

## Resting behaviour and blood meal sources of *Anopheles gambiae* in two areas of Côte d'Ivoire

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### Abstract

Several countries in tropical Africa, including Côte d'Ivoire, are affected by malaria. To prevent this disease, vector control measures including the use of LLINs is recommended. In this context, the Ivorian government has initiated national mass distribution campaigns of LLINs to help control the densities of malaria vectors and decrease the incidence of malaria. This study was carried out to investigate the resting behaviour and host preference of malaria vectors particularly *An. Gambiae* in Man and Abengourou, two areas of Côte d'Ivoire. In 2015 and 2019, after spraying insecticide in selected houses in the morning, indoor resting anopheline mosquitoes were sampled in urban and rural areas during the dry and rainy seasons in the two sites. The origin of blood meals of the mosquitoes from these collections was then investigated using the blood meal ELISA technique. The culicidian fauna inventory showed a diversity of mosquitoes (*Anopheles*, *Aedes*, *Culex* and *Mansonia*) with the predominance of the *An. Gambiae* species in each locality except the urban area of Man where the *Culex* genus predominated. The feeding behaviour of *An. Gambiae* in Man was more diverse in 2015 than those harvested in 2019, which are anthropophilic. In Abengourou, the behaviour of *An. Gambiae* remained unchanged from 2015 to 2019. The results show that in areas of massive distribution of LLINs, feeding behaviour of *An. Gambiae* can undergo changes.

**Keywords:** Malaria, *Anopheles Gambiae*, LLINs, blood meal, Côte d'Ivoire

### Introduction

Malaria remains a significant health challenge in West Africa, characterized by its high morbidity and mortality rates with 95% of cases and 96% of mortality recorded worldwide (WHO, 2022) [1]. Despite considerable efforts to combat the disease, factors such as climate, socio-economic status, healthcare limitations, and resistance to treatment and vector control continue to hinder eradication efforts (Sossouhounto *et al*, 2024) [2]. In Côte d'Ivoire, a west African country, malaria remains a major public health problem. In this country, the parasites responsible for human malaria are mainly transmitted by primary vector species such as *Anopheles Gambiae*, *An. Funeustus* and *An. Nili* (Betsi *et al*, 2012; Wiebe *et al*, 2017; Ossè *et al*, 2019) [3, 4, 5]. Nevertheless, *An. Gambiae* is the most anthropophilic and therefore the cause of the majority of malaria cases in Côte d'Ivoire (PSN, 2015) [6]. This species is well adapted to various types of breeding sites such as puddles, shallow wells, footprints, rice paddies that are generally common (Tia *et al*, 2016) [7].

To reduce malaria vector transmission, the National Malaria Control Program (NMCP) of Côte d'Ivoire, following the example of many African countries, adopted preventive control based essentially on the use of LLINs (WHO, 2017; WHO, 2018) [8, 9]. Thus, through mass distribution campaigns, more than 29 million LLINs (14 million in 2014 and 15 million in 2017) were distributed throughout the national territory with a coverage rate of 93% (MSHP, 2019) [10]. These actions have begun to have a positive influence on malaria mortality and morbidity rates. In fact, the number of deaths due to malaria fell from 3222 in 2017 to 1316 in 2020 e.i. a mortality rate down by around 50%. However, this success could be hampered by changes in vector activity. Indeed, various factors like host preference,

resting and feeding behaviour, adult longevity and density, human biting rate and host location strategy influence the role of mosquitoes in malaria transmission (Sindato *et al*, 2011) [11].

Understanding feeding and resting preferences, as well as the transmission potential of adult vectors in the region, is essential to effectively plan and execute improved vector control measures. Controlling adult vector populations can only be effective by understanding resting and feeding preferences. As Côte d'Ivoire is part of this context of malaria control based on the use of mosquito nets, we sought to identify any behavioural changes that might occur in the main malaria vectors by setting the objective of assessing the effect of mass distribution of insecticide-treated mosquito nets on the feeding and resting preferences of *An. Gambiae* in urban and rural areas of Man and Abengourou.

