

Bionomics aspects of major arbovirus vectors, *Aedes aegypti* and *Aedes albopictus* in different ecoclimatic environments of Cameroon

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Abstract

Background

Aedes mosquitoes transmit dengue, Zika and chikungunya viruses, which remain major public health concerns globally and in Africa. Effective prevention and control rely on a sound understanding of vector bio-ecology. In Cameroon, little is known about the bio-ecology and behaviour of *Aedes* vectors. This study aimed to update the geographical distribution, resting behaviour, host-seeking behaviour and natural infections by arboviruses in *Aedes* spp. vectors in Cameroon.

Methods

Sampling was carried out in eight locations across the country. Immature stages of *Aedes* were collected in seven localities, transported to the insectary, reared to adult, identified, counted, and grouped by species to determine their distribution. Resting behaviour was assessed through inside houses and outdoor aspirations using Prokopack and CDC backpack aspirators. Host-seeking activity was evaluated with BG-Sentinel traps. Natural infections with dengue, chikungunya and Zika viruses were determined by real-time quantitative Multiplex PCR and RT-PCR. Chi-squared test was used to compare abundances

Results

17,040 adults identified from the immature stages of *Aedes* spp. collected. We noted 51.05% *Ae. albopictus*, 48.93% *Ae. aegypti*, and 0.02% *Ae. vittatus*. We noted the first identification of *Aedes albopictus* in Meiganga since its introduction in Cameroon. *Aedes aegypti* was present in all the localities surveyed, while *Ae. albopictus* had a distribution limited to the southern part of the country below latitude 6.4°N. Resting and biting behaviours have shown that both species are essentially exophilic and exophagous. *Aedes aegypti* preferred to rest in used tyres, while *Ae. albopictus* had a wide range of preferred resting sites (natural vegetation, used tyres, banana plants, mixed resting places). Both species of mosquito bite throughout the day between 6 a.m. to 7 p.m., with essentially unimodal host-seeking activity, with a major peak of activity in the afternoon between 1 p.m. and 6 p.m. Natural infections by dengue, chikungunya and Zika viruses showed one positive pool of *Ae. aegypti* for serotype 3 of dengue virus (DENV-3).

Conclusion

These results provide an important knowledge base on the bionomics of *Aedes* mosquitoes in different localities in Cameroon and also establish evidence base essential for designing and implementing targeted arboviral diseases surveillance and vector control strategies.

Background

Arboviral diseases such as dengue, Zika, chikungunya, and yellow fever have increasingly become a major public health concern around the world, and specifically in Africa. Previous studies reported a widespread of morbidity and mortality assigned to these arboviruses, especially in sub-Saharan Africa, where around 70% of the population is at risk of contracting one of these diseases. These diseases are transmitted to humans by *Aedes* mosquitoes [1], of which *Aedes aegypti* and *Aedes albopictus* are the primary competent vectors. Although dengue, chikungunya, and Zika viruses have been reported repeatedly in human populations across Cameroon, natural infections of local *Aedes* populations with these arboviruses have not yet been documented. In the absence of effective vaccines and specific antiviral treatments for these arboviruses, vector control remains the cornerstone of tackling these diseases. Whether based on insecticides [2, 3, 4, 5, 6], biological control agents [7, 8, 9, 10, 11], or environmental management, this control requires a good understanding of vector bionomics [12, 13].

The literature indicates that the two primary arbovirus vectors have distinct geographic origins. *Aedes aegypti* is native to Africa [14] and is now distributed throughout tropical regions worldwide, whereas *Ae. albopictus* originates from Asia [15]. However, over the past four decades, *Ae. albopictus* has undergone rapid global expansion [16]. This invasive species was first reported in Central Africa in the early 2000s, with its initial detection in Cameroon [17]. Since then, it has rapidly colonised the southern part of the country, where ongoing displacement of the indigenous species, *Ae. aegypti*, has been documented in several locations [18, 19, 20]. In these areas where both species coexist, *Ae. aegypti* prefers urban neighbourhoods with a high building density, while *Ae. albopictus* prefers peri-urban neighbourhoods surrounded by vegetation [19, 20, 21, 22, 23].

Both *Aedes* species are essentially diurnal, resting indoors or outdoors in a wide range of resting sites [24, 25, 26]. However, in Asia and South America, *Ae. aegypti* is known to be a highly endophilic species, resting almost exclusively indoor in Bangkok, Thailand [27], in Panama [28], in Trinidad [29], and in Acapulco, Mexico [30]. Although some studies showed variations in the preference for resting places in Sri Lanka [24], in Panama [28], in Costa Rica [31], in the Dominican Republic [32], in Puerto Rico [33], and in Mexico [30]. In Africa, *Ae. aegypti* prefers to rest in a wide range of indoor and outdoor resting places. A recent study in Senegal revealed that, *Ae. aegypti* rests both indoors (including rooms and kitchens) and outdoors in vegetation, used tyres, bricks, and scrap metals [34, 35]. However, there are two main peaks of host-seeking activity: a morning peak and an evening peak starting in the afternoon [36, 37, 38].

There is a dearth of information on *Aedes* behaviour in Cameroon. Studies carried out in Yaoundé showed that the two major vectors bite throughout the day, with a peak of activity starting in the afternoon from 1 p.m. towards the evening at 7 p.m. [39] for *Ae. aegypti* and between 3 to 7 p.m. for *Ae. albopictus* [40].

In this study, we therefore aimed to characterise the spatial distribution, resting places, and host-seeking activity of *Aedes* mosquitoes in Cameroon, as well as their natural infections to dengue, chikungunya, and Zika viruses. The study of *Aedes* bionomics and the arboviruses detection arboviruses in these vectors is crucial, as it will help health authorities understand how these vectors interact with human populations and transform scientific knowledge into concrete public health actions adapted to the ecological reality of a rapidly environment changing.

Methods

Setting and study sites

Entomological surveys of immature stages and adults *Aedes* spp. mosquitoes were carried out in eight locations in four ecoclimatic zones from June 2022 to November 2024 across Cameroon following the North-South transect. These locations included Maroua (10°35'27"N ; 14°18'57"E) in the Sahelian tropical zone, Garoua (09°18'05"N ; 13°23'51"E), and Meiganga (06°33'45"N ; 14°19'10"E) in the Sudanian tropical zone, Yaoundé (03°50'29"N ; 11°29'31"E), Obala (04°10'00"N ; 11°31'59"E), and Bertoua (04°34'38"N ; 13°41'04"E) in the Guinean equatorial zone, and Douala (04°2'53"N ; 09°42'15"E), and Manjo (04°50'34"N, 09°49'18"E) in the Cameroonian equatorial zone (Fig. 1). The choice of these study locations and the neighbourhoods within each location was guided by referring to work previously conducted in Cameroon, in which the authors reported: (1) the presence of *Ae. aegypti* and/or *Ae. albopictus*, the distribution and abundance of *Aedes* spp. larval habitats [19, 20, 21, 22, 23]; (2) the arboviruses circulation in the human [41, 42, 43], and (3) general knowledge of the resting sites of *Aedes* spp. [24, 44]. Certain localities, such as Obala and Manjo, were included without any data initially available for these locations. The sites were selected based on their different environmental settings; urban and peri-urban areas (Maroua, Garoua, Bertoua, Yaoundé and Douala), semi-urban and rural areas (Obala and Manjo). Urban environments are located within cities with a high density of buildings, peri-urban environments are area bordering urban centre, semi-urban environments are transitional areas between town and rural. Rural environments are located at edge of forests with a high density of vegetation, mainly made-up of plantations [45].

