliano [25]). For a given species, the outcomes of these interactions can be negative due to predation by upper trophic levels and/or through competitive interactions with controphic species exploiting the same resource. They are beneficial when two controphic species share a common predator (i.e., apparent mutualism) or when the exploitation of a resource by one species enhances access to another resource for the other species (i.e., indirect mutualism), such as through processing chains (Lounibos [31]; Riback et al. [44]; Camara et al. [7]; Talaga et al. [48]).

The aim of this study is to investigate the relative influence of biotic and abiotic parameters on immature populations of the mosquito *Aedes aegypti* (L.), the main vector of the chikungunya, dengue, yellow fever and Zika viruses. This species is native to Africa where a 'wild, forest form,' still present, uses small natural water holes as rearing sites for immatures and adult females prefer to bite non-human animals. This ancestral form gave rise to a highly anthropophilic 'domesticated form' that uses mostly human-generated water containers, whereas females primarily bite humans indoors. By taking successive blood meals from multiple hosts, they quickly spread diseases when infected. Through the human transport of its eggs that can survive without water for more than one year, this form first spreads outside Africa with the Atlantic slave trade in the sixteenth and seventeenth centuries and then throughout all tropical and subtropical regions, causing major public health issues (Powell and Tabachnick [40]; Epelboin et al. [14]; Cunze et al. [10]; Powell et al. [41]).

We hypothesized that, as a result of warmer and drier microclimatic conditions related to greater human-mediated perturbation, a higher degree of urbanization negatively affects the native aquatic macroinvertebrates but favors the abundance of *Ae. aegypti* immature populations through biotic interactions resulting in different outcomes. Therefore, we tested whether the number of biotic interactions decreases when the degree of urbanization increases, thereby giving more relative importance to abiotic fluctuations in explaining the abundance of *Ae. aegypti* immatures.

Methods

Study area

This study was conducted in French Guiana within the city of Kourou between October 2013 and October 2014. The area is characterized by an equatorial monsoon climate corresponding to an average of 2500 mm of yearly rainfall distributed over 210 days. There is a major drop in rainfall from mid-July to mid-November (the dry season) and a short, irregular dry period in March. Minimum and maximum monthly mean temperatures vary between 25.2 and 30.6 °C.

Kourou is a small Neotropical city (26,522 inhabitants; 26.4 km^2) that was built on a former swampy area at the mouth of the Kourou River (Quézédé [42]; Fig. 1a). Soil was transported to level out many parts of the city, but most places are only 2–3 m above the mean sea level. Water management efforts explain the presence of three lakes (surface at about sea level) connected to the sea by channels and of many ditches distributed throughout the city to gather water during the rainy season (Géoportail 2019). Furthermore, numerous human-generated water containers (hereafter artificial water containers) and natural water containers are distributed throughout the city. The latter mostly include tree holes and epiphytic tank bromeliads that are frequent in gardens and green spaces like in some other Neotropical cities (Mocellin et al. [38]; Talaga et al. [49]). Note that the city's lakes, channels and ditches as well as the Kourou River do not influence the aquatic communities of the water containers studied here that represent a very different type of ecosystem, each having its own aquatic community (Juliano [25]).

We selected three experimental sites based on increasingly impervious surface coverage (Fig. 1). The first site was located in a fragment of secondary forest situated more than 100 m from the nearest building and had an impervious surface coverage of 0.65% (hereafter, slightly urbanized site; 5.1731° N, − 52.6554° W). The second site was located in a residential area with numerous gardens and green spaces and had an impervious surface coverage of 33.80% (hereafter, moderately urbanized site; 5.1703° N, − 52.6534° W). The third site was located in the old city center and had an impervious surface coverage of 86.60% (hereafter, highly urbanized site; 5.1553° N, − 52.6433° W). All geographic coordinates are expressed in decimal degrees using the World Geodetic System 84.

The percentages of impervious surface coverage were established within a radius of 70 m from each experimental site (Fig. 1b) because this distance encompasses the direct dispersal capabilities of most adult aquatic container-inhabiting amphibiotic insects. In open areas, less than 10% of *Ae. aegypti* females disperse beyond 60 m, the distances are much shorter (Maneerat and Daudé [33]). The adults of the non-mosquito dipterans in this study are poor flyers with short flights (Psychodidae; Griffith and Gillet-Kaufman 2018) or disperse using the wind (Ceratopogonidae and Chironomidae; Mellor et al. [35]; Armitage et al. [3]). Most veliid heteropterans are micropterous that disperse by walking; a few long-winged individuals disperse by flying (Lancaster and Downes [27]). The Oligochaeta noted in this study are dispersed through phoresy mostly via frogs and lizards (Lopez et al. [30]).