### Material and methods

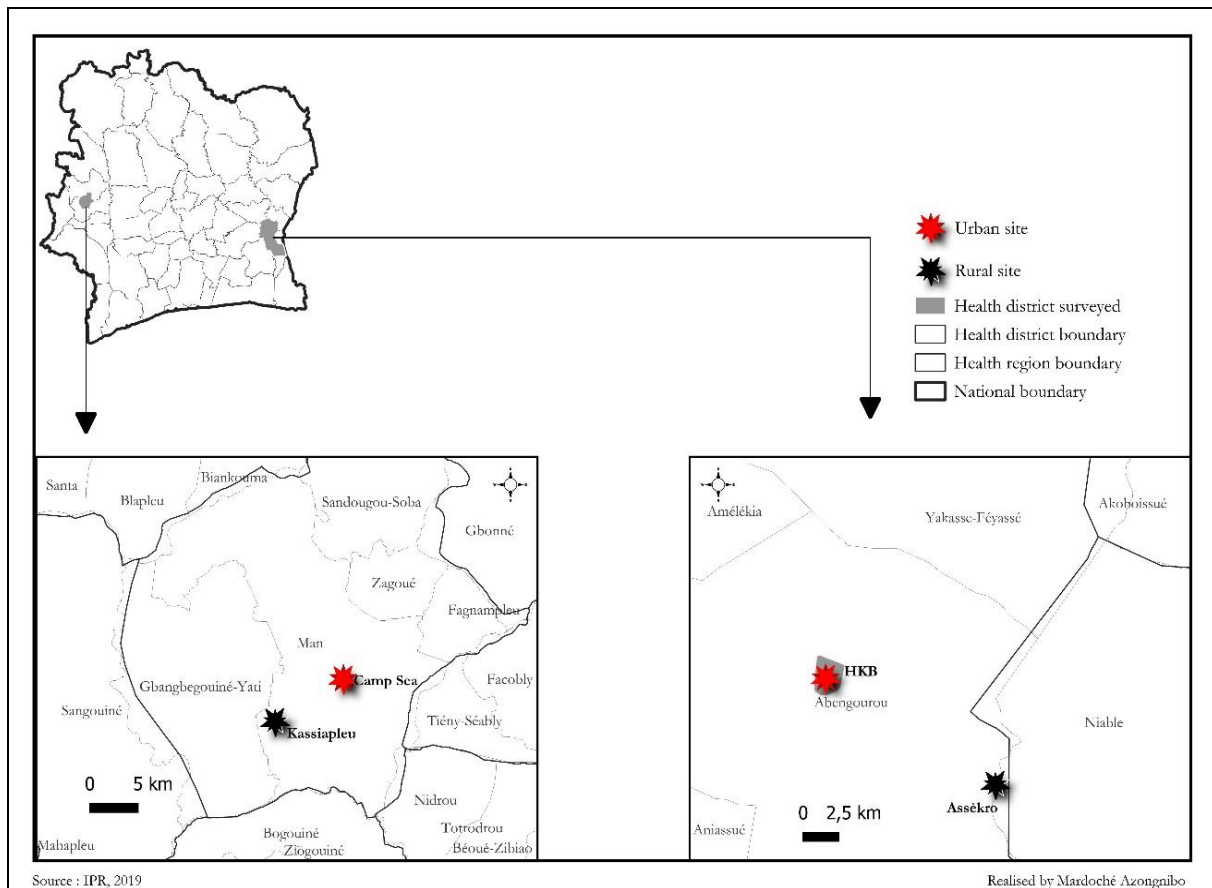
#### Study sites

The Mosquitoes were collected from two localities in urban and rural settings: Man (7° 24'45" N, 7° 33'13" W) in the western region and Abengourou (6° 43'46" N, 3° 29'47" W) in the eastern region (Fig. 1). These localities were chosen because they are part of the sentinel sites of the NMCP and therefore included in the malaria epidemiological surveillance program. Moreover, according to the NMCP, the coverage rate in these localities is over 80%. In fact, 83,660 households were counted in the western zone of Man, 78,142 households had at least one LLIN and 234,904 LLINs were distributed, suggesting a coverage rate of 93.45%. In Abengourou, 89,298 households were counted, 73,305 households had at least one LLIN and 207,630 LLINs were distributed, with a coverage rate of 82.09%.

The two areas are also characterized by two seasons: a long rainy season and a short dry season. In Man, western forest zone, the maximum temperature in Man averages 32°C over the year (from 27°C in August to 35°C in February). It rains 3496mm over the year, with a minimum of 36mm in January and a maximum of 552mm in September. On average, the hottest months are January to May and December. There are two seasons in the year in this locality: the rainiest months are September and October. (March–November) and a short dry season (December–February). In Abengourou, eastern forest zone, the maximum temperature averages 34°C over the year (from 30°C in August to 37°C in February). It rains 1909mm over the year, with a minimum of 19mm in January and a maximum of 290mm in October. In this locality, the rains generally last for 8 months, from March to October. For a better appreciation of the realities of the field and a good interpretation of the results, two catch areas were chosen in each of the sentinel sites, one in an urban area and the other in a rural area. Thus, the neighbourhoods of Camp Sea and H.K.B were chosen in urban area of Man and Abengourou, respectively. The villages of Kassiapleu and Assékro were chosen in rural areas of Man and Abengourou respectively (Fig. 1).