Evaluation of the distribution of *Aedes* mosquitoes in Cameroon

To ensure a representative sample, three neighbourhoods were selected in each environmental setting for the collection of immature *Aedes* stages. In each selected neighbourhood, all potential *Aedes* larval habitats found were prospected for immature stages of *Aedes*, mainly along main roads, in garages, tyre stores, tyres gluers, in premises and their surroundings. These larval habitats include domestic larval habitats (water storage containers, animal troughs, etc.), peri-domestic larval habitats (used tyres, discarded containers, car wrecks, etc.), and natural larval habitats (tree holes, snail shells, dead leaves, etc.). When immature stages were present, they were collected with a ladle or pipette and transferred with water from larval habitats into labelled bottles, and transported to the insectary of the Centre for Research in Infectious Diseases. In the insectary, larvae were isolated from predators such as *Lutzia tigripes*, reared under optimised insectary conditions (temperature of $28 \pm 2^\circ\text{C}$ and relative humidity of $80 \pm 10\%$) and feed with larval food until adult stage. After emergence, adult mosquitoes was morphologically identified using specific taxonomic keys [46, 47], counted, and grouped by species in cages.

Identification of resting place of *Aedes aegypti* and *Aedes albopictus*

In order to identify resting places of *Aedes* mosquitoes, a certain number of neighbourhoods located in the urban, peri-urban, semi-urban, and rural was selected in four locations across the country (Douala, Yaoundé, Garoua, and Obala). In each neighbourhood, mosquitoes were collected inside houses and outdoor (around houses, in used tyres, and vegetation) between 7 to 12 a.m. For indoor collections, 20 houses were randomly selected per neighbourhood, in which aspirations was performed in saloon, bedrooms, kitchen (under chairs, tables, bed, corners, walls, wardrobes, etc.) for 15 to 20 min using improved Prokopack aspirator model 1419 (John W. Hock Company, USA) powered by battery (12 volts, 9 amps). For outdoor collections, other resting sites (used tyres, vegetation...) were also prospected to search for resting adult mosquitoes. CDC back-pack model 1412 (John W. Hock Co., Gainesville, FL, USA) and improved Prokopack were used to capture mosquitoes in flight or in rest. The mosquito capture site was noted on each collection box. Mosquitoes collected were then brought back alive in the insectary, knock down in -20°C and identified on ice (to avoid the death of the mosquito and therefore the degradation of RNA), using specific morphological identification keys [46, 47].

Determination of host-seeking behaviour of *Aedes aegypti* and *Aedes albopictus*

BG-Sentinel traps were used to assess fluctuations abundance, host-seeking activity and biting location (inside houses/outdoor) of *Aedes* mosquitoes in different neighbourhoods selected in same locations mentioned above. Guinea pigs were used as baits. The trap is essentially a collapsible container with a white lid and a black entrance funnel in the middle. Inside there is a small electrical fan that sucks air through the trap and draws any approaching mosquitoes into a catch bag [48]. Odour released by guinea pig inside trap is spread by the fan and attracts host-seeking mosquito, which are thus trapped in the net attached to the box. Collections took place between 6 a.m. to 7 p.m. In each neighbourhood, three traps were placed in three randomly selected houses and five traps were placed outdoor near houses (courtyards, vegetation) in places shaded from the sun. Nets collecting mosquitoes were changed every hour, and mosquitoes collected were morphologically identified in the field using specific identification keys [46, 47], counted and grouped separately by species (*Ae. albopictus* or *Ae. aegypti*), and sex (male or female) into cups and stored at -20°C .

Adults *Aedes* mosquitoes conservation

On ice, unfed females collected in different setting were pooled in pools of 10 individuals and males in pools of 30 individuals in 0.5 mL Eppendorf tubes containing RNA *later*. The bloodfed females were conserved in 1.5 mL Eppendorf tubes containing silicagel and analysed to detect the bloodmeal source [49]. Once in the laboratory, mosquitoes in the RNA *later* were stored at -80°C used for the virus detection and those in tubes containing silicagel at -20°C used for the blood meal detection.

Multiplex quantitative PCR for dengue virus detection and serotyping using real-time RT-PCR

Total RNA in mosquitoes was extracted using the QIAamp-Viral RNA mini kit, Qiagen [50] following manufacturer's recommendations. Extracted RNA was used to perform multiplex quantitative PCR using the "Co-Dx™ Vector Smart® Zika, Dengue, and Chikungunya (VZDC) kit" CO-DIAGNOSTICS, INC. Amplification was performed in a final volume of 15 µL containing 5 µL of RNA and 10 µL of Master Mix (kit Co-Dx™ Vector Smart® Zika, Dengue, and Chikungunya (VZDC)). During amplification reaction, reverse transcription was performed for 15 minutes at 45°C, followed by initial denaturation for 2 minutes at 95°C and 50 amplification cycles (3 seconds at 95°C and 32 seconds at 55°C). cDNA for dengue virus-positive samples in multiplex qPCR was synthesised using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California, USA) according to the manufacturer's recommendations. Dengue serotype-specific real-time RT-PCR was performed using the TaqMan Universal PCR Master Mix kit (Applied Biosystems, Foster City, California, USA) to determine the serotype of these samples using four different sets of 5' UTR and capsid-specific primers and probes [51] "Additional file 1 Table S1". The PCR reaction was performed under the following conditions: denaturation at 50°C for 2 minutes followed by 10 minutes at 94°C, then amplification and fluorescence quantification were carried out for 45 cycles lasting 15 seconds at 94°C and 1 minute at 60°C. All experiments were performed on the Stratagene VERSANT™ kPCR qPCR machine (Molecular System AD, SIEMENS).

Data analysis

The spatial distribution of *Aedes* species identified from emerged immatures stages collections across different localities and the resting places preferences of adults *Aedes* mosquitoes according to environmental setting and sampling season were compared using Chi-square tests. Differences were considered statistically significant when *P*-value was less than 0.05.

Results

Distribution of *Aedes* spp. mosquitoes in urban, peri-urban, and semi-urban environments in Cameroon

A total of 17,040 specimens of *Aedes* were identified following emergence from collected immature stages, comprising 51.05% (8,700/17,040) of *Ae. albopictus*, 48.93% (8,337/17,040) of *Ae. aegypti*, and 0.02% (03/17,040) of *Ae. vittatus*. *Aedes albopictus* was the predominant species among the three ($\chi^2 = 119.78$; $P < 10^{-3}$) (Table 1). Other mosquitoes' genera were found in association with the *Aedes* spp., including *Anopheles*, *Culex* and *Eretmapodites*. *Aedes aegypti* was found in all the locations prospected, while *Ae. albopictus* distribution was limited to the southern part of country below 6°4'N (Table 1).

In all localities where the two species are sympatric, *Ae. albopictus* was more abundant except in Douala, where *Ae. aegypti* was predominant. Analyses by environment (peri-urban versus urban) showed that *Ae. albopictus* was the dominant species in urban and peri-urban environments in all sympatric areas ($n = 14,440$; 58.01%) except in Yaoundé, where *Ae. aegypti* was dominant in urban area ($n = 7,774$; 51.23%). Analyses also revealed that, *Ae. albopictus* was more abundant and outcompeted the native species *Ae. aegypti* in certain locations such as Bertoua ($n = 2,929$; 69.23% versus 30.77%, respectively) and Meiganga ($n = 928$; 74.57% versus 25.43% respectively). Others *Aedes* species such as *Aedes vittatus* were identified only in the peri-urban area of Garoua. Surprisingly, our results reported for the first time the presence of *Ae. albopictus* in Meiganga (N 06°33'45"; E 14°19'10") since its first introduction in Cameroon in the 2000s.