Because the dispersal of flying insects is strongly influenced by wind speed and direction, we provide the wind rose of the city of Kourou in Fig. 1a (Beaulant and Minvielle [4]). Indeed, insect flight speed drops to zero when headwind velocities reach their maximum speed; the maximum speed, 5 km/h for certain *Culex* mosquitoes (Gillies and Wilkes [19]), reaches 5.4 km/h for *Ae. aegypti* (Foster and Walker [17]). However, wind speeds above 1.08 km/h

already reduce the flight success of mosquitoes (Verdonschot and Besse-Lototskaya [50]). Thus, the experimental sites studied are more isolated from each other than only due to the distance separating them because they are almost perpendicular to westward winds (Fig. 1a).
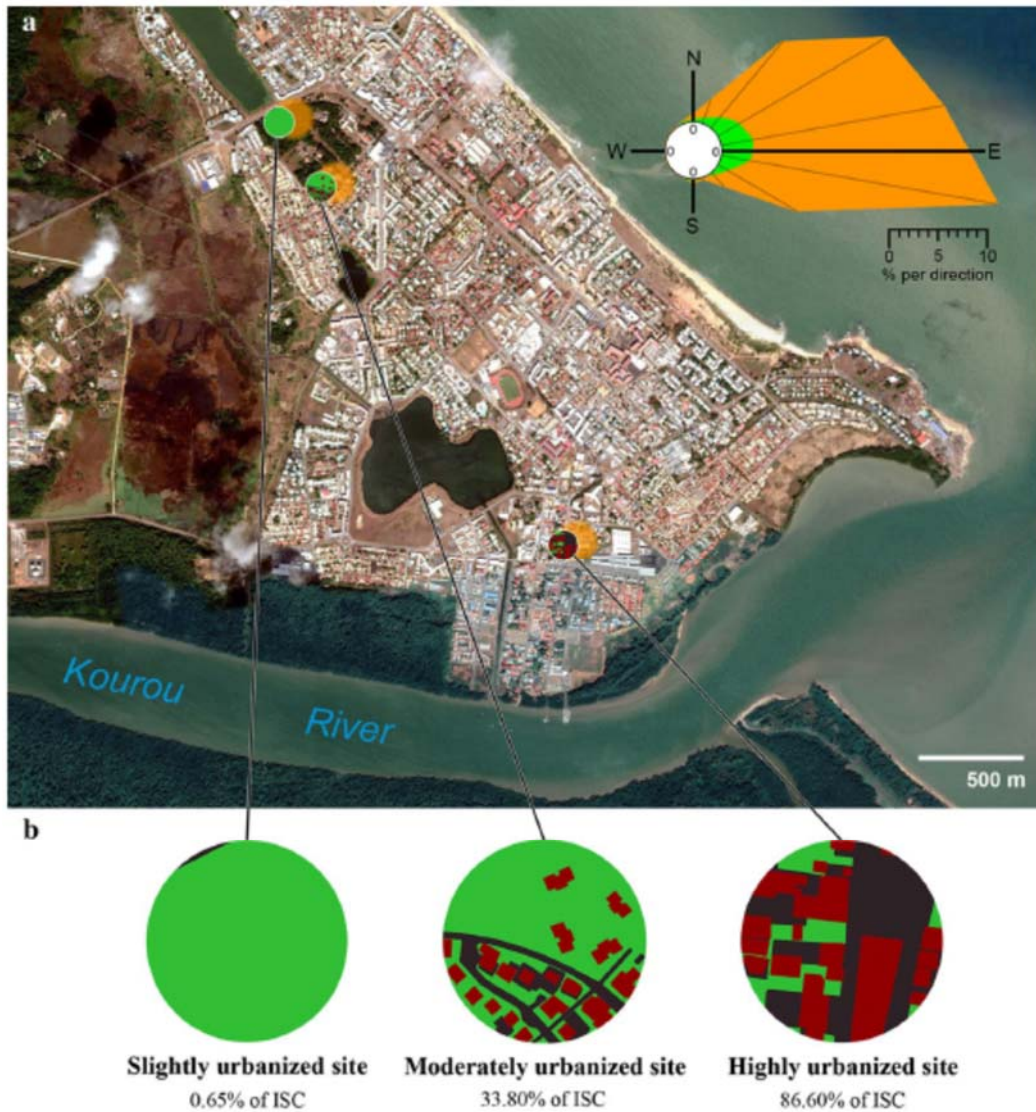

Fig. 1 a Location of the slightly, moderately and highly urbanized experimental sites situated within the city of Kourou, French Guiana. In the wind rose, the green plume represents wind speed up to 16.2 km/h, the orange plume represents wind speed > 16.2 km/h up to 28.8 km/h, and the white circle represents the value zero for wind speed. b Impervious surface coverage (ISC; charcoal: pavement; red: buildings) relative to pervious surface coverage (green) established within a radius of 70 m for each experimental site

Air temperature and percentage of relative humidity were recorded every hour by data loggers (iLog, Escort, New Zealand) installed at 1.5 m in height under a protective shelter at each experimental site. Daily precipitation records were obtained from a meteorological station situated in the city of Kourou. Mean temperature (Tm7; in °C), precipitation (Prm7; in mm) and relative humidity (RHm7; in %) were calculated at one- or two-week intervals before each sampling date. The microclimatic characteristics for each experimental site are presented in Table 1.