**Mosquito collection and morphological identification**

Repeated cross-sectional surveys were conducted in 2015 and 2019 every three months to carry out this study. A total of four surveys was carried out in each of the two years during the dry and wet seasons. Malaria vectors resting indoors were sampled in 40 bedrooms of each study site in the morning from 0600 to 0730 hours using pyrethrum spray catches (PSC) (Githinji EK, 2020) [12]. The mosquitoes were collected petri dishes containing numbers and lined at the bottom with a layer of cotton soaked in water to prevent desiccation and brought back to the laboratory. They were then identified and sorted according to sex, gender and species based on of morphological criteria using the taxonomy and identification keys to female Afrotropical anophelines (Coetzee, 2020) [13]. An identification form was drawn up to record the mosquitoes captured and their physiological state. Sampled mosquitoes were further classified according to abdominal status as unfed, fed, half-gravid and gravid (Fig. 2). Mosquitoes collected were stored in separate labelled vials. Samples were stored at the laboratory of the Institut Pierre Richet of Bouaké, Côte d’Ivoire until required for further processing.

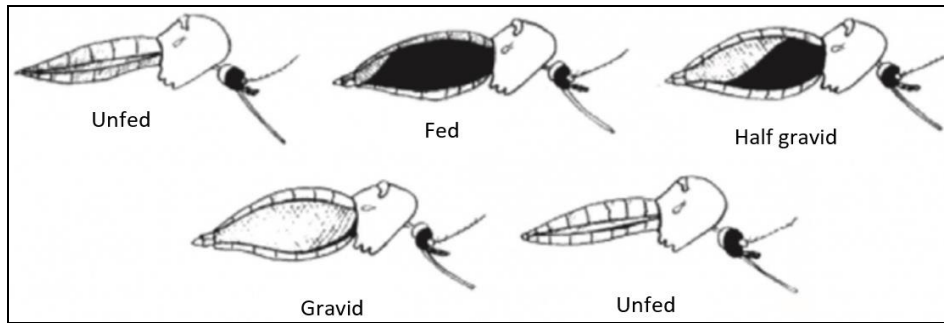


**Fig 1:** Map of Côte d’Ivoire showing the locations of the study sites

**Detection of blood meal sources**

A direct enzyme-linked immunosorbent assay (ELISA) was used to identify the source of mosquito blood meals (Beier *et al*, 1988) [14]. In fact, the blood of engorged vector females was screened for potential host antigen reacting it with host specific antibodies. The choice for antibodies tested was based on the presence of humans and animals in

the study area. In our study, monoclonal antibodies from humans, beef, sheep and chicken were tested. These antibodies were all labelled with peroxide and stored at -20 °C. Dilutions of blood meals were carried out with human sera at 1/2000, beef at 1/1000, sheep at 1/500 and chicken at 1/2000.



**Fig 2:** Physiological status of malaria vectors (Detinova, 1962)

**Data analysis**

Densities of resting anopheline mosquitoes were calculated as the number of female mosquitoes per room per day (FRD). The Kruskal-Wallis test was used to compare the proportions of feeding, gravid and other physiological state females collected in 2015 and 2019 at each site. It was also used to compare the mean resting densities in the environments and localities in 2015 and 2019. Human blood index (HBI) was calculated as the proportion of blood-fed mosquito samples that had fed on humans relative to the total tested for blood meal origin. The Pearson Chi-Square Test was used to compare HBI in 2015 and 2017. The confidence interval was 95%.

**Results**

**Mosquito species composition**

PSC culicid fauna represents all mosquitoes at rest inside dwellings. This method of capture resulted in the collection of 130 mosquitoes in 2015, i.e. 62 and 68 respectively in urban and rural areas. In 2019, 654 mosquitoes were collected, 326 and 328 in urban and rural areas respectively (Table 1). Overall, 784 mosquitoes were collected using PSC.

