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# Larval ecology of *Aedes* mosquitoes and risk of arbovirus transmission at the port of San-pedro (Côte d'ivoire)

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## Abstract
Mosquitoes of the genera *Aedes* are vectors of arboviruses. Through trade at the entry points, they spread to new areas where they can cause arbovirus epidemics. The lack of vaccine and treatment for most arboviruses makes vector control the only means of prevention. This study aimed to determine the level of infestation of *Aedes* mosquitoes and the risk of arbovirus by describing their larval ecology. All water containers found in activity and residential areas of the port of San Pedro were inspected for immature mosquitoes once a month from March to July 2018. Out of 227 identified potential breeding sites, 102 (44.93%) contained *Aedes* larvae. Abandoned containers constituted 53.92% of the sites colonized by *Aedes* and represented 73.43% of productivity. Sites associated with turbid water, organic debris, and those with vegetation around were more preferred by *Aedes* for oviposition. The house (20.80%), Breteau (27.20%) and Container (44.93%) Indices indicated a high level of entomological risk on the WHO density scale for both the activity area and the residential area. This study shows that *Aedes* uses more anthropogenic sites preferably abandoned containers and that its presence constitutes a risk of transmission of arboviruses in the port of San Pedro.

**Keywords:** *Aedes*, mosquitos, ecology, arbovirus, San-Pédro, Côte d'Ivoire

## Introduction
Mosquitoes of the genera *Aedes*, notably *Aedes aegypti* (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1894), are highly invasive. These two species are a major public health concern, as they are the main vectors of the most important arboviral diseases, including yellow fever, dengue, chikungunya, and Zika [1, 2]. The current incidence of these diseases is increasing alarmingly due to the ability of these mosquitoes to survive unfavorable periods, thanks to their eggs which are resistant to desiccation after being transported over long distances [3].
Globalization, industrialization, and the expansion of port cities are factors in the spread and proliferation of *Aedes* mosquitoes in countries [4]. The introduction of *Aedes albopictus* in Italy [5], the USA, Brazil [6], and South Africa [7] has been possible through the transport of old tires by ship [4]. The introduction and settlement of mosquitoes in a new environment occasioned the emergence of autochthons arboviruses. Thus the emergence of dengue and chikungunya cases has been observed following the introduction of *Aedes albopictus* in Italy, France, Croatia [8], Cameroon, Gabon, and Equatorial Guinea [9].
In Côte d'Ivoire, since the joint circulation of yellow fever and dengue three viruses in 2008 in Abidjan [10], arboviruses cases have been continuously recorded in several localities of the country between 2010 and 2019 [11, 12, 13,14]. In addition, entomological surveillance initiated at the port of Abidjan in 2009 revealed the presence of *Aedes albopictus* [15]. This species also was highlighted between 2013 and 2014 in the commune of Treichville, home to the autonomous port of Abidjan [16]. The increase in the potential for transmission of vector-borne diseases is linked develop the speed and scale of trade [4]. Therefore, it was crucial to extend surveillance of invasive *Aedes* mosquitoes in Côte d'Ivoire's second port city to assess the health situation and develop vector control measures. In the absence of curative treatment for arboviruses and effective vaccines, except for yellow fever, environmental and ecosystem management remains an important strategy to prevent the introduction of exotic vectors [4].

In addition, according to the International Health Regulations, international entry points to countries such as seaports, airports, railways, and bus stations must be vector-free within 400 meters [17]. It is in this context that a study was undertaken in 2018 at Côte d'Ivoire's second port, in the city of San-Pedro. The study aimed to know the level of infestation of mosquitoes of the genera *Aedes* and the entomological risk of arboviruses through the description of their larval ecology.

## Material and Methods

### Study area

This study took place in the port area of San-Pedro (4° 44′ 41″ north and 6° 38′ 23″ west), located in southwestern Côte d'Ivoire [18] at 368 km from the city of Abidjan. The climate is subequatorial with two rainy seasons (March to June and September to November) and two dry seasons (December to February and July to August). The average annual rainfall range from 1,200 to 1,500 mm, and the annual temperature varies from 24 to 27 °C [19]. With a surface area of 2,000 hectares, the activity zone of the port of San-Pedro is composed of three sectors, including the commercial port, the fishing port, and the industrial and commercial [20] Largely activities are dominated by exports with average annual growth rates of 11.31% [21] This traffic is with Europe (67%), America (25%), and Asia (8%) [19]. In this port area, six sites were selected, including two (DG quarters and Rade quarters) in a residential area and four (SEPBA, fishing port, Space Under Customs, health center) in the activity area (Figure 1). All the (25) dwellings in the DG (5) and Rade quarters (20) were included in the study as these quarters contained very few.