Table 1. Microclimatic conditions and aquatic characteristics for three degrees of urbanization and four types of water containers

	Slightly urbanized site	Moderately urbanized site	Highly urbanized site	P
	(N = 486)	(N = 486)	(N = 486)	
Temperature (°C)	[a]26.4 ± 0.15	[b]27.12 ± 0.15	[b]27.22 ± 0.18	< 0.001
Relative humidity (in %)	[a]97.65 ± 0.39	[b]94.64 ± 0.74	[c]93.34 ± 0.58	< 0.001
Daily precipitation (in mm)	*5.52 ± 0.33	*5.52 ± 0.33	*5.52 ± 0.33	-
Water volume (in mL)				
Bromeliad	[a]213.83 ± 13.04	[a]192.49 ± 15.95	[b]552.74 ± 38.22	< 0.001
Bamboo	[a]332.60 ± 20.73	[b]261.44 ± 24.20	[b]304.78 ± 24.93	< 0.001
Ovitrap	244.49 ± 13.12	215.99 ± 13.78	205.42 ± 16.53	NS
Tire	[a]1269.66 ± 89.50	[a]1233.39 ± 87.83	[b]903.26 ± 78.15	< 0.001
pH				
Bromeliad	[a]5.93 ± 0.05	[b]5.71 ± 0.06	[a]5.79 ± 0.04	< 0.01
Bamboo	[a]5.65 ± 0.12	[b]6.48 ± 0.11	[b]6.54 ± 0.08	< 0.001
Ovitrap	[a]6.41 ± 0.05	[b]6.73 ± 0.06	[b]6.81 ± 0.05	< 0.001
Tire	[a]6.87 ± 0.05	[b]6.62 ± 0.05	[a]7.04 ± 0.05	< 0.01
Conductivity (in μS)				
Bromeliad	[a]189.63 ± 26.64	[b]313.79 ± 37.44	[a]129.01 ± 17.51	< 0.001
Bamboo	[a]374.39 ± 35.36	[b]1225.43 ± 206.71	[a]456.49 ± 53.33	< 0.001
Ovitrap	[a]96.51 ± 9.00	[b]301.48 ± 35.19	[a]155.16 ± 15.06	< 0.001
Tire	[a]176.96 ± 22.50	[b]449.09 ± 87.98	[a]173.255 ± 26.57	< 0.001

*Data obtained from the same meteorological station The significant effect of the degree of urbanization was tested using a one-way ANOVA. The exponent letters indicate pairwise significance between sites at *P* < 0.05 (*P* adjusted for multiple comparisons)

Setup

The natural water containers used in this study consisted of native tank bromeliads (BR; *Aechmea aquilega*; see Talaga et al. [46]) and dry stumps of bamboo (BA; *Bambusa vulgaris*). The artificial water containers consisted of CDC ovitraps (OV; black 500-mL plastic cups) and car tires all of the same size (TI; 175/70R14). At each experimental site, the containers (six tires and 10 of each of the other types; a total of 108 containers) were installed following a random block design within an area of ca. 100 m^2 and filled with 250 mL of rainwater at the beginning of the experiment in mid-October 2013.

Every 2 weeks, we sampled one half of each container type at each experimental site (54 containers each time). The second half was sampled two weeks later and so forth for one year. Therefore, the water in each container type was poured out every month, permitting us to mitigate the priority effect relative to the colonization of such habitats and to eliminate temporal dependency between sampling dates (Alford and Wilbur [1]).

Sampling

Sampling began two weeks after the experiment was set up. At each sampling date, the water held by the containers was poured out and filtered through a 150-µm mesh. The residue containing organic matter and aquatic organisms was preserved in a formaldehyde solution at 4%. Then, the water volume (WV) was assessed (in milliliters) using a graduated cylinder and the values for pH and conductivity (Cond; in microsiemens) were measured with a portable multimeter (Multi 3410, WTW GmbH, Germany). Finally, the filtered water was returned in its entirety to its container. The water volume, pH and conductivity for each of the four types of water containers and for the three urbanized sites are presented in Table 1.

In the laboratory, all aquatic and semi-aquatic macroinvertebrate organisms were extracted from the organic material under a stereomicroscope at 10× constant magnification. The individuals collected were separated into morphospecies or species, enumerated and preserved in 70% alcohol. Mosquitoes were identified to species level using Lane's keys ([28]). Individuals belonging to other families were identified using Merritt and Cummins' keys ([36]). For the sake of convenience, both morphospecies and species are regrouped under the term 'taxa' in the rest of the text. The macroinvertebrates were classified into three functional groups relative to *Ae. aegypti*: (1) controphic, mosquito taxa (hereafter: primary competitors; CompI), (2) controphic taxa other than mosquitoes (hereafter: secondary competitors; CompII), and (3) taxa from a higher trophic level able to prey upon *Ae. aegypti* immatures (predators; Pred).

Statistical analyses

To determine the structural differences between the aquatic macroinvertebrate communities in the different sites and types of container habitat, we conducted non-metric multidimensional scaling (NMDS) ordinations. Then, permutational multivariate analyses of variance (PERMANOVAs) were used to test the effect on the structure of communities across sites and type of water containers when controlled for site variation. Both the NMDS ordinations and PERMANOVAs were based on the Bray–Curtis distance.

To explain the abundance of *Ae. aegypti* immatures, we used zero-inflated negative binomial (ZINB) models to take into consideration the excess number of zero counts (Cheung [8]).