In urban area, 4 mosquitoes were collected in 2015 and 237 in 2019 in the Camp Sea neighbourhood. Seven species were collected in both years with *Cx quinquefasciatus* dominating, followed by *An. Gambiae*. Furthermore, two of these species were common in 2015 and 2019, with proportions of 25 and 8% for *An. Gambiae* and 25 and 0.4%

for *Cx. cinereus* respectively. *Aedes aegypti* (50%) was only identified in 2015. *An. Funestus* (0.4%), *Cx. decens* (0.4%), *Cx. quinquefasciatus* (90.4%) and *Man. africana* (0.4%) were only collected in 2019 (Table 1). In the HKB neighbourhood, 58 mosquitoes were collected in 2015 and 89 in 2019. Four species were identified with *Cx quinquefasciatus* and *An. Gambiae* predominating. Among the species encountered, two were common in 2015 and 2019, with proportions of 53.4 and 49.5% for *An. Gambiae* and 46.5 and 48.3% for *Cx. quinquefasciatus* respectively. The species *Cx. nebulosus* (1.1%) and *Man. africana* (1.1%) were only identified in 2019.

In rural areas, 52 and 266 mosquitoes were identified in 2015 and 2019 respectively in the Kassiapleu village of Man. Four species were identified with *An. Gambiae* dominating, followed by *An. Funestus*. These two species are common to both years of sampling, with proportions of 98.1% and 82.3% for *An. Gambiae* and 1.9% and 16.2% for *An. Funestus* respectively. The species *Cx. cinereus* (0.4%) and *Cx. quinquefasciatus* (1.1%) were only identified in 2019 (Table 1). In Assekro, 16 and 62 mosquitoes were collected in 2015 and 2019 respectively. Three species were identified and *An Gambiae* was the only common species in 2015 and 2019, with proportions of 81.3% and 98.4% respectively. The species *Cx. quinquefasciatus* (18.7%) was only identified in 2015 and *Cx. cinereus* (1.6%) in 2019 (Table 1). Overall, *An. Gambiae* was the only species present in all environments in 2015 and 2019.

**Table 1:** Culicidian fauna collected in the localities of Man and Abengourou between 2015 and 2019

| Area  | Site            | Genus                | Species                     | Culicidian fauna |      |      |      |     |
|-------|-----------------|----------------------|-----------------------------|------------------|------|------|------|-----|
|       |                 |                      |                             | 2015             |      | 2019 |      |     |
|       |                 |                      |                             | n                | %    | n    | %    |     |
| Urban | Camp Sea        | <i>Anopheles</i>     | <i>An. Gambiae</i>          | 1                | 25   | 19   | 8.0  |     |
|       |                 |                      | <i>An. funestus</i>         | 0                | 0    | 1    | 0.4  |     |
|       |                 | <i>Aedes</i>         | <i>Ae. Aegypti</i>          | 2                | 50   | 0    | 0    |     |
|       |                 | <i>Culex</i>         | <i>Cx. cinereus</i>         | 1                | 25   | 1    | 0.4  |     |
|       |                 |                      | <i>Cx. decens</i>           | 0                | 0    | 1    | 0.4  |     |
|       |                 |                      | <i>Cx. quinquefasciatus</i> | 0                | 0    | 214  | 90.4 |     |
|       | <i>Mansonia</i> | <i>Man. africana</i> | 0                           | 0                | 1    | 0.4  |      |     |
|       | Total           |                      |                             |                  | 4    | 100  | 237  | 100 |
|       | HKB             | <i>Anopheles</i>     | <i>An. Gambiae</i>          | 31               | 53.4 | 44   | 49.5 |     |
|       |                 |                      | <i>Cx. nebulosus</i>        | 0                | 0    | 1    | 1.1  |     |
|       |                 | <i>Culex</i>         | <i>Cx. quinquefasciatus</i> | 27               | 46.6 | 43   | 48.3 |     |
|       |                 |                      | <i>Man. africana</i>        | 0                | 0    | 1    | 1.1  |     |
|       |                 | Total                |                             |                  |      | 58   | 100  | 89  |
|       | Total           |                      |                             |                  | 62   | 100  | 326  | 100 |
| Rural | Kassiapleu      | <i>Anopheles</i>     | <i>An. Gambiae</i>          | 51               | 98.1 | 219  | 82.3 |     |
|       |                 |                      | <i>An. funestus</i>         | 1                | 1.9  | 43   | 16.2 |     |
|       |                 | <i>Culex</i>         | <i>Cx. quinquefasciatus</i> | 0                | 0    | 3    | 1.1  |     |
|       |                 |                      | <i>Cx. cinereus</i>         | 0                | 0    | 1    | 0.4  |     |

|       |         |           |                             |     |     |      |     |      |
|-------|---------|-----------|-----------------------------|-----|-----|------|-----|------|
|       | Assekro | Total     |                             | 52  | 100 | 266  | 100 |      |
|       |         | Anopheles | <i>An. Gambiae</i>          |     | 13  | 81.3 | 61  | 98.4 |
|       |         |           | <i>Cx. quinquefasciatus</i> |     | 3   | 18.7 | 0   | 0    |
|       |         | Culex     | <i>Cx. cinereus</i>         |     | 0   | 0    | 1   | 1.6  |
|       | Total   |           | 16                          | 100 | 62  | 100  |     |      |
| Total |         | Total     |                             | 68  | 100 | 328  | 100 |      |