Resting place of *Aedes* spp. mosquitoes in Cameroon

The resting place of *Aedes* spp. mosquitoes was investigated across four localities in Cameroon (Yaoundé, Obala, Douala, and Garoua). A total of 8,217 mosquitoes were collected from both inside houses and outdoor. Of these, 50.54% (4,153/8,217) were identified as *Culex* spp., 47.57% (3,909/8,217) as *Aedes* spp., 1.49% (122/8,217) as *Anopheles* spp., and 0.40% (33/8,217) as *Eretmapodites* spp. "Additional file 2 Table S2". Among the 3,909 *Aedes* mosquitoes collected, *Ae. albopictus* was the predominant species, accounting for 80.96% (3,165/3,909) of all *Aedes* specimens collected, whereas *Ae. aegypti* represented only 19.06% (744/3,909). A highly significant difference was observed in the distribution of the two mosquito species across resting places ($\chi^2 = 686.15$, $df = 6$, $p < 0.0001$) (Table 2). Analysis of resting places preferences showed that *Ae. aegypti* rested predominantly in used tyres, which harboured nearly half of all individuals recorded (46.91%), followed by natural vegetation (31.85%), flower gardens (7.12%), banana plants and mixed habitats (6.18% each), cocoa trees (0.95%), and inside houses (0.81%). In contrast, *Ae. albopictus* was captured primarily in natural vegetation (39.27%), followed by mixed resting sites (32.35%), used tyres (9.73%), banana plants (9.07%), flower gardens (5.94%), cocoa trees (3.51%), and inside houses (0.13%) (Table 2). Seasonal variation was also significant for both species. For *Ae. aegypti*, most individuals were collected during the rainy season (74.19%) compared with the dry season (25.81%) ($\chi^2 = 36.37$, $df = 6$, $p < 0.0001$) (Table 2). Conversely, *Ae. albopictus* was more abundant during the dry season (66.45%) than during the rainy season (33.55%) ($\chi^2 = 117.77$, $df = 6$, $p < 0.0001$). Collectively, these findings highlight distinct resting place preferences and seasonal population dynamics, with *Ae. aegypti* being more closely associated with artificial habitats and rainy conditions, whereas *Ae. albopictus* predominates in vegetated environments and remains the dominant species during both seasons (Table 2).

Resting place preferences of *Aedes aegypti* and *Aedes albopictus* by environment and season in Cameroon.

The results demonstrate significant variation in the resting place distribution of *Ae. aegypti* and *Ae. albopictus* across urban, peri-urban, and semi-urban/rural environments, with highly significant differences observed between species in all ecological settings (urban: $\chi^2 = 737.35$, $df = 5$, $p < 0.0001$; peri-urban: $\chi^2 = 16.49$, $df = 3$, $p = 0.0009$; semi-urban/rural: $\chi^2 = 13.01$, $df = 3$, $p = 0.0046$) (Table 3). Overall, *Ae. albopictus* was the dominant species in every environment, representing 79.88%, 80.95%, and 87.99% of specimens collected in urban, peri-urban, and semi-urban/rural areas, respectively. In urban area, *Ae. aegypti* was primarily associated with used tyres (60.50%) and natural vegetation (18.67%), whereas *Ae. albopictus* predominantly occupied mixed habitats (46.31%) and natural vegetation (29.76%). In peri-urban areas, both species showed a strong preference for natural vegetation, accounting for 67.36% of *Ae. aegypti* and 73.04% of *Ae. albopictus* collections. Similarly, in semi-urban/rural environments, natural vegetation represented the principal resting place for *Ae. aegypti*

(83.72%), while *Ae. albopictus* was distributed mainly between natural vegetation (40.35%) and cocoa trees (32.46%). Seasonal effects were also evident. *Aedes aegypti* was consistently more abundant during the rainy season in urban (76.12%), peri-urban (68.06%), and semi-urban/rural (69.77%) areas (Table 3). In contrast, *Ae. albopictus* was significantly more abundant during the dry season in urban (63.64%), peri-urban (80.72%), and semi-urban/rural (59.06%) areas, although its resting places preferences shifted seasonally, particularly towards mixed habitats in urban areas during the rainy season. Collectively, these findings highlight marked ecological segregation between the two species, with *Ae. aegypti* showing a stronger association with artificial and rain-dependent habitats, whereas *Ae. albopictus* exhibits broader ecological plasticity, predominates in vegetated environments, and remains the dominant species across all landscape types throughout the year.

Resting place of *Aedes aegypti* and *Aedes albopictus* across distinct ecological zones in Cameroon.

The results reveal marked differences in the distribution and seasonal abundance of *Aedes aegypti* and *Aedes albopictus* across the three ecological zones investigated. Overall, *Ae. albopictus* was the dominant species in the equatorial zones, accounting for 92.56% of specimens in Yaoundé and 77.79% in Douala, whereas *Ae. aegypti* predominated in sudanian tropical zone (Garoua), representing 97.24% of all individuals collected (Table 4). In Yaoundé, both species were primarily associated with natural vegetation, which accounted for 66.67% and 63.74% of *Ae. aegypti* and *Ae. albopictus* collections, respectively, and no significant interspecific difference in resting place distribution was detected ($\chi^2 = 9.11$, $df = 4$, $p = 0.0584$). In contrast, highly significant differences were observed in Douala ($\chi^2 = 850.91$, $df = 5$, $p < 0.0001$), where *Ae. aegypti* rested predominantly in used tyres (64.87%), while *Ae. albopictus* was mainly found in mixed resting place (63.02%). In Garoua, both species were strongly associated with natural vegetation, although the low number of *Ae. albopictus* specimens resulted in no significant difference between species ($\chi^2 = 1.57$, $df = 2$, $p = 0.457$) (Table 4). Seasonal patterns were also evident. *Ae. aegypti* was consistently more abundant during the rainy season in both Yaoundé (72.92%) and Douala (67.03%). Conversely, *Ae. albopictus* was more abundant during the dry season in Yaoundé (76.80%) and Douala (60.55%), although significant shifts in resting-site use between seasons were observed for both species (Table 4). Collectively, these findings highlight strong ecological segregation between the two vectors, with *Ae. aegypti* exhibiting a greater affinity for anthropogenic habitats, particularly used tyres, and benefiting from rainy-season conditions, whereas *Ae. albopictus* is more closely associated with vegetated and mixed resting place and remains the dominant species throughout the equatorial zone.

Host-seeking behaviour of *Aedes aegypti* and *Aedes albopictus* in urban, peri-urban, semi-urban and rural areas of Cameroon

BG-Sentinel traps were used to assess host-seeking behaviour of adult females *Ae. aegypti* and *Ae. albopictus* inside houses and outdoor between 6 a.m. to 7 p.m. in four localities in Cameroon (Yaoundé, Obala, Douala, and Garoua) belonging to three different climatic zones. The results showed that no *Aedes* mosquito were collected inside houses. However, outdoors results show that both species bite throughout the day from 6 a.m. to 7 p.m., with one or two activity peaks according to the location “Additional file 3 Table S3”.

