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# Water sources selected for immature development of some African rainforest dwelling mosquitoes under different landscapes in Cameroon

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Little is known about the behaviors of African equatorial rain forest mosquito species and their potential role as sylvatic and bridge-vectors of various pathogens of animal and public health. In 2016 and 2017, the diversity and sources of water supporting immature development of mosquitoes in Talangaye Rainforest (South West Cameroon) before, during and after deforestation were investigated. Mosquito eggs, larvae and pupae were collected from 12 natural, seminatural, and artificial water sources and reared to adults. A total of 595 adult mosquitoes belonging to seven genera and at least 43 species were identified. *Culex* was the most abundant (56.3%) and was encountered in the majority in bamboo pots. *Aedes* and *Uranotaenia* species were mostly found in rock pools, while *Anopheles* and *Hodgesia* species solely prefer stream pools. In terms of mosquito abundance, rock pools were the most productive (29.91%) followed by bamboo pots (24.7%). Natural sites such as rock pools, tree holes, and stream pools recorded a greater number of species ($S = 21$, 14 and 12 respectively). During the rainy season, rock pools (46.23%) and bamboo pots (18.7%) were the most productive water bodies, while in the dry season, bamboo pots (35.71%) and stream pools (35.71%) harbored the most mosquitoes. The disturbed and pristine-like habitats had the greatest number of mosquitoes and breeding sites compared to palm plantation. This study provides some useful data on water sources used for immature development of forest mosquito species in Southwest Cameroon and how some species might adapt to changing landscapes, especially due to deforestation.

*Key words:* breeding site, forest mosquitoes, season, deforestation, Cameroon

## Introduction

Data on Afrotropical Forest mosquito species diurnal behavior patterns, choices of resting site microhabitat, oviposition, and immature development sites, and blood feeding propensities is substantially lacking. Additionally, relatively little is known about their role as animal and human disease vectors in pristine and disturbed forest environments. According to a World Health Organization (WHO) fact sheet in March 2020 (https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases) (WHO. 2020), vector-borne diseases account for more than 17% of all infectious diseases, causing more than 700,000 deaths annually. Most of these deaths are caused by dengue and malaria, both vectored by African mosquito species (*Aedes aegypti* and *Anopheles gambiae* s.l. among others) that were originally likely forest dwelling or derived from forest dwelling ancestors and gradually domesticated to exist in closer associations with humans (Coluzzi et al. 1979, 1985, Brown et al. 2022). Furthermore, previously benign viruses lurking for millennia in African forests that have in recent times become more dramatic globally because of host and vector switching due to minor viral genome mutations include Zika and Chikungunya viruses (Caglioti et al. 2013, Vest 2016). When human encroachment disrupts the natural habitat and especially forests, it affects the classical interactions existing between vectors, hosts, and parasites bringing human and other animal populations closer to novel sources of pathogens, which might lead to the emergence and spread of new diseases (Lafferty 2009, Lee et al. 2011). Multiple examples exist that implicate the direct linkage between deforestation and the emergence of certain human infectious diseases (Epstein et al. 2006, Jones et al. 2008, Plowright et al. 2011, Berazneva and Byker 2017, Tollefson 2020). In the south-eastern part of Côte d'Ivoire, where large parts of rainforest have been converted into oil palm plantations, several outbreaks of yellow fever and dengue have been documented (Komono 2012) and around 60 examples of linkage between deforestation and land-use changes and increase in mosquito populations and malaria risk were noted by Yasuoka and Levins (2007). Further outbreaks and emergence of diseases beyond their original geographic locations are facilitated by combinations of global trade, climate change, human mobility, and human-induced modifications of terrestrial ecosystems (Gould 2009, Leisnham et al. 2012, Kweka et al. 2016).

The little that is known about African forest species bionomics and phenology comes from observations and collections performed for systematics mostly done in the first 60 years of the 20th century in mainland Africa equatorial forests in parts of Cameroon, Uganda, Nigeria, Côte d'Ivoire, Kenya, Democratic Republic of the Congo, Gabon Central, African Republic and Northern Angola. Most noteworthy sources of these bionomic information can be found in systematic reviews and accompanying biological notes for Anophelinae by Gillies and De Meillon (1968), Gillies and Coetzee (1987), Toxorhynchitinae and Culicinae in Edwards (1941), Hopkins (1952) and Service (1990). Additionally, many publications focusing on arboviral surveys in African forests (cited in review by Braack et al. 2018, Bamou et al. 2021) report snippets of biological observations on some species. Forest focused longitudinal mosquito bionomic studies before logging were done by Doucet (1962) in Banco Forest (Côte d'Ivoire), by Mattingly (1949) in Ogun River Forest (Old Oyo Forest) (Nigeria), by Haddow et al. (1947) and Smithburn et al. (1946) in Bwamba County, such as Semliki Forest (Uganda), and in Zika Forest (Uganda) by Goma (1965). Such studies on Neotropical Forest mosquitoes have increased in popularity (Mayi et al. 2019, Barrientos-Roldán et al. 2022, Rakotonirina et al. 2023), and are probably driven by the desire to capture mosquito diversity and bionomics and to examine and predict implications on diseases before these forests disappear due to logging and other anthropogenic activities. In fact, the global rate of tropical deforestation is considerable and Africa had the largest annual rate of net forest loss in 2010–2020 at 3.9 million ha. During 2021, the Congo Basin area experienced increasing deforestation, growing by almost 5% in a 12-month period (Cavallito 2022). The second highest rate of deforestation among the Congo Basin Countries occurs in Cameroon, with an equivalent of about 1.0% of annual forest cover loss (FAO 2015). Knowledge of the behaviors of mosquitoes in forests before, during and after deforestation may give insights into which species may adapt to new human landscape modifications (agricultural and urban settings) and facilitate disease transmission. An understanding of all these behaviors, especially environments used for immature development, will assist in implementing successful vector control measures (Ijumba and Lindsay 2001, Killeen et al. 2002, Olayemi et al. 2010, Tadesse et al. 2011, Wilke et al. 2019, Ntoumba et al. 2020, Müler et al. 2022).

All mosquitoes oviposit and complete larval and pupal development in aquatic habitats (Afolabi et al. 2019). Consequently, access to appropriate aquatic habitats plays a crucial role in the maintenance of mosquito-borne diseases (Correia et al. 2015). Mosquitoes exploit almost all types of aquatic habitats, both natural and artificial, for immature development. Yet, most mosquito species specialize on particular types of larval microhabitats such as stagnant pools, abandoned tires, fresh or saltwater marshes, the edges of streams and rivers (Afolabi et al. 2013), tree holes, and plant leaf axils (Kitching 2001). Traditionally, studies refer to mosquitoes breeding in water. However, a large segment of their natural cycle occurs in nonaquatic environments, notably mating, and so we prefer not to refer to water bodies as breeding sites but rather places of immature sites and development. Bates (1949) classified forest mosquito immature sites into four major types; permanent or semi-permanent standing water, running water, transient ground pool, and container habitats. The former three types tend to be less frequently encountered in forests, but the fourth type includes prolific opportunities or sources in the form of tree-rot-holes, leaf axils and bases, flowers, fallen leaves, and fruits and empty snail shells. Following the Aedini phylogenetic classification nomenclature (Reinert et al. 2009), the subgenera *Catageiomyia, Diceromyia*, and *Elpeytonius* comprise all species that are ostensibly found in equatorial rain forests and in enclaves of moist montane and river bank forests in savannah regions (Edwards 1941, Hopkins 1952, Service 1990). Most species within *Toxorhynchites, Malaya, Hodgesia, Eretmapodites,* and *Uranotaenia* are restricted to equatorial rain forests. *Eretmapodites, Mimomyia*, and *Malaya* forest species tend to select container habitats such as epiphyte plant leaf axils, fallen fruit husks, and empty snail shells. *Catageiomyia, Diceromyia*, and *Elpeytonius* are more restricted to tree or rot holes. *Uranotaenia* prefer vegetated edges of slow running streams, vernal pools, and crab holes. *Culiciomyia* are found in more diverse sites such as vernal pools, crab holes, tree-holes and plant leaf axils. *Hodgesia* in more "marshy" areas along rivers and vernal pools (Edwards 1941, Hopkins 1952, Service 1990).