N: number of mosquito collected

**Physiological status of *An Gambiae***

PSC methods resulted in the collection of 96 female *An. Gambiae* mosquitoes, including 32 in urban area and 64 in rural area in 2015, and 324 females, including 44 in urban area and 280 in rural area in 2019 (Table 2). A total of 420 *An. Gambiae* were collected using the PSC method.

In urban setting, in the bedrooms of the Camp Sea neighbourhood, a total of 01 female *An. Gambiae* in 2015 and 19 in 2019 were collected at rest. In 2015, there was only 01 unfed female (100%). In 2019, gravidas *An. Gambiae* females were the most abundant (47.4%), followed by fed females (42.1%) and, the least represented were females in other physiological status (i.e unfed or half gravid, 10.5%) (Table 2). No significant difference was found between the proportions of fed females (KW= 0.500; df=1; p= 0.4795) in 2015 and 2019. The same was true for gravid females in 2015 and 2019 (KW= 0.500; df=1; p= 0.4795) and females in other physiological status in 2015 and 2019 (KW= 0.229; df=1; p= 0.6319). In HKB neighbourhood, a total of 31 *An. Gambiae* females in 2015 and 44 in 2019 were caught at rest. In 2015, gravid *An. Gambiae* females were the most abundant (80.7%). Gravid females and females in other physiological status represented 16.1% and 3.2% of the population of females caught, respectively. In 2019, gravid females were the most abundant (61.4%), followed by those in other physiological states (27.2%). Gravid females were the least represented (11.4%) (Table 2). In this area, there are many females likely to be laying eggs, suggesting a high risk. However, no significant difference was found between the proportions of gravid females in 2015 and 2019 (KW= 0.229; df=1; p= 0.6319). The same result was obtained for gravid females in 2015 and 2019 (KW= 0.254; df=1; p= 0.6141) and those in other physiological status in 2015 and 2019 (KW= 0.229; df=1; p= 0.6319). In addition, during 2019, there were more

females resting in the HKB area than in the Camp Sea area (KW= 3.996; df=1; p= 0.0445).

In the rural settings, overall, many fed females were encountered. In fact, in Kassiapleu, 51 *An. Gambiae* females were collected in 2015 and 219 in 2019. In 2015, the proportion of gravid mosquitoes was highest, at 68.6%. Gravid females represented 31.4% of the population of females collected. In 2019, gravid *An. Gambiae* females were the most abundant (43.8%). Fed females and females in other physiological status represented 21% and 35.2% of the population of females collected, respectively (Table 2). The proportions of fed *An. Gambiae* females in 2015 and 2019 were not significantly different (KW= 0.229; df=1; p= 0.6319). The same was true for gravid females in 2015 and 2019 (KW= 0.229; df=1; p= 0.6319) and females in other physiological status in 2015 and 2019 (KW= 0.500; df=1; p= 0.4795).

In the village of Assekro, 13 *An. Gambiae* females were collected in 2015 and 61 in 2019. In 2015, only fed and gravid *An. Gambiae* females were collected, with a high proportion of fed females (92.3%). Gravid females represented 7.7% of the population of females captured. Among *An. Gambiae* females, the proportion of gravid mosquitoes was highest in 2019, at 80.3%. Gravid females and those in other physiological status represented 11.5% and 8.2% of the population of females collected respectively (Table 2). The proportions of fed females with *An. Gambiae* in 2015 were not significantly different from those in 2019 (KW= 0.229; df=1; p= 0.6319). It was the same for gravid females in 2015 and 2019 (KW= 0.229; df=1; p= 0.6319) and those in other physiological status in 2015 and 2019 (KW= 0.500; df=1; p= 0.4795). In addition, during 2019, there were more females resting in the village of Kassiapleu than in Assekro.