In *Ae. aegypti*, host-seeking activity exhibited a predominantly unimodal pattern across nearly all sampling sites, with a major activity peak occurring during the afternoon between 2 p.m. to 6 p.m. in both seasons (Fig. 2). At some sites, including New-Bell (urban Douala), Camp Chinois, and Bocklé (urban and peri-urban Garoua, respectively), this principal afternoon peak was preceded by a less pronounced morning peak occurring between 6 a.m. to 11 a.m. (Fig. 2d, 2g, and 2i). Substantial spatial variation in host-seeking activity was observed in the urban area of Douala, particularly at the Bisseké site, where biting activity was highest during the early morning, peaking between 7 a.m. to 8 a.m. in the dry season and between 8 a.m. to 9 a.m. in the rainy season (Fig. 2c).

Overall, *Ae. albopictus* also exhibited a predominantly unimodal host-seeking activity pattern across nearly all sampling sites, with a major activity peak occurring in the afternoon between 1 p.m. to 6 p.m. during both seasons (Fig. 3). The results further showed that during the dry season, this principal afternoon peak was preceded by a less pronounced morning peak between 8 a.m. to 11 a.m. at some sites, including Fouda and Afanoya (urban and peri-urban areas of Yaoundé, respectively) and Bonamoutongo (peri-urban Douala) (Fig. 3a, 3c, and 3f). A bimodal activity pattern was however observed at the Bisseké site (urban area Douala), where biting activity was highest during the early morning between 6 a.m. to 9 a.m. and lower during the afternoon between 4 p.m. to 6 p.m. in both seasons (Fig. 3d). Marked variation of host-seeking activity was observed at this site, as well as at New-Bell and Ekoundoum (urban areas of Douala and Yaoundé, respectively). Overall, the findings indicate that, *Ae. albopictus* was more aggressive during the dry season than during the rainy season (Fig. 3).

Natural infections by dengue, chikungunya and Zika viruses in adult *Aedes* mosquitoes.

A total of 4,103 female *Aedes* mosquitoes collected in different environments in Cameroon were used for dengue, chikungunya and Zika virus detections. *Aedes albopictus* was more abundant (3,249; 79.19%) compared to *Ae. aegypti* (854; 20.81%). Virus detection by multiplex qPCR was carried out on a total of 408 pools of *Aedes* mosquitoes (323 pools of *Ae. albopictus* and 85 pools of *Ae. aegypti*), tested for three viruses (DENV, CHIKV and ZIKV) at the same time in both *Aedes* species. Five pools of *Ae. aegypti* from Douala samples (03 pools of Bisseké and 02 pools New-Bell) were tested positive to dengue virus. Positive serotyping analyses using real-time RT-PCR showed that the circulating dengue virus belonged to dengue serotype 3 (DENV-3). However, the low viral load did not allow sequencing of the sample, and therefore the viral genotype could not be determined.

Discussion

Distribution of *Aedes* spp. mosquito in urban, peri-urban, and semi-urban environments in Cameroon

The aim of this section was to present an updated overview of the current geographical distribution of *Aedes* spp. in urban, peri-urban, and semi-urban environments in Cameroon. Results reported the presence of three *Aedes* species comprising *Ae. aegypti*, *Ae. albopictus*, and *Ae. vittatus*. These observations contrast with those of recent studies conducted in Cameroon, in which the authors identified more than three *Aedes* mosquito species [23, 52, 53]. *Aedes aegypti* was present in all the locations surveyed, while *Ae. albopictus* has a limited distribution in the southern part of country, around 6°4'N latitude. The

predominance of *Ae. albopictus* (invasive species) over *Ae. aegypti* (native species) is also reported in almost all localities where both species are sympatric. These results corroborate those previously reported in several localities in Cameroon in 2019 [19]. This observation suggests that the environmental conditions found in most cities in the southern part of the country are more favourable to the development of *Ae. albopictus*. However, the spatial distribution between the two major species present in different locations in Cameroon could be due to differences in the environmental factors such as climate, vegetation and building density [20, 54].

This study revealed for the first time the presence of *Ae. albopictus* in Meiganga since its introduction in Cameroon in the 2000s [17]. We also noticed a predominance of this invasive species over native species *Ae. aegypti* which, 4 years ago, was the only species recorded in this locality [19]. The establishment of *Ae. albopictus* in Meiganga corroborates the dynamic process of invasion and establishment over time, as previously suggested by Simard *et al.* in 2005 [18]. However, the limited spread of *Ae. albopictus* in northern regions is likely mainly due to the dry environment and high temperatures prevailing in these areas, as previously reported [20].

Aedes aegypti was the predominant species in Douala, which corroborates the previous results observed in this city [19, 20, 21]. However, the predominance of *Aedes aegypti* in urban area of Yaoundé, contrasts with the previous study in this location highlighted the overdominance of *Ae. albopictus* in both urban and peri-urban areas [19, 22, 23, 55, 56]. Further longitudinal studies are needed to better elucidate this finding.

Resting place of *Aedes* spp. mosquitoes in Cameroon

Understanding the resting behaviours of *Aedes* mosquitoes is vital for public health. By identifying exactly where these vectors congregate, authorities can apply insecticides more strategically. This focused method reduces non-targeted use of insecticide which saves money and minimizes environmental damage while slowing the rise of pesticide-resistant populations. Identifying resting places of *Aedes* mosquitoes also makes it easier to monitor this vector and associated arboviruses [35].

In Africa, *Ae. aegypti* and *Ae. albopictus* are traditionally known as a highly exophilic mosquito species [27, 28, 29, 30] resting in the vegetation found in residential areas [57, 58], but can also be found indoor [24, 59]. Following this classical view, our results clearly showed that different populations of *Ae. aegypti* and *Ae. albopictus* in our collection sites exclusively rested outdoor, with only 0.8% of *Ae. aegypti* and 0.13% of *Ae. albopictus* collected inside houses. The collection of more adult *Ae. aegypti* mosquito outdoor than inside houses at four sites is corroborating previous studies carried out in Africa [60, 61], and elsewhere notably in Trinidad [62], in Malaysia [63, 64], and Brazil [65]. This exophilic behaviour is thought to be mainly due to their adaptation to reproduce in a wide range of artificial containers mainly located outdoors around houses [22, 66, 67].

Overall, *Ae. aegypti* was most frequently collected in greater numbers across both seasons in outdoor resting places such as used tyres and natural vegetation, similar observations previously reported in South-East Senegal [35] and recently in the Southern Afar region of Ethiopia [68]. Moreover, this result is contrasting other observations in Dire Dawa, in East Ethiopia, where outdoor collections of *Ae. aegypti* was carried out from open barrels [69]. The used tyres thus reported as main resting places of *Ae. aegypti* in the present study, could be associated with their low level of disturbance, provision of shade, protection of adults and frequent role as nearby larval habitats.

The present study revealed that, *Ae. albopictus* in all our collection sites rested mainly in natural vegetation around residential areas, suggesting that landscaping can strongly influence its distribution in the areas, in agreement with findings from others regions, particularly Florida (USA) [57] and Sri Lanka (Asia) [24, 58]. *Aedes albopictus* was also abundant in mixed resting places and coco trees, which could provide suitable larval habitats such as leaf axils and containers that can retain water and serve as oviposition sites [70, 71].

