Preliminary investigation on aggressive culicidae fauna and malaria transmission in two wetlands of the Wouri river estuary, Littoral-Cameroon

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Abstract
A study of the malaria transmission was carried out for the first time in the Manoka Island and Youpwè mainland area in Douala. Mosquitoes were sampled by human landing catches simultaneously indoor and outdoor in December 2013 and April 2014. Collected mosquitoes were morphologically identified and anopheles ovaries were dissected for parity determination. Legs and head-thorax of each dissected anopheles specimen belonging to Anopheles gambiae complex were used for molecular identification and infectivity status determination. A total of 2,827 female mosquitoes belonging to Anopheles, Culex and Aedes genera were collected in both localities. Anopheles gambiae complex and Culex pipiens was dominant respectively in Manoka (81.1%) and Youpwè (77.9%). In both localities, Anopheles gambiae s.l. had a high tendency to exophagy. Only Anopheles coluzzii was found infected by Plasmodium falciparum. Between Manoka and Youpwè, there was no significant difference in the infection rate and the entomological inoculation rate.

Keywords: Aggressive Culicidae fauna, Anopheles coluzzii, malaria transmission, littoral, Cameroun

1. Introduction
In Cameroon, malaria remains a major public health problem and the leading cause of morbidity and mortality [1]. Eco-climatic variations influence the transmission of the disease [1, 2]. The vector of this disease, the female anopheles mosquito, is constituted of groups or complexes with morphologically similar species, colonizing different environments, whose vector competence and vector capacity are very varied. Knowledge of the vector system in a given context allows establishing more appropriate control measures against the disease [3]. In Cameroon, a large part of the country (south-north transect) lies between the equatorial epidemiological facies corresponding to the forest zone of the south and the tropical area of northern savanna zone; both facies and their transition zones have already been subjected to many entomological studies [4-9]. However, no entomological data are available in the literature on peninsular and insular areas as the island of Manoka and its surroundings along the Wouri river estuary on the Atlantic South-Western coast of Cameroon. This island does commercial activities with the city of Douala and Youpwè quarter serves as the hub for the transportation of human populations and goods between the two areas; these movements could allow passive transport of anopheles and parasite exchanges. This article provides information on the diversity of aggressive culicidae fauna, feeding behavior of vectors and malaria transmission to improve vector control measures and reducing culicidae nuisance on the Manoka Island.

2. Materials and Methods
2.1 Study area
The present study was conducted at Douala (04°03′N 09°41′E), the main port and the largest industrial city of Cameroon, 260 km northwest of the capital city Yaoundé. The city sits on the Wouri river estuary in a degraded Atlantic rain forest. The climate is tropical with two main seasons, a dry season from November to February and a rainy season from March to October.

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However, the city sees rainfall throughout the year with an average of 3,600 mm rainfall per year [10]. The climate is typically warm and humid with an average annual temperature of 27.0 °C and an average humidity of 85% [11]. The city and its surrounding area had an estimated population that surpassed 3,000,000 inhabitants. Two surrounded districts of Douala located in the wetlands of the estuary have been selected for the study, Manoka and Youpwè (Figure 1). Manoka (03°47' N, 09°37' E) is an island in the south of the estuary where fishing and agriculture are main activities. Youpwè (04°00' N; 09°42' E) in the left bank of Wouri river is a landing hub for fresh fish from Manoka island and other places, and the main fish market along the Douala bay. Houses in Manoka are usually built with provisory materials while Youpwè displays high proportion of houses with cement blocks.

Fig 1: Manoka and Youpwè map showing capture points.

2.2 Adult mosquito collections and field processing
Mosquito collections were planned in December 2013 (dry season) and April 2014 (rainy season). Adult mosquitoes were sampled while landing on human volunteers from 6.00 pm to 6.00 am, simultaneously indoor and outdoor. The overall mosquito collections consisted of 48 man-nights in Manoka and 24 man-nights in Youpwè. Sampled mosquitoes were identified following morphological identification keys of Gillies & De Meillon [12] and Edwards [13] and ovaries of anopheline specimens checked for parity according to Detinova’s method [14]. Legs and head-thorax of each *Anopheles* specimen was kept separately in an individual tube with silicagel at –20 °C until the molecular identification of members of the *An. gambiae* complex and ELISA (enzyme linked immunosorbent assay) detection of *Plasmodium* parasite infection in laboratory.