**Table 2:** Physiological status of *An. Gambiae* in the localities of Man and Abengourou between 2015 and 2019

| Area  | Site       | <i>An. Gambiae</i> | 2015 |      | 2019 |      |
|-------|------------|--------------------|------|------|------|------|
|       |            |                    | n    | %    | n    | %    |
| Urban | Camp Sea   | Fed                | 0    | 0    | 8    | 42.1 |
|       |            | Gravid             | 0    | 0    | 9    | 47.4 |
|       |            | Other              | 1    | 100  | 2    | 10.5 |
|       |            | Total              | 1    | 100  | 19   | 100  |
|       | HKB        | Fed                | 25   | 80.7 | 27   | 61.4 |
|       |            | Gravid             | 5    | 16.1 | 5    | 11.4 |
|       |            | Other              | 1    | 3.1  | 12   | 27.2 |
|       |            | Total              | 31   | 100  | 44   | 100  |
| Total |            |                    | 32   | 100  | 63   | 100  |
| Rural | Kassiapleu | Fed                | 35   | 68.6 | 46   | 21   |
|       |            | Gravid             | 16   | 31.4 | 96   | 43.8 |
|       |            | Other              | 0    | 0    | 77   | 35.2 |
|       |            | Total              | 51   | 100  | 219  | 100  |
|       | Assekro    | Fed                | 12   | 92.3 | 49   | 80.3 |
|       |            | Gravid             | 1    | 7.7  | 7    | 11.5 |
|       |            | Other              | 0    | 0    | 5    | 8.2  |
|       |            | Total              | 13   | 100  | 61   | 100  |
| Total |            |                    | 64   | 100  | 280  | 100  |

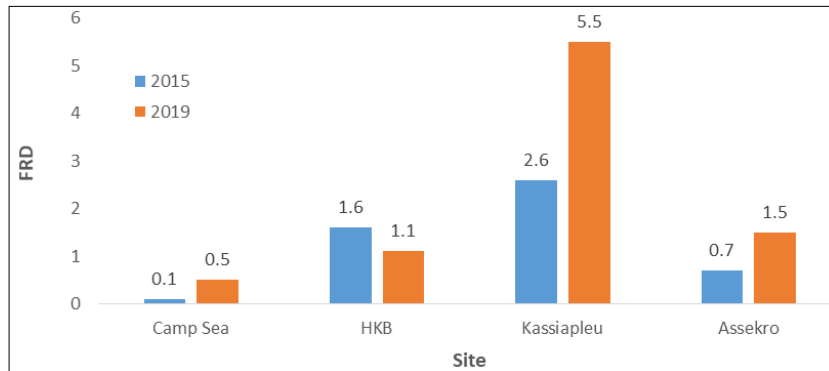
N: Number of *An. Gambiae* females collected

**Resting behaviour of *An Gambiae* females**

Densities of resting *An Gambiae* females were estimated as the number of female mosquitoes per room per day (FRD) in each site. Thus, in urban area, a total of 01 female *An. Gambiae* in 2015 and 19 in 2019 were collected in the bedrooms of Camp Sea neighbourhood, suggesting resting behaviour of 0.1 FRD in 2015 and 0.5 FRD in 2019 (Fig. 3). The resting behaviour of *An. Gambiae* collected in 2015 was significantly lower than in 2019 (KW = 4.247; df = 1; P = 0.0393). In the HKB neighbourhood, a total of 31 *An. Gambiae* females in 2015 and 44 in 2019 were collected, suggesting resting densities of 1.6 FRD in 2015 and 1.1 FRD in 2019 (Fig. 3). The resting behaviour of *An. Gambiae* in this neighbourhood have not changed between

2015 and 2019 (Kruskal-Wallis = 0.668; df=1; P = 0.4138). In rural setting, 51 *An. Gambiae* females were collected in 2015 and 219 in 2019 in the village of Kassiapleu, suggesting resting behaviour of 2.6 FRD in 2015 and 5.5 FRD in 2019 (Table 4). In this village, the resting behaviour has not changed between 2015 and 2019 (Kruskal-Wallis = 0.125; df=1; P = 0.7242).

In Assekro, 13 females in 2015 and 51 females in 2019 of *An. Gambiae* were collected i.e the average resting behaviour of *An. Gambiae* females was 0.7 FRD in 2015 and 1.5 FRD in 2019. As in Kassiapleu, resting behaviour in this village remained unchanged between 2015 and 2019 (KW= 3.696; df=1; p= 0.0545).



**Fig 3:** Resting behaviour of *An Gambiae* in Man and Abengourou between 2015 and 2019

**Blood meal sources**

The stomach contents of the mosquitoes from indoor collections were identified by ELISA. Of the *An. Gambiae* mosquitoes collected, 233 were blood-fed and tested for the human blood index (HBI) during the two sampling years i.e 97 in 2015 and 136 in 2019 (Table 3).