Seasonality also had a significant effect on the abundance of both *Ae. aegypti* and *Ae. albopictus*. *Aedes aegypti*, was predominantly collected during the rainy season, suggesting that rainfall may increase the proliferation of artificial larval habitats suitable for its development. Consistent with this hypothesis, nearly half of all *Ae. aegypti* specimens were collected from used tyres, which are widely recognised as highly productive larval habitats. This observation is consistent with the findings of Theno *et al.* [72] in Cameroon, where *Ae. aegypti* was reported to be more abundant in used tyres during the rainy season than during the dry season. In contrast, *Ae. albopictus* was significantly more abundant during the dry season. This pattern may be explained by the fact that, despite the greater availability of larval habitats in the rainy season, the vegetation that serves as the preferred resting place of *Ae. albopictus* is subjected to substantial environmental disturbances, including rainfall and winds, potentially reducing adult survival mosquito during the rainy season.

Together, these findings highlight the predominantly outdoor resting behaviour of both vectors in the study area and underscore the importance of focusing control and surveillance efforts on peri-domestic vegetation, used tyres, and plant-associated microhabitats.

Bite location and host-seeking activity of *Aedes aegypti* and *Aedes albopictus* in urban, peri-urban, semi-urban and rural environments in Cameroon

Host-seeking or biting activity was characterised in this study to determine the biting cycle of *Ae. aegypti* and *Ae. albopictus*, vectors of arboviruses in our study sites. This information is essential both for individual protection against mosquito bites and improving the timing of vector control interventions. Mosquito biting behaviour and frequencies vary considerably in time and space, as well as between species and climates. Overall, *Ae. aegypti* and *Ae. albopictus* collected for this study showed unimodal diurnal host-seeking patterns between 06 a.m. to 07 p.m., with a peak activity generally occurring in the afternoon between 01 p.m. to 06 p.m. These results are consistent with similar studies carried out in Democratic Republic of the Congo [26] and Cameroon [39, 40], where an unimodal host-seeking activity with major peak between 3 p.m. to 6 p.m. was observed. These observations contrast with studies carried out in Trinidad, [73], in Malaysia [62] and Sri Lanka [24] showing a bimodal biting behaviour during the day, with a first peak in the morning and another more significant peak of biting activity in the afternoon. However, in this study, *Ae. aegypti* from Camp Chinois and Bocklé neighbourhoods in Garoua and New-Bell neighbourhood in Douala, *Ae. albopictus* collected in dry season in Yaoundé (Fouda and Afanoya neighbourhoods) and in Douala (New-Bell and Bonamoutongo neighbourhoods) showed similar behaviour with two peaks of activity: a first peak of activity in the morning between 8 a.m. to 11 a.m., and

the second more significant peak in the afternoon between 3 p.m. and 6 p.m. These results differ from the study of nycthemeral cycle of *Ae. albopictus* previously carried out at Club France in Yaoundé, Cameroon [40] showing one activity peak in the afternoon between 3 p.m. to 7 p.m.

A significant reduction in *Ae. albopictus* host-seeking activity was observed during the rainy compared to dry seasons in Yaoundé likely due to lower temperatures that could slow blood-meal digestion, lengthen the gonotrophic cycle and consequently reduce daily mosquito biting rates [74, 75]. Similarly, a variation in biting activity between two species was observed in Douala, particularly in Bisseké (during both seasons) and New-Bell (in rainy season) and in the Ekoundoum neighbourhood of Yaoundé during the dry season. This variation could be associated with heavy rainfall during rainy season at these sites, and local atmospheric conditions (wind, temperature, rainfall, etc.) impacted by proximity to the sea at Bisseké site. These results corroborate work carried out on Island of Samui in Thailand showing that rainfall can modulate *Aedes* biting activity [76].

Natural infections by dengue, chikungunya and Zika viruses in adults *Aedes* mosquitoes

Natural infections by arboviruses (DENV, CHIKV and ZIKV) in female *Aedes* mosquitoes at different collection sites were investigated. Results showed that one pool of *Ae. aegypti* was infected with the DENV-3 virus, confirming dengue virus circulation in local vector populations. However, the viral load was too low to permit successful sequencing of the sample and, consequently, the determination of its genotype.

None of the *Ae. albopictus* pools screened tested positive for the arboviruses under investigation. This finding is consistent with vector competence studies conducted in Cameroon, which demonstrated that *Ae. aegypti* possesses markedly higher dengue virus transmission efficiency than *Ae. albopictus* [77]. In general, the detection of viruses in *Aedes* spp. populations during non-epidemic periods is rare [78]. Similar results have been observed in environments where the circulation of dengue virus in the human population has been documented [79].

Conclusion

Study findings identified three species of *Aedes* in the different urban, peri-urban, and semi-urban environments prospected: *Ae. aegypti*, *Ae. albopictus*, and *Ae. vittatus*. While *Ae. aegypti* is widespread, invasive *Ae. albopictus* dominates southern regions and is expanding to Meiganga. Both species are essentially exophilic and exophagous, bite during the day with a major peak host-seeking activity between 1 p.m. and 7 p.m. *Ae. aegypti* prefers used tyres for resting, while *Ae. albopictus* has a wide range of preferred resting sites (natural vegetation, used tyres, banana plants, mixed resting places). The detection of dengue virus confirms their active circulation in *Aedes* vectors, signalling an urgent risk for future epidemics. These findings support the urgent need for strengthened, integrated entomological and epidemiological arboviral surveillance system, coupled with targeted outdoor vector control in high-risk urban and peri-urban environments in Cameroon.

Declarations

Ethical approval: The project received ethical clearance from the National Ethics Committee for Human Health Research in Cameroon (N° 2022/12/1503/CE/CNERSH/SP) and the LSHTM Ethics Committee in the United Kingdom (N° 29790, 30/10/2023). Research authorizations were also obtained from Cameroon's Ministry of Scientific Research and Innovation (N° 000135/MINRESI/B00/C00/C10/C13) and the Ministry of Public Health (N° 631-17 23).

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Tables

Table .1: Spatial distribution of *Aedes* spp. mosquitoes from different locations in Cameroon. Chi-square (χ^2) tests were used to assess differences in species abundance within each locality; χ^2 , Chi-square statistic; *P*, probability value; n/a, not applicable; Ae., *Aedes*.

Location	Environments	Identified <i>Aedes</i> species					
		<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	<i>Ae. vittatus</i>	Total	χ^2	P value
Garoua	urban	629	0	0	629	n/a	n/a
	peri-urban	583	0	03	586	583	$< 10^{-3}$
Meiganga	urban	94	232	0	326	58.42	$< 10^{-3}$
	peri-urban	142	460	0	602	167.98	$< 10^{-3}$
Bertoua	urban	887	1985	0	2872	419.77	$< 10^{-3}$
	peri-urban	14	43	0	57	14.75	$< 10^{-3}$
Yaoundé	urban	3983	3791	0	7774	4.74	< 0.05
	peri-urban	402	1829	0	2231	912.74	$< 10^{-3}$
Douala	urban	541	37	0	578	439.47	$< 10^{-3}$
Maroua	urban	1022	0	0	1022	n/a	n/a
Manjo	Semi-urban	40	323	0	363	220.63	$< 10^{-3}$
Total		8337	8700	03	17 040	119.78	$< 10^{-3}$

Table 2: Numbers and proportions of *Aedes aegypti* and *Aedes albopictus* collected from different resting places during the dry season and rainy season in Cameroon. n, number of mosquitoes collected; %, percentage; χ^2 , Chi-square statistic; P, probability value; df, degree of freedom.