Knowledge on mosquito community interactions in forest larval sources in African forests is lacking despite their ecological importance as pollinators and important food sources for many organisms and their potential vector role in the transmission and maintenance of several viruses and parasites that lie in the forest (Braack et al. 2018, Bamou et al. 2021). Most studies investigating the physical and biological properties and species-specific use of larval habitats of mosquitoes have been done in agricultural, urban, and semi-African settings (Muturi et al. 2007, Tadesse et al. 2011, Suganthi et al. 2014, Ntoumba et al. 2020, Djiappi-Tchamen et al. 2021, Djoufouna et al. 2022) on the major anthropophilic vectors *Anopheles sp.*, *Aedes aegypti*, *Aedes albopictus,* and *Culex quinquefasciatus*.

Permission was granted to examine the mosquito diversity before, during and after deforestation of a tract of land in the South West Region of Cameroon. This provided an opportunity to identify the types of aquatic habitats selected by African forest-dwelling mosquitoes and to assess the contribution of each habitat type to mosquito productivity and diversity. First investigations on the same location identified immature development habitats of *Eretmapodites* mosquitoes (Forfuet et al. 2022). Here we provide data on spatiotemporal variations of water sources used by species within seven other genera (*Culex, Aedes, Hodgesia, Anopheles, Uranotaenia, Lutzia,* and *Toxorhynchites*). Examination of larval habitats selected, both natural and artificial, also provides insights into which forest species may successfully adapt and survive deforestation and transition into palm oil plantations and increased anthropophilic environs.

## Materials and Methods

### Study Area

This study was carried out in the Talangaye Rainforest of Nguti Sub-Division, of the South West Region of Cameroon (Fig. 1).

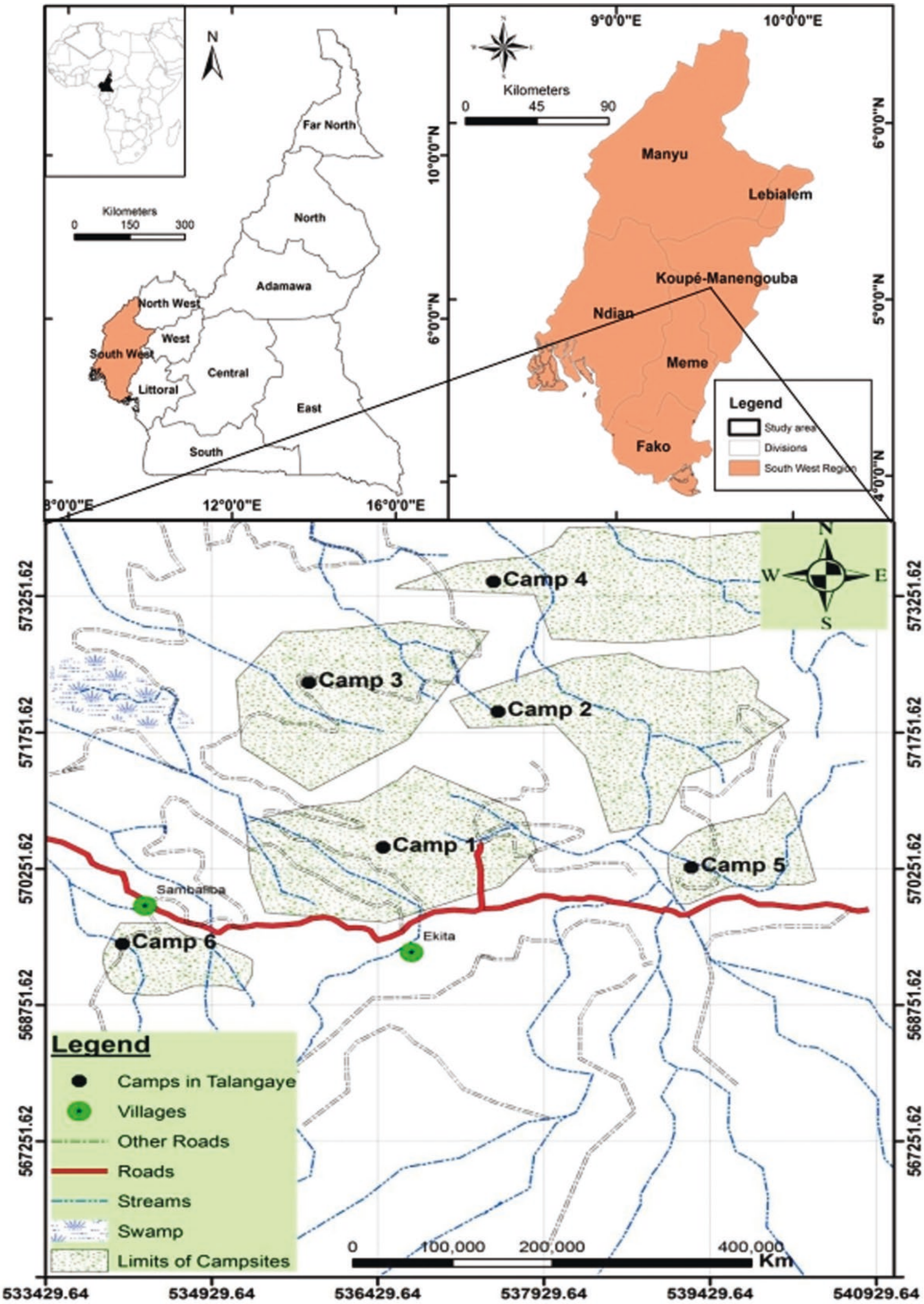

**Fig. 1.** Map showing the study area and sampling sites (camps).

It is a rainforest corridor located between four protected areas in Cameroon (Korup National Park, Bayang Mbo Wildlife Sanctuary, Rumpi Hills Forest Reserve, and the Bakossi Mountains Reserve). This area is located within the equatorial climatic zone with an average annual rainfall of 3,000 mm and experiences two main seasons: a dry season (mid-October to mid-March) and a rainy season (mid-March to mid-October). Temperatures range from 25 °C to 35 °C for most of the year and the relative humidity is usually above 75% (CVUC-UCCC, 2017). Talangaye Rainforest is a hilly area, covered with lowland broad leaf forest trees and bushes; most of the forest canopy is created by multiple high valued Mahogany and Sapele hard wood timber trees, and a variety of broad leaf plants predominated below the canopy. Some parts of the Talangaye Rainforest have undergone modifications as a result of human activities and have become compromised. Four types of landscapes were then defined: (i) unlogged forest: pristine forest showing no evidence of logging with mature and tall trees of about seven to ten meters in height forming a continuous canopy of about 70%; (ii) pristine-like forest: unlogged areas surrounded by small cocoa and banana plantation; (iii) disturbed forest: forest slightly fragmented due to selective logging of especially hard wood trees; the canopy was slightly fragmented and allowed sunlight penetration; (iv) Palm plantation: young palm trees (less than a year old) with windrows of deadwood and grass bordered by forest patches as buffers along rivers. There were forest patches at a close distance (≤150 m) from the plantation.

### Classification and Description of Immature Development Habitats

A total of twelve different water bodies were surveyed in pristine, disturbed, and palm plantation habitats inside the Talangaye Rainforest. They were classified in three major categories; "natural," "seminatural," and "artificial" and described as follows:

Natural water bodies included: (i) large curled up dead leaves on the ground (fallen large dry leaves holding a small amount of water); (ii) rock pools with large quantity of slimy/clean water with dead leaf debris; (iii) stream pools along edges of small river inlets with clean water; (iv) tree holes from different tree species holding a small amount of water.