In urban settings, in Camp Sea neighbourhood, no blood meals were taken in 2015. In 2019, 7 meals were collected from *An. Gambiae* females. In this neighbourhood, the majority of blood meals analysed were taken from humans, i.e. 85.7%, and 14.3% from animals (Table 3). In HKB neighbourhood, out of 25 meals of *An. Gambiae* females tested in 2015 and 26 in 2019. In this neighbourhood, the majority of meals tested were taken on animals, i.e. 56% of meals, and the other 44% of meals were taken on humans in 2015. In 2019, the majority of meals tested were taken by humans, i.e. 80.8% and 19.2% of the animals (Table 3). Thus, *An. Gambiae* females in 2019 had a significantly higher human blood index than those collected in 2015 ( $\chi^2 = 8.8870$ ; df= 1; P = 0.003). However, *An. Gambiae* females that had taken their meals on animals in 2015 had a significantly higher proportion ( $\chi^2 = 7.3714$ ; df= 1; P = 0.007) than those in 2019. Finally, it should be noted that the human blood index has not changed in 2019 in the two neighbourhoods sampled.

In rural areas, 46 meals of *An. Gambiae* females in 2015 and 54 meals in 2019 were analysed in Kassiapleu. In 2015, 67.4% or the majority of meals were taken on humans and 32.6% on animals. In 2019, 96.3% of meals were taken on humans and 3.7% on animals (Table 3). Thus, *An. Gambiae* females collected in 2015 had a significantly lower human blood index ( $\chi^2 = 14.7086$ ; df = 1; P = 0.000) than those in 2019. However, in 2015, the proportion of female *An. Gambiae* having taken their meals on animals is significantly higher ( $\chi^2 = 14.7086$ ; df = 1; P = 0.000) than in 2019. In Assekro, 26 meals of *An. Gambiae* females in 2015 and 49 meals in 2019 were analysed. The majority of the meals tested were taken on animals (73.1%) in 2015. The remaining 26.9% of meals were taken on humans. In 2019, the majority of females (93.9%) took their meals on humans. The remaining 6.1% of females was taken on animals (Table 3). Thus, *An. Gambiae* females from 2015 had a significantly lower HBI ( $\chi^2 = 36.7360$ ; df = 1; P = 0.000) than those collected in 2019. However, the proportion of *An. Gambiae* females having taken their meals on animals in 2015 is significantly higher ( $\chi^2 = 36.7360$ ; df = 1; P = 0.000) than in 2019. Like in an urban setting, the human blood index has not changed in 2019 in the two villages sampled.

**Table 3:** Blood meal origins of *An Gambiae* from Man and Abengourou localities between 2015 and 2019

| Area  | Site     | Year | N  | Blood meal origins |      |      |   |       |   |         |   |       |      |
|-------|----------|------|----|--------------------|------|------|---|-------|---|---------|---|-------|------|
|       |          |      |    | Human              |      | Beef |   | Sheep |   | Chicken |   | Other |      |
|       |          |      |    | N                  | %    | N    | % | N     | % | n       | % | N     | %    |
| Urban | Camp Sea | 2015 | 0  | 0                  | 0    | 0    | 0 | 0     | 0 | 0       | 0 | 0     | 0    |
|       |          | 2019 | 7  | 6                  | 85.7 | 0    | 0 | 0     | 0 | 0       | 0 | 1     | 14.3 |
|       | HKB      | 2015 | 25 | 11                 | 44   | 0    | 0 | 0     | 0 | 0       | 0 | 14    | 56   |
|       |          | 2019 | 26 | 21                 | 80.8 | 0    | 0 | 0     | 0 | 0       | 0 | 5     | 19.2 |

|       |            |      |    |    |      |   |   |   |   |   |   |    |      |
|-------|------------|------|----|----|------|---|---|---|---|---|---|----|------|
| Rural | Kassiapleu | 2015 | 46 | 31 | 44   | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 32.6 |
|       |            | 2019 | 54 | 52 | 96.3 | 0 | 0 | 0 | 0 | 0 | 0 | 2  | 3.7  |
|       | Assekro    | 2015 | 26 | 7  | 26.9 | 0 | 0 | 0 | 0 | 0 | 0 | 19 | 73.1 |
|       |            | 2019 | 49 | 46 | 93.9 | 0 | 0 | 0 | 0 | 0 | 0 | 3  | 6.1  |