	Total <i>Ae aegypti</i>		Total <i>Ae albopictus</i>		Between species	<i>Ae aegypti</i> by season					<i>Ae albopictus</i> by season				
	n	%	n	%		P	Dry Season		Rainy season		P	Dry Season		Rainy season	
Inside houses	6	0.81	4	0.13	$\chi^2 = 686.15$	1	0.52	5	0.91	$\chi^2 = 36.37$	4	0.19	0	0	$\chi^2 = 117.77$
Outside															
- Banana palms	46	6.18	287	9.07	df = 6	17	8.85	29	5.25	df = 6	173	8.23	114	10.74	df = 6
- Cocoa trees	7	0.95	111	3.51	p < 0.0001	0	0	7	1.26	p < 0.0001	72	3.42	39	3.67	p < 0.0001
- Flower gardens	53	7.12	188	5.94		27	14.06	26	4.71		129	6.13	59	5.56	
- Natural vegetation	237	31.85	1243	39.27		42	21.88	195	35.33		932	44.32	311	29.28	
- Used tyres	349	46.91	308	9.73		99	51.56	250	45.29		230	10.94	78	7.34	
Mixed	46	6.18	1024	32.35		6	3.13	40	7.25		563	26.77	461	43.41	
Total	744	19.06	3165	80.96		192	25.81	552	74.19		2103	66.45	1062	33.55	

Table 3: Numbers and proportions of adult *Aedes aegypti* and *Aedes albopictus* collected in urban, peri-urban, and semi-urban/rural areas preferences of *Aedes aegypti* and *Aedes albopictus* in dry and rainy in Cameroon. n, number of mosquitoes collected; %, percentage; χ^2 , Chi-square statistic; P, probability value; df, degree of freedom; n/a, not applicable.

	Total <i>Ae aegypti</i>		Total <i>Ae albopictus</i>		Between species P	<i>Ae aegypti</i> by season					<i>Ae albopictus</i> by season					
						Dry Season		Rainy season		Between seasons	Dry Season		Rainy season		Between seasons	
	n	%	n	%	P	n	%	n	%	P	n	%	n	%	P	
URBAN environments (n=2768)																
Inside houses	2	0.36	2	0.1	$\chi^2 = 737.35$	1	0.75	1	0.24	$\chi^2 = 68.02$	2	0.14	0	0	$\chi^2 = 116.53$	
Outside																
- Banana palms	15	2.69	108	4.88	df = 5	0	0	15	3.54	df = 5	43	3.06	65	8.08	df = 5	
- Cocoa trees	-	-	-	-	p < 0.0001	-	-	-	-	p < 0.0001	-	-	-	-	p < 0.0001	
- Flower gardens	53	9.52	188	8.5		27	20.3	26	6.13		129	9.17	59	7.34		
- Natural vegetation	104	18.67	658	29.76		0		104	24.53		490	34.83	168	20.9		
- Used tyres	337	60.5	231	10.45		99	74.44	238	56.13		180	12.79	51	6.34		
- Mixed	46	8.26	1024	46.31		6	4.51	40	9.43		563	40.01	461	57.34		
Total urban	557	20.12	2211	79.88		133	23.88	424	76.12		1407	63.64	804	36.36		
PERI-URBAN environments (n=756)																
Inside houses	4	2.78	1	0.16	$\chi^2 = 16.49$	0	0	4	4.08	$\chi^2 = 15.17$	1	0.2	0	0	$\chi^2 = 18.34$	
Outside																
- Banana palms	31	21.53	138	22.55	df = 3	17	30.96	14	14.29	df = 3	108	21.86	30	25.42	df = 3	
- Cocoa trees	-	-	-	-	p = 0.0009	-	-	-	-	p = 0.0017	-	-	-	-	p = 0.00037	
- Flower gardens	-	-	-	-		-	-	-	-		-	-	-	-		-
- Natural vegetation	97	67.36	447	73.04		29	63.04	68	69.39		372	75.3	75	63.56		
- Used tyres	12	8.33	26	4.25		0		12	12.24		-	-	-	-		
- Mixed	-	-	-	-		-	-	-	-		13	2.63	13	11.02		
Total peri-urban	144	19.05	612	80.95		46	31.94	98	68.06		494	80.72	118	19.28		
SEMI-URBAN and RURAL environments (n=385)																
Inside houses	0	0	1	0.29	$\chi^2 = 13.01$	0	0	0	0	n/a	1	0.5	0	0	$\chi^2 = 11.94$	
Outside																
- Banana palms	0	0	41	11.99	df = 3	0	0	0	0		22	10.89	19	13.57	df = 4	
- Cocoa trees	7	16.28	111	32.46	p = 0.0046	0	0	7	23.33		72	35.64	39	27.86	p = 0.0178	
- Flower gardens	-	-	-	-		-	-	-	-	-	-	-	-	-		
- Natural vegetation	36	83.72	138	40.35		13	100	23	76.67	70	34.65	68	48.57			
- Used tyres	0	0	51	14.91		0	0	0		37	18.32	14	10			
- Mixed	-	-	-	-		-	-	-	-		-	-	-	-		
Total semi-urb/rural	43	12.01	342	87.99		13	30.23	30	69.77		202	59.06	140	40.94		

Table 4: Numbers and proportions of adult *Aedes aegypti* and *Aedes albopictus* collected from different ecological zones during the dry and rainy season in Cameroon. n, number of mosquitoes collected; %, percentage; χ^2 , Chi-square statistic; P, probability value; df, degree of freedom; n/a, not applicable.

	Total <i>Ae aegypti</i>		Total <i>Ae albopictus</i>		Between species P	<i>Ae aegypti</i> by season					<i>Ae albopictus</i> by season				
						Dry Season		Rainy season		Between seasons	Dry Season		Rainy season		Between seasons
	n	%	n	%	P	n	%	n	%	P	n	%	n	%	P
YAOUNDE: Capital city, Guinean equatorial zone (n=1290)															
Inside houses	0		2	0.17	$\chi^2 = 9.11$	0	0	0	0	$\chi^2 = 18.36$	2	0.22	0	0	$\chi^2 = 22.27$
Outside															
- Banana palms	6	6.25	200	16.75	df = 4	0	0	6	8.58	df = 3	130	14.18	70	25.27	df = 4
- Cocoa trees	-	-	-	-			-	-	-		-		-	-	
- Flower gardens	15	15.62	130	10.89	p = 0.0584	10	38.46	5	7.14	p = 0.00037	103	11.23	27	9.75	p = 0.00018
- Natural vegetation	64	66.67	761	63.74		16	61.54	48	68.57		609	66.41	152	54.87	
- Used tyres	11	11.46	101	8.45		0	0	11	14.71		73	7.96	28	10.11	
- Mixed	-	-	-	-							-	-	-	-	
Total	96	7.44	1194	92.56		26	27.08	70	72.92		917	76.8	277	23.2	
DOUALA: Port city, Cameroonian equatorial zone (n=2089)															
Inside houses	3	0.65	1	0.06	$\chi^2 = 850.91$	1	0.65	2	0.64	$\chi^2 = 12.45$	1	0.1	0	0	$\chi^2 = 69.09$
Outside															
- Banana palms	40	8.62	46	2.83	df = 5	17	11.11	23	7.4	df = 5	21	2.13	25	3.9	df = 5
- Cocoa trees	-	-	-	-			-	-	-		-		-	-	
- Flower gardens	38	8.19	58	3.57	p < 0.0001	17	11.11	21	6.75	p = 0.029	26	2.64	32	4.99	p < 0.0001
- Natural vegetation	82	17.67	340	20.92		13	8.5	23	7.4		253	25.71	87	13.57	
- Used tyres	301	64.87	156	9.6		99	64.71	202	64.95		120	12.2	36	5.62	
- Mixed	0	-	1024	63.02		6	3.92	40	12.86		563	57.22	461	71.92	
Total	464	22.21	1625	77.79		153	32.97	311	67.03		984	60.55	641	39.45	
GAROUA: Soudanian tropical zone (n=145) – rainy season only															
Inside houses	3	2.13	0	0	$\chi^2 = 1.57$	n/a	-				n/a	-			
Outside															
- Banana palms	-	-	-	-	df = 2	n/a	-				n/a	-			
- Cocoa trees	-	-	-	-			n/a	-				n/a	-		
- Flower gardens	-	-	-	-	p = 0.457	n/a	-				n/a	-			
- Natural vegetation	101	71.63	4	100		n/a	-				n/a	-			
- Used tyres	37	26.24	0	0		n/a	-				n/a	-			
- Mixed	-	-	-	-		n/a	-				n/a	-			
Total	141	97.24	4	2.76		n/a	-				n/a	-			