Seminatural water bodies included: (i) fallen cocoa pods containing a small amount of water and (ii) banana leaves (axils fallen leaves). Bamboo pots hung in trees or placed on the forest floor were also provided as "seminatural" water sources to mimic water logged tree holes that could be accessed a few feet above ground. Bamboo pots consisted of bamboo stems that were cut into one-foot sections, the bases of which were cut just below the nodes so it could hold water. Each bamboo pot was filled with rainwater almost to the top.

Artificial water bodies included: (i) discarded cooking pots filled with rainwater and (ii) ground pools of clean water created by bulldozer tracks (tyre tracks) directly exposed to sunlight in the palm plantation. Water bodies that we placed on the forest floor as supplement for mosquitoes to lay eggs in were also classified as "artificial." These included: (i) coconut pods; (ii) blue tarps; (iii) plastic cups and were filled with rainwater and other surrounding water sources and placed in different parts of the forest.

### Mosquito Sampling and Identification

Collections of mosquitoes from 12 natural, seminatural, and artificial water sources were done in the forest for two to three weeks each, during the months of October and January (dry season), and April and July (rainy season) in 2016 and 2017. In each sampling month, different numbers and types of water bodies were recorded from different forest landscapes. In October, investigations were done in pristine unlogged areas (Camp 5) and in a small palm plantation (Camp 6) inside a remnant forest from four sources (bamboo pots, plastic cups, tree holes, and artificial pools from bulldozer tracks). In January (Camp 2), investigations were done in unlogged and fragmented areas from five sources (bamboo pots, tarps, leaves on the ground, stream pools, and coconut pods). In April (Camp 1), investigations were done in partially logged or fragmented areas from five sources (bamboo pots, tarps, plastic cups, rock pools, and tree holes). In July (Camp 4), investigations were done in pristine unlogged areas surrounded by small cocoa and banana plantations from seven sources (bamboo pots, cooking pots, plastic cups, tree holes, banana leaf axils, cocoa pods, and stream pools).

Larvae, pupae, and mosquito eggs were collected using dipping and suction with pipettes and plastic scoops and were then held for further development in plastic cups filled with water from the source and labeled according to water source type and collection date. The pupae were removed and placed in individual tubes until adult emergence. Larvae were given a supply of food consisting of finely ground TetraMin fish food (Tetra Holding (USA Inc. Blacksburg VA)) for their development. After emergence, mosquito adults were immobilized with triethylamine and identified to genera and to species using morphological keys (Edwards 1941, Service 1990). It should be mentioned that we did not have very good success rearing larvae and pupae because of lack of providing appropriate nutrient and micro-organism requirements for these mosquitoes.

### Data Analysis

Statistical analyses were performed using the software R version 4.2.2 ((2022-10-31 ucrt) R Foundation for Statistical Computing Platform 2022). Mosquito abundance or the number of individuals of each species captured was determined per immature development habitat type, habitat type, and sampling month. Differences in mosquito abundance across water sources, landscapes, and months were analyzed using a Kruskal Wallis test. Shannon-Wiener (H) and Simpson's (DS) indices were used to determine the diversity of mosquitoes per immature development habitat type.

## Results

### Mosquito Abundance Per Genus

From the immatures collected in a total of 12 natural, seminatural, and artificial water bodies, 595 adult mosquitoes belonging to seven genera and at least 43 species were morphologically identified. A total of 15 individuals were not identified because of partial emergence. It should be mentioned that 184 mosquitoes (52 *Aedes*, 125 *Culex*, and seven *Uranotaenia*) could only be identified to genera and groups because only females were successfully reared that are member species of morphologically identical and similar subgenera and groups. Of the different genera, *Culex* was the most abundant (56.3%), followed by *Aedes* (24.37%) (Fig. 2 and Table 1).

Of the species that emerged, *Cx. (Culiciomyia) nebulosus* (15.63%) and *Cx. (Culiciomyia)* species (13.95%) were by far the most abundant. The remaining other species were collected in relatively low abundances, varying from 0.17% to 8.23%. Important vector species of arboviral diseases such as *Ae.* (*Stegomyia*) *aegypti* (L.) (2.52%) and *Ae. africanus* (1.01%) were infrequently encountered (Table 1).

### Water Body Preferences of Forest Mosquito Species

Overall, rock pools were the most productive water bodies (29.91%), followed by bamboo pots (24.7%), while the least productive were dead leaves (0.17%) and coconut pods (0.33%) (Table 1).

The majority of *Aedes* species were found in rock pools (e.g., *Ae.* (*Aedimorphus*) *tarsalis*, *Ae.* (*Stegomyia*) *aegypti* (L.)), tree holes (e.g., *Ae. (Finlaya) longipalpis*, *Ae. (Aedimorphus) simulans*) and bamboo pots (e.g., *Ae.* (*Stegomyia*) *corneti*, *Ae.* (*Stegomyia*) *africanus*). *Anopheles* (e.g., *An. (Anopheles) obscurus*) and *Hodgesia*

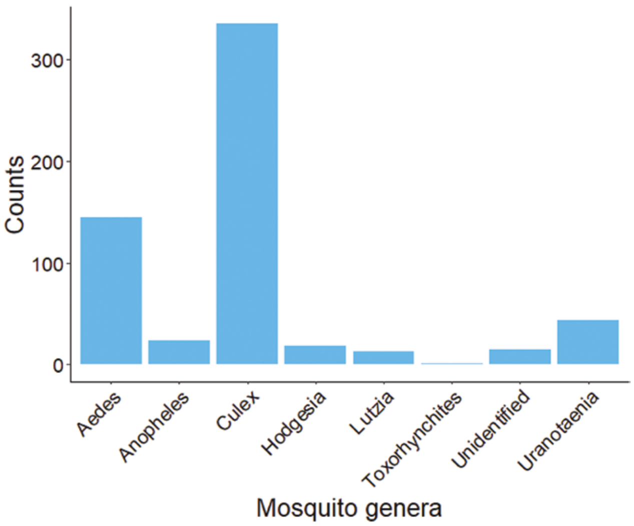

**Fig. 2.** Number of mosquitoes (counts) recorded per mosquito genus in Talangaye Rainforest during the study period.

(*Ho. cuptopous*) preferred vegetated stream pools along river edges. Most species of *Culex* were found in bamboo pots (e.g., *Cx. (Culiciomyia) nebulosus*) and stream pools along river edges (e.g., *Cx. (Eumelanomyia) wigglesworthi*). *Lutzia* (*Lt. tigripes*) and *Uranotaenia* (e.g., *Ur. (Pseudoficalbia) fusca*) were mostly found in rock pools (Table 1 and Fig. 3). Importantly, only *Cx. (Culex) argenteopunctatus* were found in artificial pools of water created by bulldozer tracks (tyre tracks) in the palm plantation (Table 1).

### Productivity of Natural vs Artificial Water Bodies

In total, 12 types of immature microhabitats were surveyed. Natural water bodies such as rock pools, tree holes, and stream pools recorded a greater number of species ($S = 21$, 14 and 12 respectively) and similar higher diversities ($H' = 2.7$, 2.27, and 2.01 respectively) and evenness (**DS** = 0.92 and 0.86 respectively). The least diverse water source types included dead leaves and tyre tracks, from which only one species was sampled (Table 2).

### Seasonal Distribution of Mosquitoes and Immature Development Habitats

The highest number of mosquito individuals ($n = 385$, 64.71%) and mosquito species ($S = 36$) was recorded in the rainy season compared to the dry season ($n = 210$, 35.29%; $S = 21$) (Table 3). Likewise, a high variety of water bodies were encountered during the same season (Table 3 and Fig. 4).