2.3 Laboratory procedures
The ELISA technique was performed on head and thorax of each anopheline specimen to detect the *P. falciparum* parasite circumsporozoite protein following the method described by Burkot et al. [15], modified by Wirtz et al. [16]. DNA extracts from legs of *An. gambiae* specimens were proceed for polymerase chain reaction (PCR) identification of sibling species belonging to the *An. gambiae* complex as described by Fanello et al. [17].

2.4 Data analyses
The following parameters were considered: i) species diversity expressed by the number of species recorded per location and per season, ii) the human biting rate (HBR) as the number of bites/person/night (b/p/n) is the product of number of mosquito collected and number of man-night, iii) the parity rate (PR) as the proportion of parous specimens over the total number of mosquitoes checked for parity, iv) the infection rate (IR) as the proportion of infected mosquitoes over the total number of mosquitoes checked for parasite infection, and v) the daily entomological inoculation rate (EIR) as the number of infected bites/person/night (ib/p/n) was the product of the infection rate and the biting rate. The monthly EIR was estimated by multiplying the daily EIR by an average of 30 days. The R software (version 2.15.2) [18] was used for statistical analyses at risk α = 0.05.

2.5 Ethical consideration and informed consent
This study was approved by the ethic committee of the University of Douala. The purpose of the study was clearly explained to the community with the help of headquarters and opinion leaders. The community members were informed that participation in the research was voluntary without penalty for the give up. The heads of each household was asked to sign the consent form for their household to participate in the study. The participants in the study were remunerated agreeing adults. At the first suspicion of malaria, volunteers were provided with the curative treatment recommended by the National Malaria Control Programme in Cameroon.

3. Results
3.1 Diversity of aggressive culicidae fauna
A total of 2,827 mosquitoes were collected during the two periods in Manoka (1,202) and Youpwè (1,625). The number of species recorded between 7 in December 2013 and 6 in April
2014 in Manoka, but was invariable (3 species) in Youpwè (Table 1). In Manoka, the mosquito fauna consist of at least 12 species belonging to genus *Anopheles* (81.1%, n=975), genus *Culex* (14.5%, n=174) and genus *Aedes* (4.4%, n=52). Among *An. gambiae* complex, two species were found, the most predominant *An. coluzzii* (98%, n = 192) and *An. melas* (2%, n = 4). One specimen of *An. tenebrosus* was also found. The remaining mosquito fauna was represented by *Culex quinquefasciatus* (7.07%, n = 85), *Culex poicilipes* (0.67%, n = 8), *Culex gr decens* (0.5%, n = 6), *Culex duttoni* (1.58%, n = 19), *Aedes aegypti* (2.91%, n = 35), *Aedes albopictus* (0.42%, n = 5), *Aedes vexans* (0.83%, n = 10) and non-identified specimens (4.83%, n=58) because of their degraded state. In Youpwè, 3 mosquito species including *Culex quinquefasciatus* (77.9%, n = 1,265), *An. coluzzii* (21.4%, n = 348) and *Aedes aegypti* (0.7%, n = 12) were collected.

### Table 1: Diversity of aggressive culicidae fauna in Manoka and Youpwè in December 2013 and April 2014

<table>
<thead>
<tr>
<th>Species</th>
<th>Total</th>
<th>December 2013</th>
<th>April 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td><em>Anopheles gambiae s.l.</em></td>
<td>1,323</td>
<td>46.8</td>
<td>344</td>
</tr>
<tr>
<td><em>Anopheles tenebrosus</em></td>
<td>1</td>
<td>0.1</td>
<td>1</td>
</tr>
<tr>
<td><em>Aedes aegypti</em></td>
<td>47</td>
<td>1.6</td>
<td>10</td>
</tr>
<tr>
<td><em>Aedes albopictus</em></td>
<td>5</td>
<td>0.2</td>
<td>4</td>
</tr>
<tr>
<td><em>Aedes vexans</em></td>
<td>10</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td><em>Aedes sp</em></td>
<td>2</td>
<td>0.1</td>
<td>2</td>
</tr>
<tr>
<td><em>Culex duttoni</em></td>
<td>19</td>
<td>0.6</td>
<td>19</td>
</tr>
<tr>
<td><em>Culex gr decens</em></td>
<td>6</td>
<td>0.2</td>
<td>6</td>
</tr>
<tr>
<td><em>Culex quinquefasciatus</em></td>
<td>1,350</td>
<td>47.7</td>
<td>74</td>
</tr>
<tr>
<td><em>Culex poicilipes</em></td>
<td>8</td>
<td>0.4</td>
<td>0</td>
</tr>
<tr>
<td><em>Culex sp</em></td>
<td>56</td>
<td>1.9</td>
<td>56</td>
</tr>
<tr>
<td>Total</td>
<td>2,827</td>
<td>100</td>
<td>516</td>
</tr>
</tbody>
</table>