Full models were constructed with nine explanatory variables related to (1) microclimatic conditions (i.e., temperature, humidity and precipitation), (2) the characteristics of the aquatic habitat (i.e., WV, pH and Cond), and (3) the different groups of taxa (i.e., CompI, CompII and Pred). The type of water container (TWC) was added as a three-level factor. Because the containers were consecutively sampled every month, we also added the container type as a random factor to all of the models. Due to the strong collinearity between microclimatic variables calculated at one- or two-week intervals, distinct models were computed and compared using the Akaike information criterion (AIC). In addition, all explanatory variables were tested a posteriori for multicollinearity using the variance inflation factor (VIF) for each site. Since the variables were computed for each site, we considered three different datasets, each including the nine above-cited variables. Because tank bromeliads are highly compartmented, the effects of biotic interactions are likely lower. We thus created a model with a binomial distribution and the same explanatory variables as previously, but we replaced the type of water container by the type of site as a three-level factor. Finally, we used a multimodel inference approach to examine the relative effects of the parameters on the abundance or presence of *Ae. aegypti* immatures. For each dataset, all possible models were ranked using the AIC, and the Akaike weights were computed for all models (noted w_i for model i). Since the Akaike weights are probabilities, we were able to estimate the relative importance of a given variable by summing up the Akaike weights across all models where this variable occurred (noted $w_+(j)$ for variable j). So, $w_+(j)$ is the probability that the variable j will appear in the most appropriate model. The larger $w_+(j)$, the higher variable j is relative to the other variables. Finally, the direction and magnitude of effect size is given by the sign and value of the estimated parameters (Burnham and Anderson [6]).

Statistical analyses were conducted in the R software (R Development Core Team [43]) using the 'car,' 'glmmADMB,' 'MuMIn' and 'vegan' packages.

Results

Structure of the aquatic macroinvertebrate communities

A total of 174,840 aquatic and semi-aquatic macroinvertebrate individuals belonging to 25 taxa were sorted out from the water containers sampled all throughout the year (Table 2). Except for a few Coleoptera, Heteroptera and Oligochaeta, most of the specimens were immature Diptera. Among the latter, the Culicidae family represented 54.43% of all individuals and *Ae. aegypti* alone accounted for 30.97% of the total number of individuals. The percentage of occurrence of *Ae. aegypti* and *Culex pleuristriatus* Theobald increased from the slightly urbanized site to the highly urbanized site. On the contrary, native mosquito species such as *Limatus durhamii* Theobald and two *Wyeomyia* species decreased with increasing urbanization.

The structure of the aquatic and semi-aquatic macroinvertebrate communities was significantly different between the three urbanized sites (PERMANOVA; $N = 820$; $F = 38.73$; $R^2 = 0.09$; $P < 0.001$; Fig. 2a) and between the type of water container (PERMANOVA; $N = 820$; $F = 69.99$; $R^2 = 0.20$; $P < 0.001$; Fig. 2b, c, d).

Table 2. List of the aquatic and semi-aquatic macroinvertebrate morphospecies or species (hereafter, 'taxa') occurring in water containers in Kourou, French Guiana

Class	Order / Sub-order	Family	Sub-family	Morphospecies/species	Group	Slightly urbanized site % of occur	No. of rank	Moderately urbanized site % of occur	No. of rank	Highly urbanized site % of occur	No. of rank
Insecta	Coleoptera	Scirtidae	Scirtinae	Cyphon sp.	CompII	1.06	18	3.95	20	1.33	13
	Diptera										
	Brachycera			Brachycera spp.	CompII	46.92	3	45.83	4	28.44	5
	Nematocera	Ceratopogonidae	Ceratopogoninae	Bezzia sp.1	CompII	2.55	12	25.44	8	8.89	12
				Bezzia sp.2	CompII	1.27	15	1.32	16	2.67	10
				Ceratopogoninae sp.	CompII	1.91	16	2.19	18	0.44	18
				Dasyhelea sp.	CompII	–	–	15.13	7	18.67	7
			Forcipomyiinae	Forcipomyiinae sp.2	CompII	3.18	9	2.19	17	24.22	3
				Forcipomyiinae sp.3	CompII	–	–	–	–	0.22	15
		Chironomidae	Chironominae	Chironominae sp.	CompII	20.81	6	35.31	2	26.67	2
			Tanypodinae	Tanypodinae sp.	Pred	–	–	–	–	0.89	9
		Culicidae	Culicinae	Aedes aegypti (L.)	–	15.29	5	57.89	1	57.33	1
				Culex sp.1	CompI	–	–	0.66	15	–	–
				Culex sp.2	CompI	–	–	0.88	14	–	–
				Culex bonnei Dyar	CompI	0.85	14	–	–	–	–
				Culex mollis Dyar & Knab	CompI	2.55	7	5.70	6	0.22	17
				Culex quinquefasciatus Say	CompI	0.42	11	7.24	5	2.22	8

Class	Order Sub-order	Family	Sub-family	Morphospecies/species	Group	Slightly urbanized site		Moderately urbanized site		Highly urbanized site	
						% of occur	No. of rank	% of occur	No. of rank	% of occur	No. of rank
				Culex pleuristriatus Theobald	CompI	0.21	20	3.73	13	20.44	6
				Limatus durhamii Theobald	CompI	42.89	1	5.92	10	0.67	14
				Toxorhynchites haemorrhoidalis (F.)	Pred	7.64	13	21.71	12	0.44	16
				Trichoprosopon digitatum (Rondani)	CompI	0.64	10	–	–	–	–
				Wyeomyia aporonoma Dyar & Knab	CompI	7.22	8	0.44	21	–	–
				Wyeomyia pertinans (Williston)	CompI	16.77	4	7.89	11	–	–
		Psychodidae	Psychodinae	Telmatoscopus spp.	CompII	31.42	2	46.05	3	24.44	4
	Heteroptera	Veliidae		Veliidae sp.	Pred	1.06	19	9.87	9	7.11	11.00
Oligochaeta				Oligochaeta sp.	CompII	0.21	17	1.32	19	–	–