During the rainy season, rock pools (46.23%) and bamboo pots (18.7%) were the most productive water bodies, while during the dry season, bamboo pots (35.71%), stream pools in forest (35.71%) and tyre tracks in palm plantations (21.43%) harbored the most mosquitoes (Fig. 4). The differences in mosquito abundance across months (Kruskal-Wallis chi-squared = 2.6954, $df = 3$, $P$-value = 0.441) and water bodies (Kruskal-Wallis chi-squared = 15.224, $df = 11$, $P$-value = 0.1725) were not significant.

### Spatial Distribution of Mosquitoes and Immature Development Habitats

The disturbed habitat (348 mosquitoes belonging to 34 species from seven water sources) and pristine-like forest (97 mosquitoes of 37 species from seven water sources) had the greatest number of mosquitoes and water sources compared to the pristine forest (91 mosquitoes of 21 species from three water sources) and palm plantation (59 mosquitoes of 11 species from four water sources) (Fig. 5). No significant difference in mosquito abundance across landscapes was obtained (Kruskal-Wallis chi-squared = 0.85257, $df = 3$, $P$-value = 0.8369).

## Discussion

### Mosquito Abundance and Productivity of Immature Development Habitats

Afrotropical forests provide a wide range of larval habitats, resting and mating places, and the availability of nectar and blood-food sources for mosquitoes (Diallo et al. 2012a, 2012b). From an ecological perspective, mosquito diversity hotspots occur in tropical forests (Foley et al. 2007) and longitudinal studies in forests would be useful to examine influences of climatic changes such as across dry and wet seasons and average temperature variations. Over a two-year period in 2016 and 2017, a high diversity of species was collected in the Talangaye Rainforest, including approximately 38 *Culex* species collected as adults (Mayi et al. 2019), 32 *Eretmapodites* species collected as adults and immatures (Forfuet et al. 2022) and over 120 species of other genera (not yet published). In this study, 43 species were collected as immatures.

Species from the genera *Culex* and *Aedes* were the most abundant in the study area. These mosquitoes are cosmopolitan, and many seem to be less discriminate in selection of water bodies to lay eggs and complete immature development in, as they were encountered in the majority of the water sources sampled. Many authors have suggested that they are versatile nonspecific mosquitoes that adapt to a variety of immature habitats (Edwards 1941, Service 1990, Adebote et al. 2006, Afolabi et al. 2010, Ahmad et al. 2011, Mayi et al. 2019). This plasticity in water source selection habits confer a high potential degree of adaptability and potential to survive in different environments, including more open habitats. This is problematic as some species from these genera are well-known vectors of many pathogens including many viruses that likely originate from sylvatic (forest) environments (Brottes et al. 1966, Salaun et al. 1969, Braack et al. 2018, Bamou et al. 2021).

Access to appropriate larval habitat types plays important roles in determining mosquito abundance and distribution, which in turn affects disease incidence and overall vectorial capacity (McCrae 1984). Rock pools, tree holes, and stream pools were the most productive water sources and habitats of Talangaye Rainforest mosquito species. This can be explained by the fact that different mosquito species of different genera co-existed in those water bodies sites (Muturi et al. 2007, Djiappi-Tchamen et al. 2021). The coexistence of mosquitoes in these habitats is likely attributable to the availability of shade and sufficient organic material for larval feeding (Guagliardo et al. 2014). In 2011, Ahmad et al. discovered that the most common sites where mosquito larvae co-existed were in rock pools. Similar observations of rock pools producing high diversity of mosquito species were made by Dida et al. (2018), Obi et al. (2020) and Müler et al. (2022). Fallen leaves on the ground were among the least productive natural habitats, probably because they held too small a volume of water. Interestingly, despite their artificial nature, the introduced plastic cups and tarps were found to attract many forest species, especially those of the genus *Culex*.

### Water Body Preferences of Forest Mosquito Species

*Culex* species were collected in all recorded water sources in Talangaye Rainforest. *Cx.* (*Eumelanomyia*) *wigglesworthi* (found in the majority in stream pools in the Talangaye Rainforest) is a

**Table 1.** Distribution of mosquito species in different water sources in Talangaye Rainforest

| | BP | T | CP | DL | PC | RP | SP | TH | CnP | BL | TT | CkP | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Aedes*** **(*n* = 24.37%)** | | | | | | | | | | | | | |
| *Ae. (Aedimorphus) apicoannulatus* | 0 | 0 | 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 24 |
| *Ae. (Aedimorphus) tarsalis* | 0 | 0 | 0 | 0 | 0 | 14 | 1 | 1 | 0 | 0 | 0 | 0 | 16 |
| *Ae. (Neomelaniconion) taeniasrostris* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *Ae. (Stegomyia) corneti* | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 10 |
| *Ae. (Stegomyia) aegypti* (L.) | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 15 |
| *Ae. (Stegomyia) africanus* | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| *Ae. (Aedimorphus) simulans* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 4 |
| *Ae. (Finlaya) longipalpis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 7 |
| *Ae. (Aedimorphus) boneti* | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 7 |
| *Ae. (Aedimorphus)mutilus* | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Ae. (Aedimorphus) leptolabis* sp. *talangayensis* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Ae. (Aedimorphus) Tarsalis* gp. | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 16 |
| *Ae. (Stegomyia)* sp. | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 2 |
| *Ae.* sp. | 6 | 0 | 0 | 0 | 0 | 14 | 0 | 7 | 0 | 7 | 0 | 0 | 34 |
| **Total** | 22 | 0 | 0 | 0 | 0 | 93 | 1 | 22 | 0 | 7 | 0 | 0 | 145 |
| ***Anopheles*** **(*n* = 4.03%)** | | | | | | | | | | | | | |
| *An. (Anopheles) obscurus* | 0 | 0 | 1 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 23 |
| *An. (Cellia) cinctus* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **Total** | 1 | 0 | 1 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 24 |
| ***Culex*** **(*n* = 56.3%)** | | | | | | | | | | | | | |
| *Cx.(Culex) argenteopunctatus* | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 45 | 0 | 49 |
| *Cx. (Eumelanomyia) albiventris* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *Cx. (Eumelanomyia) wigglesworthi* | 0 | 0 | 0 | 0 | 0 | 1 | 23 | 1 | 0 | 0 | 0 | 0 | 25 |
| *Cx. (Culex) ornatothoracis* | 0 | 1 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 1 | 0 | 0 | 10 |
| *Cx. (Eumelanomyia) simpliciforceps* | 0 | 0 | 0 | 0 | 0 | 7 | 16 | 0 | 0 | 0 | 0 | 0 | 23 |
| *Cx. (Culiciomyia) nebulosus* | 47 | 3 | 1 | 1 | 33 | 2 | 0 | 4 | 0 | 0 | 0 | 2 | 93 |
| *Cx. (Culiciomyia) muspratti* | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Cx. (Eumelanomyia)horridus* | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 1 | 0 | 0 | 0 | 1 | 5 |
| *Cx. (Culiciomyia)* sp. | 63 | 8 | 3 | 0 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 5 | 83 |
| *Cx. (Culex) guiarti* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| *Cx. (Culiciomyia) subaequalis* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Cx.* sp. | 12 | 0 | 1 | 0 | 1 | 22 | 5 | 1 | 0 | 0 | 0 | 0 | 42 |
| **Total** | 124 | 13 | 5 | 1 | 40 | 36 | 52 | 9 | 1 | 1 | 45 | 8 | 335 |
| ***Hodgesia*** **(*n* = 3.02%)** | | | | | | | | | | | | | |
| *Ho. cuptopous* | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 18 |
| **Total** | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 18 |
| **Lutzia (*n* = 2.18%)** | | | | | | | | | | | | | |
| *Lt. tigripes* | 0 | 3 | 0 | 0 | 0 | 9 | 0 | 0 | 1 | 0 | 0 | 0 | 13 |
| **Total** | 0 | 3 | 0 | 0 | 0 | 9 | 0 | 0 | 1 | 0 | 0 | 0 | 13 |
| ***Toxorynchites*** **(*n* = 0.17%)** | | | | | | | | | | | | | |
| *Tx. brevipalpis brevipalpis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| **Total** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |

**Table 1.** Continued

| | BP | T | CP | DL | PC | RP | SP | TH | CnP | BL | TT | CkP | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Uranotaenia* (*n* = 7.39%)** | | | | | | | | | | | | | |
| *Ur.* (*Uranotaenia*) *balfouri* | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Ur.* (*Pseudoficalbia*) *mashonaensis* | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 |
| *Ur.* (*Pseudoficalbia*) *fusca* | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 12 |
| *Ur.* (*Pseudoficalbia*) *montana* | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Ur.* (*Uranotaenia*) *caerulocephala* | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Ur.* (*Pseudoficalbia*) *pseudohenrardi* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Ur.* (*Pseudoficalbia*) *ornata* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 4 |
| *Ur.* (*Pseudoficalbia*) sp. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 |
| *Ur.* (*Pseudoficalbia*) *yovani* | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| *Ur.* (*Uranotaenia*) *chorleyi* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Ur.* sp. | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 2 | 0 | 0 | 7 |
| **Total** | 0 | 0 | 0 | 0 | 0 | 26 | 9 | 0 | 0 | 9 | 0 | 0 | 44 |
| **Unidentified (*n* = 2.52%)** | 0 | 0 | 0 | 0 | 0 | 14 | 1 | 0 | 0 | 0 | 0 | 0 | 15 |
| **Grand Total** | 147 | 16 | 6 | 1 | 40 | 178 | 103 | 32 | 2 | 17 | 45 | 8 | 595 |

Abbreviations: BP, bamboo pots; T, tarps on the ground; CP, cocoa pods; DL, large curled up dead leaves on the ground; PC, plastic cups; RP, rock pools; SP, stream pools (along edges of small river lets); BL, banana leaf axils; TH, tree holes; CnP, coconut pods; TT, Tyre tracks; CkP, discarded cooking pots; n, total number of adult mosquitoes recorded expressed in percentage; sp., species; gp., group.

widely distributed species usually found in more wooded areas in forests and wooded moist enclaves in savannah regions, and whose larvae are common in shallow pools along rivers and in rock pools (Hopkins 1952). Nothing is known about the biology and disease transmission of this species. *Cx.* (*Eumelanomyia*) *albiventris* immatures (found in a tree hole in the Talangaye Rainforest) have been reported elsewhere in water filled rot holes in tree branches and trunks (Hopkins 1952). Ntaya virus (NTAV) and Arumowot virus (AMTV) have been isolated from this species (Brottes et al. 1966, Braack et al. 2018, Bamou et al. 2021). *Cx.* (*Culex*) *ornatothoracis* larvae and pupae in this study were mainly found in stream pools but also occasionally in banana leaf axils and a tarp on the ground, suggesting this species selects sites quite opportunistically. *Cx.* (*Eumelanomyia*) *simpliciforceps* were found in Talangaye Rainforest in stream pools and rock pools. Hopkins (1952), found the larvae on the edges of swamps on the White Nile in Sudan. It has a wide distribution, extending from forests into drier savannah areas in South Sudan, Kenya, Tanzania and South Africa (Edwards 1941, Jupp 1996). This suggests it is not strictly a forest dwelling species. Nothing is known about its behavior and disease transmission and has never been reported to be a biting nuisance to humans. Likewise, *Cx. (Eumelanomyia) horridus* (collected in rock pools and tree holes in the Talangaye Rainforest) is not a strictly forest-dwelling species and is widely distributed in Africa south of the Sahara Desert. They do not feed on humans and don't have any public health importance. Egg rafts of *Culiciomyia* were frequently laid in bamboo pots (63 + 47 + 2 + 1 emerged as adults). On 47 occasions, at least one male successfully emerged and could be identified as *Cx. (Culiciomyia) nebulosus*. The other 63 occasions were recorded as *Culiciomyia* sp (Table 1). *Cx. (Culiciomyia) nebulosus* is the most common of all *Culiciomyia* in most parts of rural sub Saharan Africa but will occasionally be found in large garden trees in urban areas (AJC personal observations). No species of *Culiciomyia* have been implicated as a vector of human pathogens. However, NTAV and Yaounde virus (YAOV) have been isolated from these mosquitoes in Cameroon (Brottes et al. 1966). A single adult *Cx.*(*Culex*) *guiarti* successfully emerged from a fallen cocoa pod, which is an atypical microhabitat for this species as it is usually found in vegetated clean water borrow-pits, water holes and swamp edges according to Hopkins (1952). It is worth noting that our identifications of adult mosquitoes were solely based on morphology using the keys of Edwards (1941) and Service (1990), which to date are the only sources of identification of Afrotropical mosquitoes. Therefore, in the absence of updated versions of the identification keys and the ability to confirm species identification using molecular techniques, misidentification could have occurred and led to such an unusual scenario. *Cx. (Culex) guiarti* has a wide distribution but is confined to moist equatorial forests and riverine forests in savannah regions. It is not known to feed on humans and thus far never been implicated in disease transmission. *Cx.* (*Culex*) *argenteopunctatus* immatures (solely encountered in artificial pools in Talangaye Rainforest) likely occur in ground pools (Hopkins 1952). This species has no role in human disease transmission. The selection of water body preference by *Cx. argenteopunctatus* is interesting as they were solely found in water sources in degraded and open areas (plantations).

*Aedes* species were collected in rock pools, tree holes, bamboo pots, stream pools, and banana leaves in the Talangaye Rainforest. Surprisingly, *Ae.* (*Aedimorphus*) *apicoannulatus* was found only in rock pools, while it is usually found in tree-holes (Hopkins 1952). Again, this unusual situation may be the result of misidentification (due to the limited resources available for mosquito identification in many parts of Africa) or genetic evolution/environmental adaptation