**Fig 1:** Map of the San Pedro port area showing collection sites

### A sampling of mosquito larvae

All water containers found in activity and residential areas of the port of San Pedro were inspected for immature mosquitoes once a month from March to July 2018. Breeding sites were identified based on the presence (positive) or absence (negative) of larvae. For each breeding site, its nature, and factors influencing its positivity (presence of vegetation, presence of organic debris, exposure to sunlight, and water quality) were recorded. The larvae and pupae were then collected from the positive containers and stored in jars labelled according to the area and nature of the breeding site. These immature stages were sent to the insectarium of the National Institute of Public Health (NIPH). At the insectarium, L3, L4 stage larvae, and pupae were counted. Containers were classified according to criteria related to human habitat and their mode of impoundment [22] into water storage containers (WSC), abandoned containers (AC), tires, natural breeding sites (NBS), and other breeding sites (OBS). These immature populations were reared to the adult stage and identified to the genera and species level using the keys of [23, 24].

### Data analysis

Data were analyzed with SPSS 25.0 software. Fisher's exact test was used to compare the proportions of breeding sites in the different sites. A bivariate analysis was used to identify. factors influencing the positivity of breeding sites. The productivity of each breeding site was estimated according to WHO [22]. The usual larval indices (Container, Habitation, and Breteau) were considered to assess the level of risk on the WHO density scale: "house" index (HI) = percentage of houses containing at least one positive *Ae. aegypti* site, "container" index (CI) = percentage of containers containing larvae of *Ae. aegypti* larvae, "Breteau" index (BI) = the number of positive sites per 100 houses visited [25]. According to WHO [26], the risk of an epidemic is low when container index < 3%, house index < 4%, and Breteau index < 5. It is moderate when container index ≥ 3%, house index ≥ 4, and Breteau index ≥ 5. It is high when container index ≥ 20%, house index ≥ 35, and Breteau index ≥ 50 [25].

## Results

### Culicidae fauna obtained after rearing larvae collected at the port of San Pedro

A total of 1453 mosquitoes, including 710 (48,86%) females and 743 (51,14%) males, were obtained after larval rearing. These mosquitoes belong to three genera (*Aedes*, *Anopheles*, and *Culex*) and six species (*Ae. aegypti*, *An. gambiae*, *Cx. fraseri*, *Cx. nebulosus*, *Cx. quinquefasciatus*, and *Cx. rima*). *Aedes aegypti* accounted for 67.38% of the emergences, of which 49.23% were females, followed by *Cx. quinquefasciatus* (31.18%) with 49.45% were females (Table 1).

**Table 1:** Culicidian fauna obtained after rearing larvae collected at the port of San Pedro from March to July 2018

| Species | Activity area | | Residential area | | Total | |
|---|---|---|---|---|---|---|
| | Females | Males | Females | Males | Females | Males |
| *Aedes aegypti* | 278 | 233 | 204 | 264 | 482 | 497 |
| *Anopheles gambiae* | 2 | 2 | 0 | 0 | 2 | 2 |
| *Culex fraseri* | 0 | 10 | 0 | 0 | 0 | 10 |
| *Culex nebulosus* | 0 | 0 | 1 | 0 | 1 | 0 |
| *Culex quinquefasciatus* | 89 | 88 | 135 | 141 | 224 | 229 |
| *Culex rima* | 0 | 0 | 1 | 5 | 1 | 5 |
| Overall total | 369 | 333 | 341 | 410 | 710 | 743 |

### The nature of the breeding sites

A total of 227 potential breeding sites consisting of abandoned containers (39.21%), water storage containers of less than 15 Litres (18.94%), tires (17.62%), other breeding sites (14.10%), water storage containers of more than 15 Litres (7.93%) and natural breeding sites (2.20%) were collected (Table 2). Of these potential breeding sites, 110 (48.45%) were positive, whose 102 (92.72%) were colonized by *Aedes aegypti*. In the activity area, 60.78% (n = 62) of the *Aedes aegypti* breeding sites were identified compared to 39.21% (n = 40) in the residential area. More than half of the *Aedes aegypti* breeding sites (53.92%) were abandoned containers. In both activity and residential areas, these abandoned containers accounted for more than half of the *Aedes aegypti* breeding sites (50%) and (60%) (Table 3). In addition, these abandoned containers were the most productive *Aedes aegypti* breeding sites pupal, a productivity of 73.43% (Table 4).