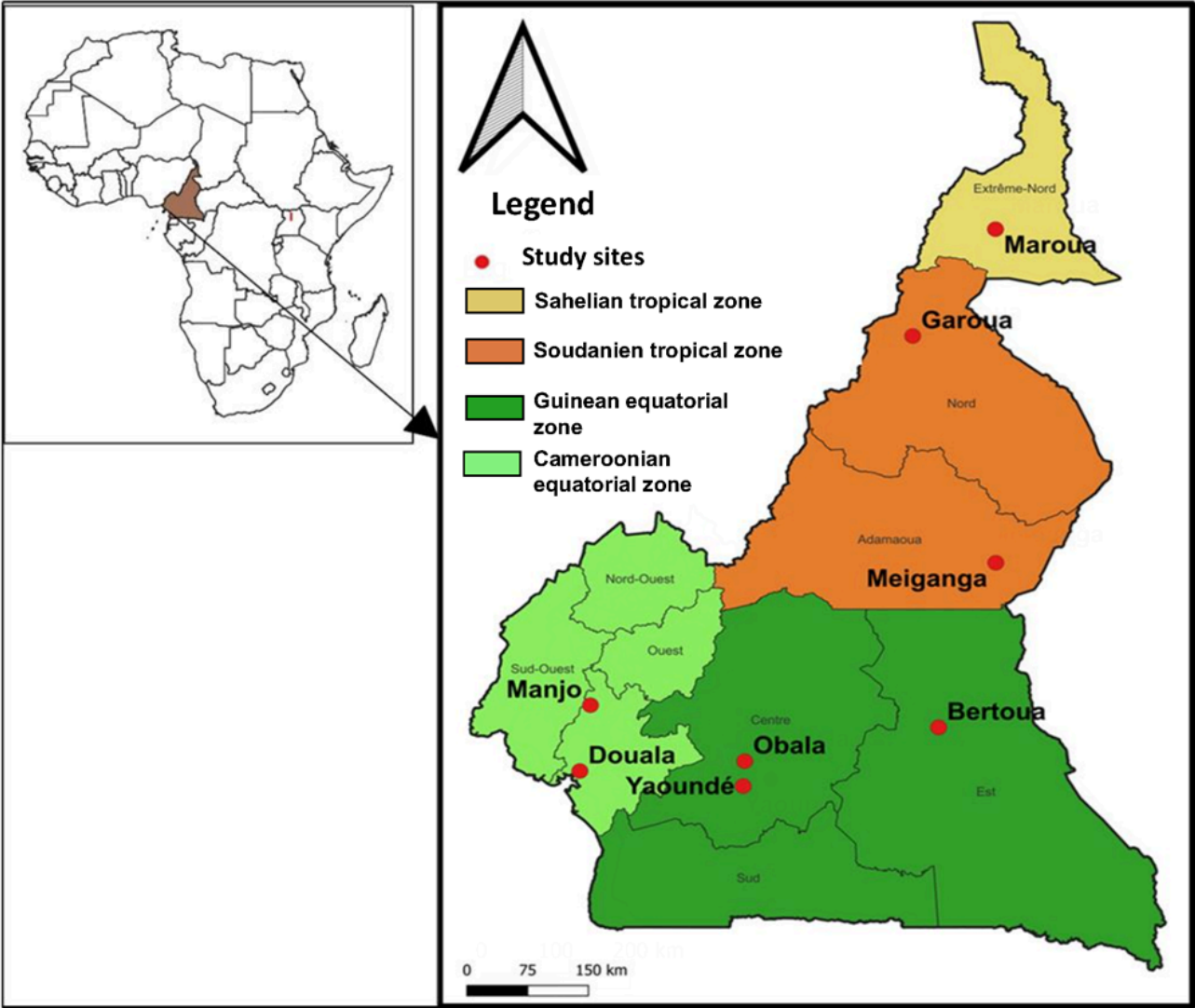


Figure 1

Map of Cameroon showing different collections sites. Red dots indicate study sites