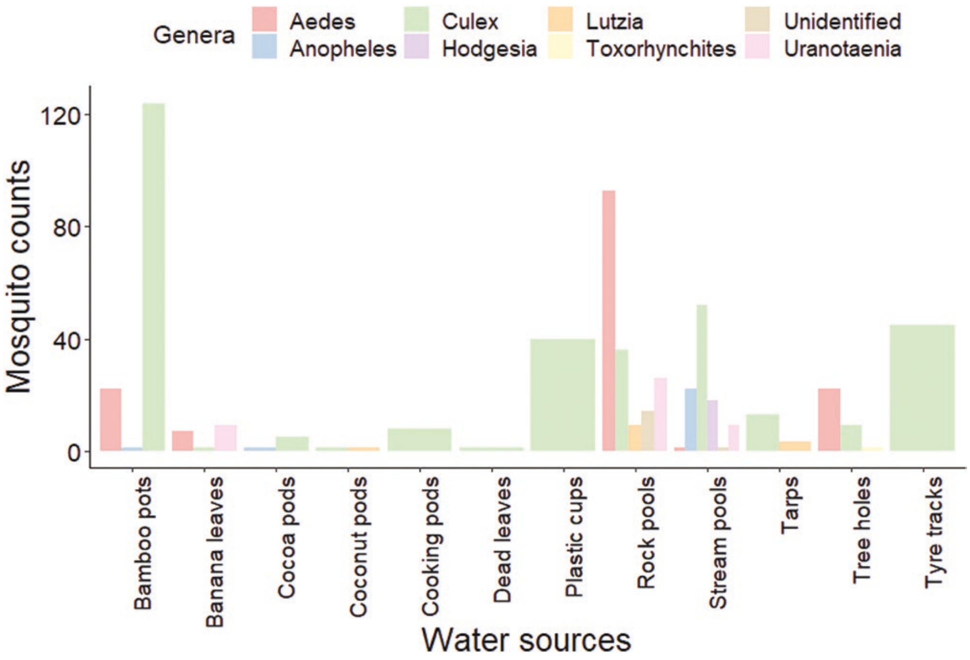

**Fig. 3.** Distribution of mosquito genera and abundances across different water sources in Talangaye Rainforest.

of the mosquito species. Nothing is known about the role of *Ae.* (*Aedimorphus*) *apicoannulatus* in disease transmission and behavior. *Ae.* (*Aedimorphus*) *tarsalis,* mostly found in rock pools in this study, is a rock pool "breeder" according to Hopkins (1952). Species from the Tarsalis group have been implicated in the transmission of Middelburg virus (MIDV), Wesselsbron virus (WESV), Kedougou virus (KEDV), Pongola virus (PGAV), and Zika virus (ZIKV) (Braack et al. 2018). *Ae.* (*Stegomyia*) *corneti* were collected mostly in bamboo pots in Talangaye and according to Huang (1986), this species was collected in cut bamboo about 0.33 m above ground in partially shaded areas in Sierra Leone, in bamboo pots in Forest de Banco in Nigeria and in tree holes in Saoua, Cerce de Sassandara (Côte d'Ivoire). Disease vector status of this species is unknown, but as a member of the Africanus subgroup, *Ae.* (*Stegomyia*) *corneti* may serve as a sylvatic disease vector of yellow fever, Chikungunya, Rift Valley fever, Zika and Bouboui viruses (Huang 1986). The presence of *Aedes* (*Stegomyia*) *aegypti* (L.) in rock pools in partially logged (fragmented) areas of Talangaye may suggest that it is not the forest ancestral type (*Ae. aegypti formosus* (*Aaf*), the dark-colored sylvatic form) but the more domesticated type (*Ae. aegypti aegypti* (*Aaa*), the light-colored domestic form) that invaded the area, or that the forest ancestral type may have adapted to the deforestation. *Aedes* (*Stegomyia*) *aegypti* (L.) is a known vector of yellow fever virus (YFV), dengue virus (DENV), ZIKV, Chikungunya virus (CHIKV), and Rift Valley fever (RVFV) (Braack 2018, Bamou et al. 2021). *Ae.* (*Stegomyia*) *africanus* collected in bamboo pots in Talangaye Rainforest are predominantly found in tree holes but can be found in bamboo pots and artificial containers and in depressions in granite (rock pools) (Hopkins 1952). As a more opportunistic selection of aquatic sites, this species could become more domesticated and a disease vector of note in the future. However, in mosquito community comparisons in various habitat types in Senegal, *Ae. africanus* were predominant in forest canopy and ground habitats and very rarely collected in agricultural, savannah, and outdoors in urban environments (Zahouli et al. 2017, Diallo et al. 2019) while Mayi et al. (2020) recorded higher numbers in Raffia palm bushes in rural and peri urban areas in western Cameroon. *Ae. africanus* are implicated in the transmission of YFV, ZIKV, Bouboui virus (BOUV), Babanki virus (BBKV), CHIKV, WESV, West Nile virus virus (WNV), Orungo virus (ORUV) and Bozo virus (BOZOV). *Ae.* (*Aedimorphus*) *simulans* was collected in tree holes as expected but can also be found in bamboo stumps (Hopkins 1952). They are probably restricted to forests and are unlikely to occur in drier environments. Nothing is known about its role in disease transmission and behavior. However, Bunyamwera virus (BUNV) and WESV have been isolated from this species in Cameroon (Brottes et al. 1966). *Ae.* (*Finlaya*) *longipalpis* (collected in tree holes in Talangaye) is a species only found in forest canopy in Senegal (Diallo et al. 2019) and likely unable to survive in urban settings as was shown in Côte d'Ivoire (Zahouli et al. 2017). Kumm (1932) reported that larvae survived in starved conditions for several weeks and were found in tree-holes and bamboo stumps. Nothing is known about its role in disease transmission and behavior. Larvae of *Ae.* (*Aedimorphus*) *mutilus* (collected in bamboo pots in Talangaye) have been reported in ground pools by Hopkins (1952). BUNV has been isolated from this species (Brottes et al. 1966) *Ae.* (*Aedimorphus*) *boneti* was collected in rock pools, which seem to be a typical immature development habitat for this species (Hopkins 1952). *Aedes* (*Aedimorphus*) *leptolabis ssp. talangayensis sp. nov.*, is a newly described species (Kowo et al. 2023) of which the single specimen was sampled in a bamboo pot.

The low densities of *Anopheles, Hodgesia,* and *Toxorynchites* species encountered could be due to their aquatic habitat specificities (Edwards 1941, Service 1990) and the possibility that some of the water sites could have been missed during our surveys. Most *Anopheles* species were found in stream pools. Anopheline larvae occur frequently in shallow temporary habitats (Mereta et al. 2013) and a few species have been found in tree holes or leaf axils of some plants (CDC. 2004). In this study, larvae of *An. (Anopheles) obscurus* were found in stream pools, typical for this species, but one larva was found in a fallen cocoa pod, which is not its usual habitat for immatures. According to Gillies and de Meillon (1968), it is a forest species that does not vector human malaria and immatures of which are confined to pools of water on the forest floor along

**Table 2.** Mosquito abundance and diversity in natural, seminatural and artificial water sources

| | Natural water sources | | | | Seminatural water sources | | | Artificial water sources | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DL | RP | SP | TH | CP | BL | BP | TT | CnP | T | PC | CkP |
| Abundance (N) | 1 | 178 | 103 | 32 | 6 | 17 | 147 | 45 | 2 | 16 | 40 | 8 |
| Richness (S) | 1 | 21 | 12 | 14 | 4 | 5 | 9 | 1 | 2 | 5 | 4 | 3 |
| Shannon (H′) | 0.00 | 2.7 | 2.01 | 2.27 | 1.24 | 1.43 | 1.49 | 0.00 | 0.69 | 1.32 | 0.63 | 0.9 |
| Simpson (DS) | 0.00 | 0.92 | 0.84 | 0.86 | 0.67 | 0.73 | 0.7 | 0.29 | 0.5 | 0.67 | 0.31 | 0.53 |

Abbreviations: DL, large curled up dead leaves on the ground; RP, rock pools; SP, stream pools (along edges of small river lets); TH, tree holes; BP, bamboo pots; CP, cocoa pods; BL, banana leaf axils; TT, tyre tracks; CnP, coconut pods; T, tarps on the ground; PC, plastic cups; CkP, discarded cooking pots; N, number of adult mosquitoes recorded; S, number of adult mosquito species identified.

**Table 3.** Mosquito abundance and diversity across seasons

| | Dry season | | Rainy season | |
|---|---|---|---|---|
| Months | October | January | April | July |
| Number of water sources | 4 | 5 | 5 | 7 |
| Mosquito abundance | 75 | 135 | 288 | 97 |
| Mosquito richness | 9 | 15 | 24 | 23 |