The taxa were sampled from natural (i.e., tank bromeliads and bamboo stumps) and artificial (i.e., ovitraps and tires) water containers and are grouped as follows. Taxa controphic to *Aedes aegypti* having the same feeding mode (CompI), controphic taxa that do not have the same feeding mode (CompII), and taxa from higher trophic levels able to prey upon *Ae. aegypti* (Pred). The percentage of occurrence and the rank number (the first two in bold for each site) of a taxon's relative abundance are indicated for each site
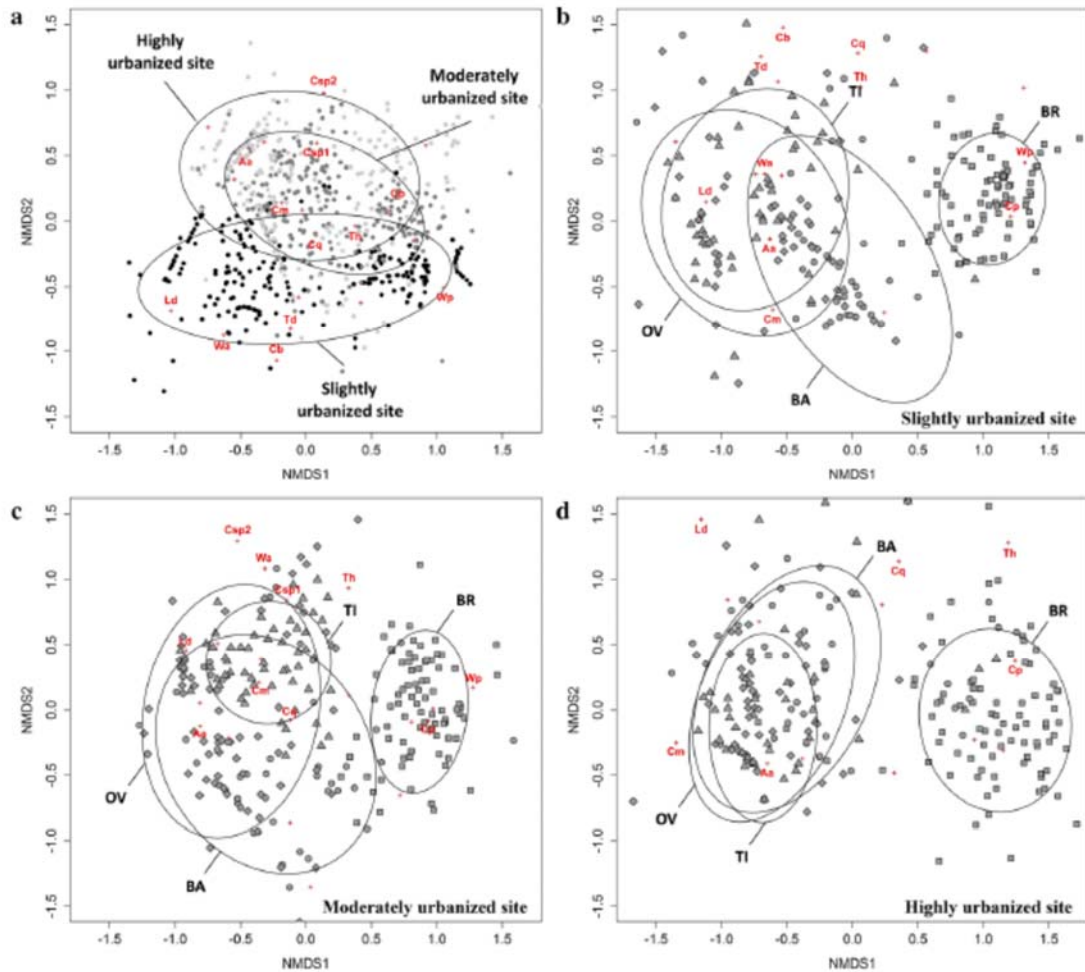
Fig. 2 Non-metric multidimensional scaling (NMDS) ordinations based on the Bray–Curtis distance showing the degree of dissimilarity of aquatic macroinvertebrate communities between the slightly, moderately and highly urbanized experimental sites (a), and between the types of containers within each site (b, c, d). Ellipses are drawn around each group with a confidence level of 95%. BR: tank bromeliads, BA: bamboo stumps, OV: ovitraps, TI: tires. Red crosses indicate aquatic macroinvertebrate taxa plotted in the same ordination space. For the sake of clarity, only mosquito taxa are shown and are abbreviated as follows. Aa: *Aedes aegypti*, Csp1: Culex sp.1, Csp2: Culex sp.2, Cb: Culex bonnei, Cm: Culex mollis, Cp: Culex pleuristriatus, Cq: Culex quinquefasciatus, Ld: Limatus durhamii, Th: Toxorhynchites haemorrhoidalis, Td: Trichoprosopon digitatum, Wa: Wyeomyia aporonoma, and Wp: Wyeomyia pertinans

Abundance of Aedes aegypti immatures according to container type and site

A total of 54,142 *Ae. aegypti* immatures were sorted out. While it ranked fifth in terms of frequency in the slightly urbanized site (Table 2), *Ae. aegypti* was the most frequent and abundant taxon in the moderately and highly urbanized sites (Tables 2, 3); the percentage of water containers sheltering its immatures followed the same pattern (Table 3). Also, *Ae. aegypti* was more abundant in artificial water containers than in natural ones regardless of the site (Table 3).