n = effective; % = genus percentage

#### 3.2 Biting habits of *An. coluzzii*

*Anopheles coluzzii* was aggressive in the study sites with an average man-bite rate of 20.29 b/p/n in Manoka and 14.50 in Youpwè. The difference in biting density between Manoka and Youpwè was significant (*P*<0.001). Night biting cycles of *An. coluzzii* (Figure 2) varied with the study site and did not vary with the season. In Youpwè, it was characterized by a peak between 12.00 pm to 02.00 am while in Manoka, it was characterized by a plateau around 12.00 pm until 06.00 am. In total, 75% of the bites of *An. coluzzii* in Manoka and 76% in Youpwè occurred after midnight. The biting behavior of *An. coluzzii* was similar in the two study sites. It was observed to bite mainly outdoor compared to indoor in both localities (table 2). In fact, the overall biting density of *An. coluzzii* was 13, 96 b/p/n indoor and 34.76 b/p/n outdoor in Manoka, and 4,75 b/p/n indoor and 24,83 b/p/n outdoor in Youpwè. In both localities, density of *An. coluzzii* was significantly higher outdoor than indoor (*P*<0.001).
3.3 Parity rate
The average percentage of parous females were 39.7% (n = 934) in Manoka and 40.5% (n = 348) in Youpwè. In general, the difference in parity rate of An. coluzzii between Manoka and Youpwè was not significant (P = 0.799). However, parity rate was significantly higher during the dry season in Youpwè (P = 0.015).

3.4 Infectivity of An. coluzzii and malaria transmission
Entomological parameters of malaria transmission in Manoka and Youpwè are shown in table 2. The average of the infection rate of An. coluzzii was 2.58% in Manoka and 5.53% in Youpwè. The average of the Entomological Inoculation Rate was 18.32 ib/p/m in Manoka and 28.18 ib/p/m in Youpwè. Between Manoka and Youpwè, there was no significant difference in the infection rate (P=0.158) and EIR (P = 0.968). However, in both locations, the highest infection rate (4% in Manoka and 6.88% in Youpwè) and EIR (31.55 ib/p/m in Manoka and 51.25 ib/p/m in Youpwè) occurred during rainy season in April (P<0.001).

Table 2: Entomological parameters of malaria transmission in Manoka and Youpwè in December 2013 and April 2014.

<table>
<thead>
<tr>
<th></th>
<th>December 2013</th>
<th>April 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>dHBR</td>
</tr>
<tr>
<td>Manoka</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoors</td>
<td>142</td>
<td>11.83</td>
</tr>
<tr>
<td>Outdoors</td>
<td>202</td>
<td>16.83</td>
</tr>
<tr>
<td>Pooled</td>
<td>344</td>
<td>14.33</td>
</tr>
<tr>
<td>Youpwè</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoors</td>
<td>11</td>
<td>1.83</td>
</tr>
<tr>
<td>Outdoors</td>
<td>39</td>
<td>6.50</td>
</tr>
<tr>
<td>Pooled</td>
<td>50</td>
<td>4.16</td>
</tr>
</tbody>
</table>

N = effective; dHBR = daily human biting rate; IR = infection rate; dEIR = daily entomological inoculation rate; mEIR = monthly entomological inoculation rate