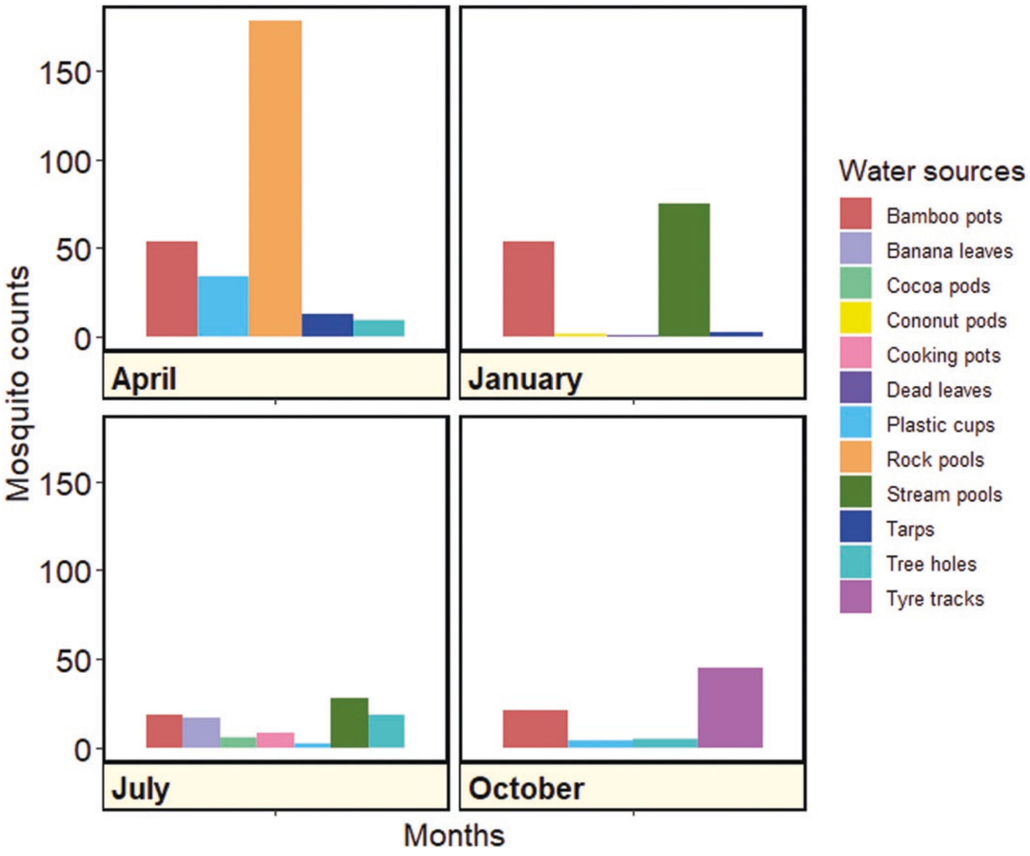

**Fig. 4.** Monthly abundance of mosquitoes in different water sources in Talangaye Rainforest.

swamp and river edges and has some salt tolerance. *An. (Cellia) cinctus* (collected in bamboo pots in Talangaye) has a wide distribution in Equatorial Africa in forests and is found in small, slowly flowing stream pools with overgrown trailing vegetation in which larvae seek shelter. This species plays no role in human disease transmission (Gillies and de Meillon 1968).

*Ho. cuptopous* were solely found in stream pools in our study area. Previous studies have reported them in swamps and marshy areas and small ground pools containing rotting leaves and other organic debris (Hopkins 1952, Service 1990). While these species are known to blood feed on humans, they have not been implicated as human disease vectors.

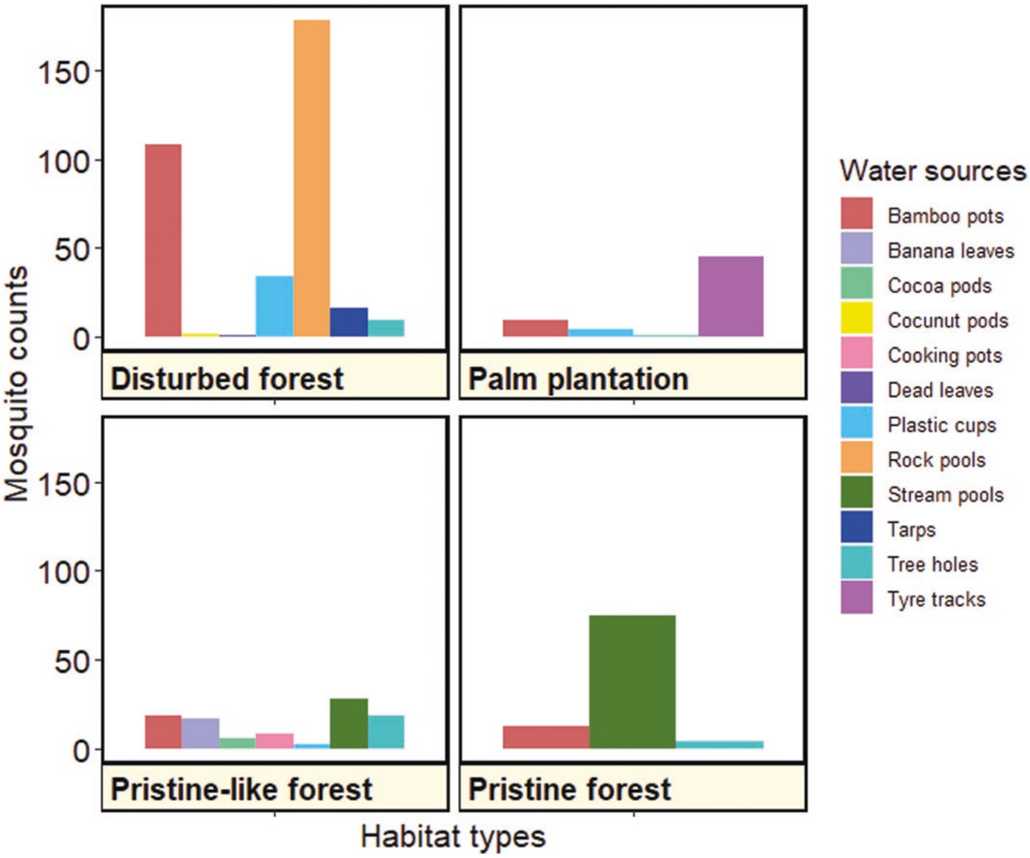

**Fig. 5.** Mosquito abundance in different water sources in Talangaye Rainforest across different habitat types.

One adult of *Tx. brevipalpis brevipalpis*, emerged from immature collected in a tree hole, which is the most usual aquatic habitat for this species (Hopkins 1952). Additionally, larvae of this species have been collected in artificial containers such as wooden barrels, discarded tins, and pots, metal water tanks and iron drums (Theobald 1904, AJC personal observations). *Toxorhynchites* larvae are predaceous and have been used for biological control of mosquito vectors such as *Ae. aegypti* (Donald et al. 2020). They have no known role in human disease transmission in nature because they do not blood feed but, interestingly, can be experimentally infected with flaviviruses such as dengue and yellow fever (Service 1990).

*Lutzia tigripes* larvae (mostly collected in rock pools and tarps in Talangaye Rainforest) are predaceous and are quite general in selection of aquatic habitats ranging from river eddies, rock pools, roadside ditches, and swamps. They generally do not select small water bodies such as tree holes, plant leaf axils, and small artificial sites tins, bottles, and plastic containers (Hopkins 1952, AJC personal observations). This species is not confined to forests but is widespread throughout sub Saharan Africa and is found in rice agro-ecosystems (Muturi et al. 2007) and a variety of large artificial containers in urban environments (Zahouli et al. 2017). This predominantly avian blood-feeding species (Snow 1983, AJC personal observations) has been found infected with NTAV, Kabuto Mountain virus (KAMV), and Mossuril virus (MOSV) (Brottes et al. 1966, Braack et al. 2018). Given their predatory behavior, *Toxorhynchites* and *Lutzia* larvae from our study site probably fed on other mosquito larvae with which they were in sympatry in the water bodies, since unfortunately all the larvae were reared together until they emerged as adults, and this may have affected the breeding success of other mosquito species.