The percentages of co-occurrence of *Ae. aegypti* with other mosquito species were significantly different between sites and decreased the more the site was urbanized, ranging from 80.66% in the slightly urbanized site to 33.58% and 25.10% in the moderately and

Table 3. Statistics relative to *Aedes aegypti* immatures for three degrees of urbanization and four types of water containers

	Slightly urbanized site	Moderately urbanized site	Highly urbanized site	P
	(N = 486)	(N = 486)	(N = 486)	
Ae. aegypti density (ind. L^{-1})	[a]6.75	[b]165.57	[b]137.02	< 0.001
Bromeliad	[a]0.05	[b]10.89	[b]6.11	< 0.001
Bamboo	[a]2.25	[b]127.04	[c]75.1	< 0.001
Ovitrap	[a]11.54	[b]220.58	[b]224.48	< 0.001
Tire	[a]17.56	[b]378.86	[b]357.61	< 0.001
% of positive container*	[a]18.32 (N = 393)	[b]73.61 (N = 360)	[b]74.64 (N = 347)	< 0.001
Bromeliad	[a]1.67	[b]36.04	[c]52.10	< 0.001
Bamboo	[a]11.76	[b]89.16	[b]85.00	< 0.001
Ovitrap	[a]23.71	[b]85.26	[b]83.52	< 0.001
Tire	[a]47.30	[b]98.59	[c]92.98	< 0.001
% of co-occurrence with CompI	[a]80.56 (N = 72)	[b]33.58 (N = 265)	[c]25.10 (N = 259)	< 0.001
Bromeliad	50.00	55.00	75.81	NS
Bamboo	50.00	10.81	8.82	NA
Ovitrap	91.30	17.28	1.32	NA
Tire	[a]85.71	[b]64.29	[c]20.75	< 0.001
% of co-occurrence with CompII	[a]77.78	[b]92.83	[a,b]87.26	< 0.01
Bromeliad	100.00	100.00	96.77	NS
Bamboo	[a]100.00	[b]89.19	[a,b]85.29	< 0.05
Ovitrap	[a]60.87	[b]86.42	[b]80.26	< 0.01
Tire	[a]80.00	[b]100.00	[a]88.68	< 0.01
% of co-occurrence with Pred	[a]5.56	[b]23.02	[a]1.93	< 0.001
Bromeliad	0.00	32.50	1.61	NA
Bamboo	0.00	12.16	0.00	NA
Ovitrap	0.00	11.11	0.00	NA
Tire	[a]11.43	[b]42.86	[a]7.55	< 0.001

*Corresponds to the percentage of occurrence of *Ae. aegypti*. Containers that had dried out were not taken into account The significant effect of the degree of urbanization was tested using a one-way ANOVA or *Chi*-square when proportions were tested. Exponential letters indicate a pairwise significance between sites at *P* < 0.05 (*P* adjusted for multiple comparisons)

highly urbanized sites, respectively (Table 3). *Aedes aegypti* also co-occurred with secondary competitors and predators, and, in both cases, the highest percentages were found in the moderately urbanized site (Table 3). In addition, regardless of the site, the percentage of co-occurrence of *Ae. aegypti* with competitors and predators was higher in water-filled tires compared to the other water containers (Table 3).

Effects of biotic and abiotic parameters on the abundance of Aedes aegypti immatures