4. Discussion
At Manoka and Youpwè, aggressive Culicidae fauna consists of three genera: Anopheles, Culex and Aedes. The frequency of the genus Anopheles is significantly higher in Manoka than in Youpwè, and vice versa for the frequency of Culex. The predominance of the genus Anopheles in Manoka could be due to the rural character of the island with natural water collections favorable for the development of Anopheles larvae. The predominance of culicinae in Youpwè could be due to uncontrolled urbanization characterized by unsafe and proliferation of waste water collections, which favors the development of the genus Culex larvae to the detriment of the Anopheles genus. These results are in agreement with those of Darriet et al. [19] in Burkina Faso and Antonio Nkondjio et al. [20] who observed a dominance of Culex in Douala. The presence of An. coluzzii in the urban area of Youpwè is in agreement with Gimnioneau et al. [21] and Kamdem et al. [22]. The observed Anopheles diversity in both localities was low. In fact, only three species were collected on the quarantine known in Cameroon [23]. This low Anopheles diversity could be due to the salty character of breeding sites. Moreover, Manoka has another time made object of oil exploitation. Only An. coluzzii has capabilities to adapt to such conditions [24]. The predominance of this species to the detriment of An. gambiae which is absent is due to its tolerance to salinity. This is consistent with previous works in Cameroon that highlight the prevalence of this species in the coastal region [25, 26]. An. melas were only collected in island area. This observation confirms that of Antonio Nkondjio et al. [27] showing that this species is present only in the coast. In fact, An. melas is a species of halophilous natural environments of the West African coastal zones i.e. mangroves. The aggressivity of Anopheles is significantly higher in Manoka than in Youpwè; that may be due to the fact that rural areas are more favorable to the proliferation of Anopheles [24]. Night biting cycles of An. coluzzii observed in Youpwè is in agreement with previous studies in Cameroon [2, 20]. The one observed in Manoka, different from that observed on the island.
of Bioko by Cano et al. [29], would be in connection with the population’s habits. In fact, the population mainly practise fishing during the night and are back in the second half of the night. In this moment, the host is available in significant quantity for the vector. In both localities, an inhabitant receives the majority of bites during the second half of the night according to the observations made in other studies [4, 20]. Whatever the ecological zone, the aggressivity of Anopheles was significantly higher at the beginning of the rainy season than during the dry season. Rainfall favors the larval development by creating the breeding sites and thus supporting the production of adults, but also supporting the biting activity and the reproduction of adult because of optimum temperature and hygrometry [29]. An. coluzzii is exophagic in both localities. This trend exophagy is an adaptation of the vector to the habits of human populations. In fact, in both localities, human populations are mainly fishermen. Preparatory activities for fishing are largely held by night, involving a late human presence outside the houses. This favors the exophagic behavior of Anopheles which is also subjected to the repulsive effect of nets (long lasting insecticidal nets) present in 95% of households. The exophagic behavior of An. coluzzii in both localities leads to associate other vector control skills to the use of nets like cleansing of the environment.

The level of malaria transmission is similar between the two localities. Our findings contradict those of Akogbeto et al [29] who reported that malaria transmission is lower in urban areas than in rural areas in Benin. In reality, high anopheles number in Manoka is offset by the high level of sporozoite index in Youpwè to balance the transmission in both localities. The level of malaria transmission in Youpwè is however relatively high compared to other reports from similar urban areas [20, 28, 30]. An. melas were not found infected with P. falciparum. This confirms earlier observations made in Cameroon where this species has never been found infected [27]. However, this species has been found weakly infected with P. falciparum in the coastal areas of Benin by Akogbeto and Romano [31] who reported a close relationship between An. melas and animals which, according to them, is the fundamental reason for its low infectivity.

The parity rate of An. coluzzii was higher during the dry season. This observation can be explained by the rarity of the larval breeding sites during this season. In fact, the rarity of the larval breeding sites during this season force mosquito to retain their eggs in the ovaries while waiting for the ideal conditions [32]. Thus, this rarity of egg-laying justifies a growing old population. However, the repetitive egg-laying during the rainy season, according to the abundance of larval breeding sites enables the emergence of young mosquitoes in great number.

5. Conclusion
Anopheles coluzzii is the malaria vector on the Manoka Island and Youpwè mainland area of Douala. These findings are of great importance for the malaria vector surveillance and for targeting malaria elimination in the Manoka Island. The exophagous behaviour of An. Coluzzii leads to associate other vector control skills to the use of nets like cleansing of the environment including larviciding and the destruction of the larval breeding sites around habitations.

6. Acknowledgements
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7. References