*Uranotaenia* species were mostly found in rock pools and stream pools. More specifically, *Ur.* (*Uranotaenia*) *balfouri* found in stream pools in Talangaye Rainforest have been collected in borrow-pits (Wesch 1910), water-holes (Ingram 1912), pools covered with *Pistia* (Macfie and Ingram 1922), grassy borders of a marsh (Schwetz, 1930b) and a swamp (Philip 1931). This species plays no known role in human disease transmission. *Ur.* (*Pseudoficalbia*) *mashonaensis* (collected in stream pools in Talangaye Rainforest) have been found in a variety of habitats in sub-Saharan Africa, including ditches, forest pools, marshes, rice fields, rock pools in a riverbed, stream margins, and swamps (Service 1990, da Cunha Ramos 1993). Although engorged females showed to have fed on humans and other animals have been found (Service 1985), they are currently not considered to be human disease vectors. *Ur.* (*Uranotaenia*) *chorleyi* larvae collected in stream pools in Talangaye Rainforest usually occur in stagnant water, marshes, ditches, and sedge-swamps often with vegetation and always with a certain amount of shade (Hopkins 1952). This species has no known role in human disease transmission. *Ur.* (*Pseudoficalbia*) *fusca* found in the rock pools of Talangaye Rainforest have nearly all previous records of being collected in rock pools (Hopkins 1952), and they play no known role in human disease transmission. While *Ur.* (*Pseudoficalbia*) *montana*, *Ur.* (*Uranotaenia*) *caerulocephala* larvae were collected in rock pools, *Ur.* (*Pseudoficalbia*) *pseudohenrardi* larvae were collected in a stream vernal pool in Talangaye Rainforest. *Ur.* (*Pseudoficalbia*) *ornata*, a known plant axil specialist, were collected in rock pools and banana leaf axils in Talangaye Rainforest. Bacot (1916) records larvae from the axils of *Dracaena* and *Colocasia*, and Connal (1931) recorded larvae in pineapple plants. In Uganda, *Ur. ornata var. musarum* occurs plentifully in the axils of wild banana (Musafecmda) (Hopkins 1952) while Doucet (1951a) collected larvae in bamboos. Although females were caught in human bait catches in Lagos Nigeria (Mattingly 1949), no vector role in disease transmission has been established for this species. The above records show that *Uranotaenia* mosquitoes are opportunist site selectors. Although relevance from a public health standpoint is poorly known, Jurga virus was isolated from a collection of *Uranotaenia* collected in Selangor, Malaysia (Barge 1975). Furthermore, *Uranotaenia* species, generally considered specialists of ectothermic hosts, have been found with eastern equine encephalitis virus in North American (Cited by Armstrong and Andreadis 2022).

Mosquito species differ in the type of aquatic habitats they prefer for oviposition selection based on location, the physicochemical conditions of water body, and the presence of potential predators (Shililu et al. 2003, Piyaratnea et al. 2005). Physicochemical factors that influence oviposition, survival and the spatio-temporal distribution of mosquito species include salts, dissolved organic and inorganic matter, degree of eutrophication, turbidity, presence of suspended mud, presence or absence of plants, temperature, light, shade, and hydrogen ion concentration (Ephantus 2008). Unfortunately, this study only identified the types of water sources used by forest mosquitoes and did not look at the physicochemical characterization of each water source, which could be considered in future studies.

### Seasonal Distribution of Forest Mosquitoes in Water Bodies

A high number of mosquito species were collected during the rainy season, with more in April (beginning of wet season) than in July (during heavy rainfall events). This was likely because of flooding that washed away the eggs and larvae in July. The lowest mosquito abundance and richness was observed in October and January when there was a notable scarcity of natural water sources. Mosquitoes are commonly associated with rainfall that creates numerous temporary breeding grounds (Russell. 1998, Patz et al. 2000, Djoufounna et al. 2022) as it was the case in April and July, where numerous immature development habitats were recorded as compared to January and October. The results of this study are in accordance with many other studies that captured more mosquitoes during the wet season than during the dry season sampling period (Meyer et al. 2016, Mayi et al. 2020, Djiappi-Tchamen et al. 2021). While rock pools and bamboo pots were mostly colonized during the rainy season, bamboo pots, stream pools, and artificial pools were mostly colonized during the dry season. Season as well as the type of water source are therefore important factors to be considered when designing mosquito control strategies. Overall, bamboo pots were the most suitable for collection of mosquito immatures during both the dry and wet seasons in this study. In Brazil, bamboos proved to be important for the maintenance of the mosquito fauna (Müler et al. 2022) that should be taken into consideration during the implementation of vector control measures, especially in places close to wild areas of Atlantic Forest.

### Spatial Distribution of Forest Mosquitoes in Water Bodies

Disturbed habitat and pristine-like forest had the greatest number of mosquitoes and aquatic habitats sources compared to the pristine forest and palm plantation. In this study, disturbed and pristine forests had in common the partial disturbance of the environment due to selective logging and agricultural practices. Such disturbance and practices possibly allow the creation of new larval biotopes and sunlight penetration, and accelerates mosquito development and survivorship (Kilpatrick et al. 2006, Tangena et al. 2016, Zahouli et al. 2016). Similar trends have been observed in other insects, especially in butterflies, which tend to be less diverse in undisturbed forests compared to disturbed forests (Schulze et al. 2004, Fermon et al. 2005, Bobo et al. 2006). Importantly, Vu et al. (2015) in their study highlighted the need for distinction between forest habitat types when studying the ecological and conservation requirements for butterflies and other tropical insects.

## Conclusion

This is the first report on aquatic habitats that immature forest mosquito species were found in, in Talangaye Rainforest, South West Region of Cameroon. Forest mosquito species were most preferentially found in rock pools, tree holes, stream pools, and bamboo pots. *Culex* species (especially *Cx. (Culiciomyia) nebulosus*, *Cx. (Eumelanomyia) wigglesworthi*, *Cx.* (*Culex*) *ornatothoracis*, *Cx. (Eumelanomyia) horridus*, *Cx.* (*Culex*) *argenteopunctatus*), the most abundant mosquitoes, were encountered in all natural and artificial water bodies. This versatile behavior of those forest mosquito species is informative, as those species would stand best chances of adapting to the more anthropogenic environmental changes and serving as disease vectors. These findings add contributions to knowledge of the biology of 43 forest dweller mosquito species and provide key information useful for the development of suitable and specific vector control measures against diseases outbreak and emergence. Further studies coupled with water quality studies considering biotic (vegetation and fauna) and abiotic (chemical and physical) factors are required to characterize the larval habitats and determine the species-specific utilization of larval habitats. It will also be interesting to undertake further research to study the vector role of forest mosquitoes. Deeper longitudinal studies in forests combining morphological, molecular, and bionomic approaches will also likely lead to the identification of new forest specialized mosquito species. For example, even after almost a century of work on the *An. gambiae* complex, a new species, *An. fontenillei* has been described (Barrón et al. 2019). Collections of male mosquitoes that were done in our investigations in Talangaye Rainforest also revealed multiple undescribed species (Mayi et al. 2019, Kowo et al. 2023).

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## Author contributions

Marie Paul Audrey Mayi (Data curation [lead], Formal analysis [lead], Investigation [equal], Methodology [equal], Project administration [equal], Visualization [lead], Writing—original draft [lead], Writing—review & editing [lead]), Cyril Kowo (Investigation [equal], Methodology [equal], Project administration [equal]), Foncha Forfuet (Investigation [equal], Methodology [equal], Project administration [equal]), Damian Anong (Conceptualization [lead], Funding acquisition [lead], Investigation [supporting], Project administration [supporting], Resources [lead], Supervision [supporting], Validation [supporting], Writing—review & editing [supporting]), Andongma Esack Fonda (Investigation [equal], Methodology [equal], Project administration [equal]), Mirabel Elad (Investigation [equal], Methodology [equal], Project administration [equal]), Charlene Djomo (Investigation [equal], Methodology [equal], Project administration [equal]), Timoléon Tchuinkam (Conceptualization [supporting], Investigation [supporting], Methodology [supporting], Project administration [supporting], Supervision [supporting], Validation [equal], Writing—review & editing [equal]), Ravinder Sehgal (Conceptualization [lead], Funding acquisition [lead], Investigation [supporting], Methodology [supporting], Project administration [supporting], Resources [supporting], Supervision [supporting], Validation [equal], Writing—review & editing [supporting]), and Anthony Cornel (Conceptualization [lead], Data curation [supporting], Investigation [supporting], Methodology [supporting], Project administration [supporting], Supervision [lead], Validation [equal], Writing—review & editing [supporting])

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