N: Number of *An. Gambiae* tested

## Discussion

Behavioural diversification in vector populations in areas with widespread use of LLINs is a threat to the efficacy of vector control strategies (Bayoh *et al.*, 2010; Coleman *et al.*, 2017; Reddy *et al.*, 2011; Derua *et al.*, 2012, Degefa *et al.*, 2017)<sup>[15, 16, 17, 18, 19]</sup>. This study investigated the behaviour of malaria vectors, specifically their resting and feeding choices. Following mass distributions of LLINs in 2015 and 2019, PSC were carried out in Man and Abengourou. The PSC method is a precise method that makes it possible to collect many endophilic mosquitoes. The results of this collection showed that the resting culicid fauna in urban and rural Abengourou and rural Man in 2015 and 2019 is dominated by the *Anopheles* genus followed by the *Culex* genus. These results are in agreement with those of studies conducted by Salem *et al.* (1994)<sup>[20]</sup> in Pikine, Senegal, and Adja *et al.* (2015)<sup>[21]</sup> in Adzopé, which showed the abundance of *An. Gambiae* in urban environments. Matubi *et al.* (2015)<sup>[22]</sup> also showed the abundance of *An. Gambiae* in urban-rural environments. As for the results obtained in 2019, in the urban environment of Man, the culicid fauna was dominated by the *Culex* genus. This abundance is thought to be linked to domestic pollution, making the surface water unsuitable for the development of other genera and favourable to the development of *Culex* genus. These results are consistent with those of Klinkenberg *et al.* (2008)<sup>[23]</sup> in Accra. The latter reported a higher proportion of *Culex* than *Anopheles*, which could be due to the high level of organic pollution.

The physiological status and resting densities of *Anopheles Gambiae* did not differ from 2015 to 2019, which would imply that the proportions of mosquitoes resting inside houses did not change significantly.

The blood meal analyses revealed that a large proportion of the malaria vectors preferred feeding on humans than on animals in almost all the sites. This preference for human hosts is of great concern for malaria elimination efforts due to the efficacy of these mosquitoes in transmitting malaria. These results are similar to those obtained by Forson *et al.*, 2022<sup>[24]</sup> and Kroko-Djahouri *et al.*, 2024<sup>[25]</sup> in Ghana and Côte d'Ivoire respectively. Furthermore, the majority of *An. Gambiae* females collected from dwellings were found to be zoophilia in 2015 and anthropophilic in 2019. The anthropophilicity and zoophagy of *An. Gambiae* were highlighted by Dossou Yovo (1998)<sup>[26]</sup> in Bouaké. Adja *et al.* (2015)<sup>[21]</sup> also showed this variation in feeding behaviour in Adzopé. Several hypotheses can be put forward to explain this situation, including the use of LLINs in these localities, which is low. The highest level of anthropophilia in 2019 is therefore likely to be the result of high levels of human-vector contact due to the low use of LLINs. The number of zoophagous mosquitoes obtained in 2019 was significantly lower than in 2015; this could be explained by the fact that LLINs have a repellent effect on mosquitoes, which will lead to changes in the feeding behaviour of some of *Anopheles*. Populations of zoophagous and endophilous *An. Gambiae*, which used to be abundant in dwellings, have changed their feeding behaviour by becoming significantly

less present inside dwellings, leading to the disappearance of zoophagous *An. Gambiae* inside dwellings. Only strict anthropophilic *An. Gambiae* populations are found inside houses, leading to a numerical increase in these. These results are similar to those of Carnevale and Robert (2009)<sup>[27]</sup> where the use of insecticide treated nets increased exophilic behaviour and mosquito emergence rates. Darriet *et al.* (2000)<sup>[29]</sup> in M'bé and Yaokoffikro also showed that the natural exophilicity of *An. Gambiae* was doubled by the use of LLINs.

## Conclusion

The culicid fauna collected at rest in dwellings is made up of two genera mainly in urban areas: *Anopheles* and *Culex* with *Cx quinquefasciatus* dominating in Man and *An. Gambiae* and *Cx quinquefasciatus* predominating in Abengourou. In rural settings, the fauna is dominated by a single genus: *Anopheles*, with two vector species: *An. Gambiae* et *An. funestus*. The proportion of *An. Gambiae* females resting inside houses did not change significantly from 2015 to 2019. Analysis of the blood contained in the abdomen of *An. Gambiae* females showed that there were both zoophagous and anthropophilous females in dwellings in 2015. However, in 2019, *An. Gambiae* females are very anthropophilic. This high anthropophilicity is thought to be due to the fact that LLINs have a selective and repellent effect that leads to the gradual disappearance of endophilic zoophagous *An. Gambiae* from homes.

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