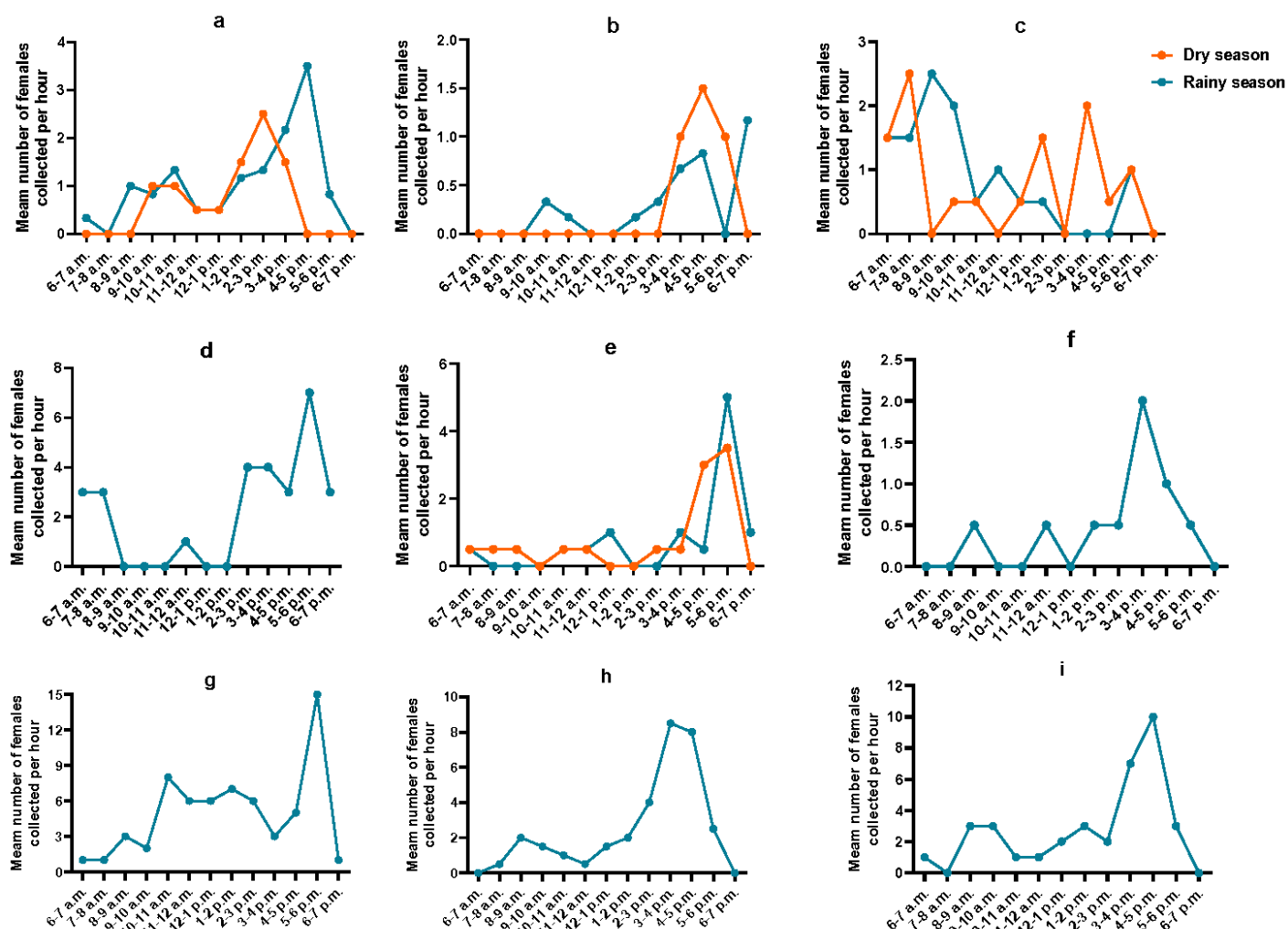


Figure 2

Diurnal host-seeking activity profiles of adult females *Aedes aegypti* in different ecological environment in Cameroon, by season. Hourly host-seeking activity of *Aedes aegypti* recorded in nine sites in Cameroon; a and b Fouda (urban and peri-urban area Yaoundé respectively); c and d, Bisseké and New-Bell (urban areas Douala respectively); e, Ngwele (peri-urban area Douala); f, Nkolowondo (rural area Obala); g and h, Camp-Chinois and Laindé (urban areas Garoua respectively) and i, Bocklé (peri-urban area Garoua). The orange and green curves represent mosquito abundances recorded during the dry season and rainy season, respectively. Values correspond to the mean number of female mosquitoes collected per hour.

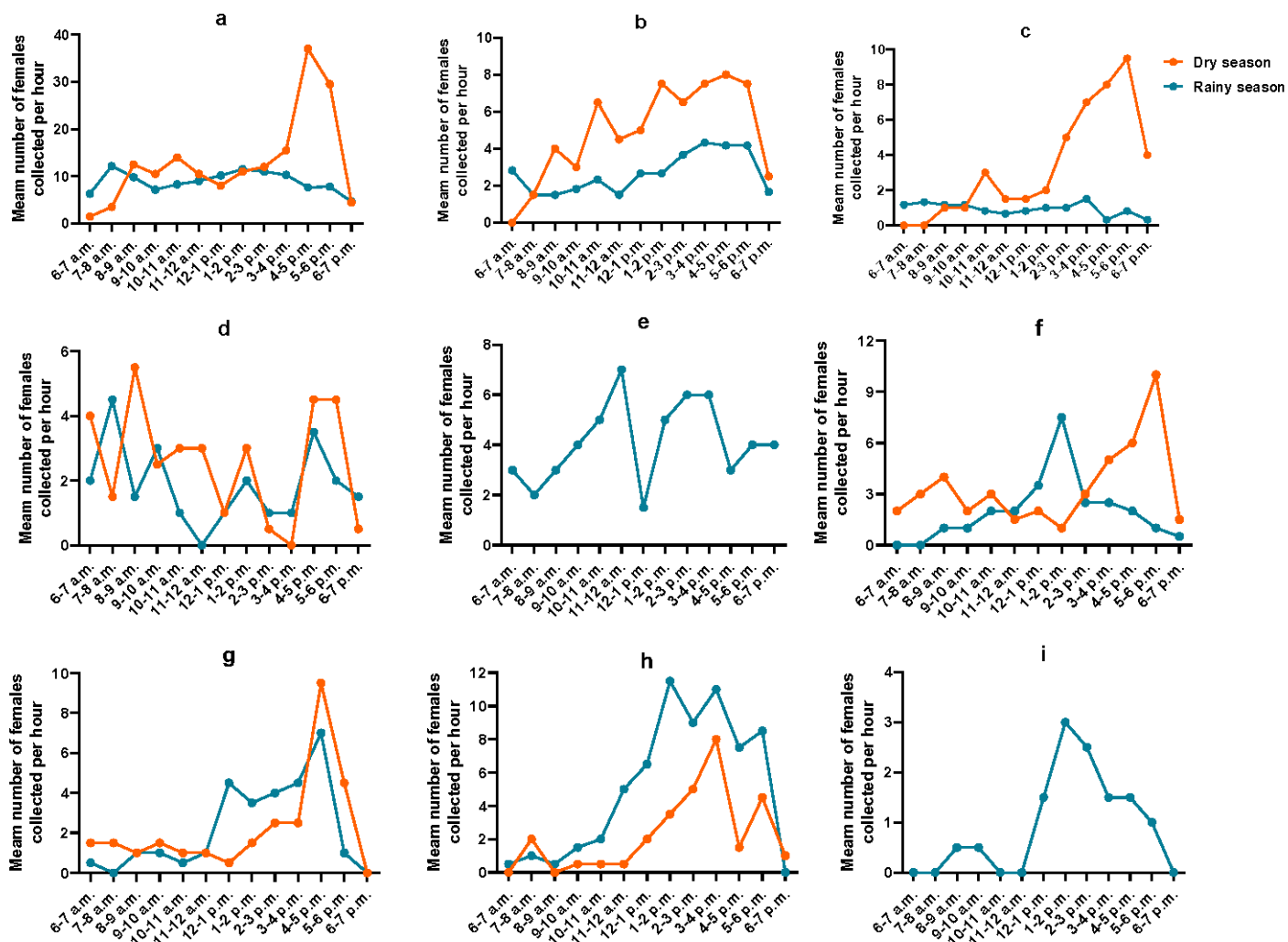


Figure 3

Diurnal host-seeking activity profiles of adult females *Aedes albopictus* in different ecological environment in Cameroon, by season. Hourly host-seeking activity of *Aedes albopictus* recorded in nine sites in Cameroon; a and b, Fouda and Ekoundoum (urban area Yaoundé respectively); c, Afanoyoa (peri-urban area Yaoundé); c and d, Bisseké and New-Bell (urban areas Douala respectively); e, Bonamoutongo (peri-urban area Douala); f and g, Elig-Bessala and Ekok-Assi (semi-urban areas Obala respectively) and i, Nkolowondo (rural area Obala). The orange and green curves represent mosquito abundances recorded during the dry season and rainy season, respectively. Values correspond to the mean number of female mosquitoes collected per hour.

Supplementary Files

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