The microclimatic variables measured in each experimental site permitted us to confirm that our selection based on increasingly impervious surface coverage resulted in warmer and drier environments as predicted by the urban heat island effect (Mohajerani et al. [39]). Models with microclimatic parameters calculated at one- or two-week intervals yielded similar results. Because the former always had the lowest AIC values, only the models with microclimatic parameters calculated at a one-week interval are presented (Fig. 3).
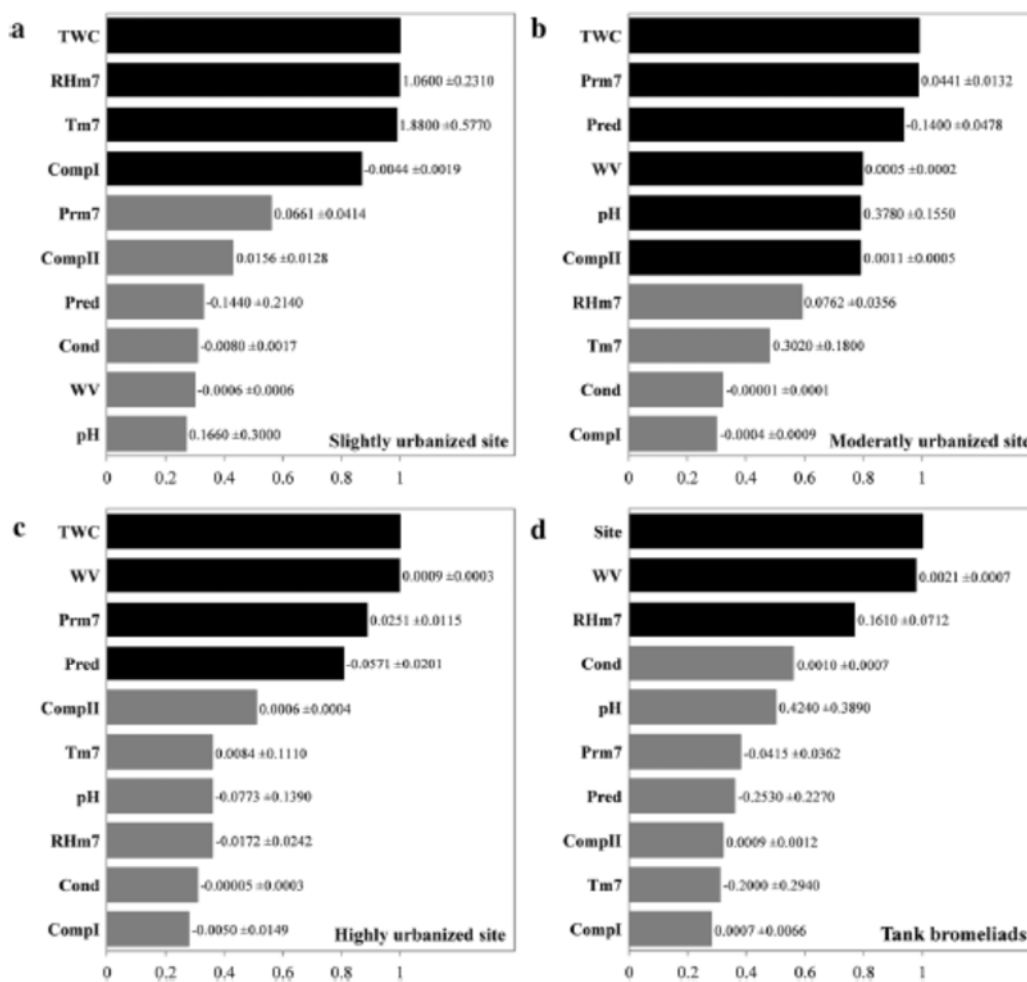

Fig. 3 Relative importance of each biotic or abiotic variable explaining the abundance of *Aedes aegypti* immatures in the slightly (a), moderately (b) and highly (c) urbanized experimental sites, and presence in tank bromeliads at those sites (d). Variables are ranked in increasing order of the sum of their Akaike weights, which are the probabilities that the given variable will appear in the best-fitting model (lowest AIC value). Black bars indicate variables with a probability higher than 0.7 of appearing in the best-fitting model. Estimated parameter values and their SE for each variable are shown to the right of each bar

The multimodel inference reveals that the type of water container (TWC) is an important parameter of the abundance of *Ae. aegypti* immatures whatever the site considered (Fig. 3a, b, c). Moreover, for the moderately and highly urbanized sites, the size of the aquatic habitat (w_+(WV) = 0.80 and w_+(WV) = 1, respectively) and the amount of precipitation (w_+(Prm7) = 0.99 and w_+(Prm7) = 0.89, respectively) showed a high relative importance and positively influenced the abundance of *Ae. aegypti* immatures (Fig. 3b, c). On the contrary, for these sites, predators negatively influenced the abundance of *Ae. aegypti* immatures with a relative importance of 0.94 and 0.81, respectively (Fig. 3b, c). In addition, for the moderately urbanized site, the pH and the abundance of secondary competitors (CompII) were also important (w_+(pH) = 0.79 and w_+(CompII) = 0.79) and positively influenced the abundance of *Ae. aegypti* immatures (Fig. 3b). For the slightly urbanized site, the relative importance of temperature and percentage of relative humidity were high (w_+(RHm7) = 1 and w_+(Tm7) = 0.99) and positively influenced the abundance of *Ae. aegypti* immatures (Fig. 3a). Also, the abundance of primary competitors was detected as an important parameter (w_+(CompI) = 0.87) for this site and showed a negative influence on the abundance of *Ae. aegypti* immatures (Fig. 3a).

In tank bromeliads, the presence of *Ae. aegypti* varied with the urbanized site considered (w_+(Site) = 1) and was positively influenced by the size of the aquatic habitat (w_+(WV) = 0.98) and the percentage of relative humidity (w_+(RHm7) = 0.77) (Fig. 3d). All the other parameters had a low relative importance and are assumed to have little or no effect.

Discussion

Our one-year-long study involving four types of water containers and three urbanized sites permitted us to obtain detailed information on the relative influence of microclimatic characteristics and biotic interactions on immature populations of *Ae. aegypti.*

Overall, most of the culicid species identified are strict container-inhabiting mosquitoes. Among the three most abundant species, *Ae. aegypti* and *Li. durhamii* use mostly artificial water containers, whereas *Cx. pleuristriatus* is a native bromeliad specialist restricted to this peculiar habitat. *Aedes aegypti* was the most frequent and abundant taxon in the two most urbanized sites (Tables 2, 3), confirming its strong domestic status (Juliano and Lounibos [26]; Mocellin et al. [38]; Powell et al. [41]), whereas precipitation promotes the abundance of its immatures.

In the slightly urbanized site, interactions with primary competitors were detrimental to *Ae. aegypti* immatures. This negative impact can be attributed to *Li. durhamii*, the dominant native mosquito species in the area that likely prevents the long-term establishment of *Ae. aegypti* through a mechanism of competitive displacement and reduction (Honório et al. [22]; Lounibos [31]; Talaga et al. [45]). In the moderately and highly urbanized sites, the abundance of *Ae. aegypti* was negatively impacted when it co-occurred with the native predator mosquito species *Toxorhynchites haemorrhoidalis* (F.), a well-known genus already used as biocontrol agent of container-inhabiting mosquitoes (Focks [16]). The same was true for a predator chironomid species of the sub-family Tanypodinae. These two species clearly play a role in the lower abundance of *Ae. aegypti* at the community scale, but their relatively low occurrence at the meta-community scale may reduce their impact on the overall population dynamics of *Ae. aegypti*. Competitive displacement and reduction as well as predation by native aquatic organisms can be considered a form of ecosystem service (Millennium Ecosystem Assessment [37]).

We also provide evidence from the moderately urbanized site that the abundance of *Ae. aegypti* immatures was enhanced when it co-occurred with secondary competitors, especially chironomid immatures (Table 2; Figs. 1, 2). This is likely due to two non-mutually exclusive possibilities. First, the presence of secondary competitors might reduce the predation pressure exerted on *Ae. aegypti* immatures by higher trophic levels, a mechanism known as an apparent mutualism (Blaustein and Chase [5]). Second, the presence of these secondary competitors might also result in a greater amount of available food resources because, by processing coarse particulate organic matter, they increase the amount of fine particulate organic matter (e.g., feces and/or uningested matter) beneficial to *Ae. aegypti* larvae (Heard [21]; Daugherty and Juliano [11]). Co-occurrence of *Ae. aegypti* with chironomid immatures is probably widespread but overlooked (Martínez et al. [34]), and thus we encourage further investigation.

Container type and size also influence *Ae. aegypti* immatures which are more abundant in artificial and large water containers. Water-filled tires, which constitute a customary habitat for many culicids, also appear in this study to be the most propitious habitat for *Ae. aegypti* (Yee [51]). Furthermore, while the aquatic community structure did not differ between tires, ovitraps and bamboo, bromeliads sheltered structurally distinct communities and also represented the least productive aquatic habitat for *Ae. aegypti*. Something likely due, at least in part, to the acidity of the water retained in their tanks (Lopez et al. [29]). Consequently, this aquatic habitat has a comparatively small role in the production of *Ae. aegypti* although immatures were more frequent in large bromeliads growing in more urbanized areas (see also Cunha et al. [9]; Mocellin et al. [38]). The presence of *Cx. pleuristriatus* in bromeliads was associated with the highly urbanized site, but its negative influence on *Ae. aegypti* was not detected. This situation contrasts with native *Wyeomyia* species that negatively impact *Aedes albopictus* (Skuse) populations living in Floridian bromeliads (Lounibos et al. [32]) but can be explained by lower larval competition due to the different feeding modes used by *Aedes* and *Culex* mosquitoes (collecting–gathering *versus* collecting–filtering, respectively) (Talaga et al. [47]). Because they can abound in Neotropical cities, a better understanding of the role of tank bromeliads in the population dynamics of *Ae. aegypti* is needed.

Overall, this study, can be used to prioritize regional control strategies directed at preventing the development of *Ae. aegypti* immatures by targeting specific areas and water container types. Immature populations of *Ae. aegypti* are negatively influenced in the slightly urbanized site by competition with native culicids and in the more urbanized sites by predators. We can image that these ecosystem services can be enhanced by the conservation and/or augmentation of natural enemies, for example, through modifications to landscape features (Talaga et al. [49]). Reinforcing the presence of natural enemies could also be used in combination with incompatible and/or sterile insect techniques with little or no downsides. Moreover, because biological insect control techniques inevitably reduce environmental niche occupancy, reinforcing the presence of natural enemies would likely improve the short- and long-term success of these methods by increasing the level of biotic interactions and reducing the risk of recolonization by the targeted species. However, this approach is in apparent contradiction with the use of water container reduction and insecticide-based techniques which likely impact natural communities as a whole but remain the most common methods for controlling *Ae. aegypti* (e.g., Epelboin et al. [15]).

In conclusion, by examining *Ae. aegypti* immatures within aquatic communities and not in isolation as is usually done, this study shows that interactions with native aquatic organisms can have a strong impact on immature populations of *Ae. aegypti*. Because these interactions

resulted in antagonistic outcomes depending on the urbanized site considered (e.g., competition and predation *versus* facilitation), they need to be better examined in the future. The conservation and/or augmentation of natural enemies should improve the short- and long-term success of incompatible and/or sterile insect techniques, thus opening up perspectives for the future of mosquito management.

Author contributions

ST, CL and YD conceived the research. ST and AD prepared the experimental setup. ST and CL conducted the experiments. ST and AD wrote the manuscript. FA prepared Fig. 1. All authors edited the manuscript and approved the final version.

Acknowledgments

Compliance with ethical standards
Conflict of interest

The authors have declared that no conflict of interest exists.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Research involving human participants and/or animals

This article does not include studies with human participants or animals (vertebrates) conducted by any of the authors.

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