**Table 2:** Nature and importance of potential breeding sites identified at the Port of San Pedro from March to July 2018

| Area | Sites | PBS | WSC <15 L | WSC >15 L | AC | Tires | NBS | OBS |
|---|---|---|---|---|---|---|---|---|
| | | | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) |
| AA | SE | 57 | 1(0.44) | 3 (1.32) | 15 (6.61) | 21 (9.25) | 5 (2.20) | 12 (5.29) |
| | FP | 46 | 5 (2.20) | 2 (0.88) | 25 (11.01) | 7 (3.08) | 0 (0.00) | 7 (3.08) |
| | SUC | 20 | 0 (0.00) | 1 (0.44) | 5 (2.20) | 2 (0.88) | 0 (0.00) | 12 (5.29) |
| | CS | 13 | 0 (0.00) | 0 (0.00) | 12 (5.29) | 1 (0.44) | 0 (0.00) | 0 (0.00) |
| | Total | 136 | 6 (2.64) | 6 (2.64) | 57 (25.11) | 31 (13.66) | 5 (2.20) | 31 (13.66) |
| RA | DG | 24 | 3 (1,32) | 2 (0.88) | 12 (5.29) | 6 (2.64) | 0 (0.00) | 1 (0.44) |
| | RC | 67 | 34 (14.98) | 10 (4.41) | 20 (8.81) | 3 (1.32) | 0 (0.00) | 0 (0.00) |
| | Total | 91 | 37 (16.30) | 12 (5.29) | 32 (14.10) | 9 (3.96) | 0 (0.00) | 1 (0.44) |
| Overall total | | 227 | 43 (18.94) | 18 (7.93) | 89 (39.21) | 40 (17.62) | 5 (2.20) | 32 (14.10) |

PBS: Potential Breeding Sites; WSC: Water Storage Containers; AC: Abandoned Containers; NBS: Natural Breeding Site; OBS: Other breeding sites; AA: Activity Area; RA: Residential Area; FP: Fishing Port; SE: SEPBA; SUC: Space Under Customs; HC: Health Center; DG: DG Quarters; RC: Rade Quarters

**Table 3:** Nature and importance of *Aedes* breeding sites identified at the Port of San Pedro from March to July 2018

| Area | Site | PBS | ABS | WSC <15L | WSC >15L | AC | Tire | NBS | OBS |
|---|---|---|---|---|---|---|---|---|---|
| | | N | N | N (%) | N (%) | N (%) | N (%) | N (%) | N (%) |
| AA | SE | 19 | 16 | 0(0.0) | 1 (6.25) | 5 (31.25) | 6 (37.50) | 2 (12.50) | 2 (12.50) |
| | FP | 30 | 27 | 3 (11.11) | 0 (0.0) | 16 (59.26) | 5 (18.52) | 0 (0.0) | 3 (11.11) |
| | SUC | 11 | 10 | 0(0.0) | 0 (0.0) | 2 (20.0) | 2 (20.0) | 0 (0.0) | 6 (60.0) |
| | HC | 9 | 9 | 0(0.0) | 0 (0.0) | 8 (88.89) | 1 (11.11) | 0 (0.0) | 0 (0.0) |
| | Total | 69 | 62 | 3 (4.84) | 1 (1.61) | 31 (50.0) | 14 (22.58) | 2 (3.23) | 11 (17.74) |
| AR | DG | 15 | 15 | 1 (6.67) | 0 (0.0) | 10 (66.67) | 3 (20.0) | 0 (0.0) | 1 (6.67) |
| | CR | 26 | 25 | 5 (20.0) | 4 (16.0) | 14 (56.0) | 2 (8.0) | 0 (0.0) | 0 (0.0) |
| | Total | 41 | 40 | 6 (15.0) | 4 (10.0) | 24 (60.0) | 5 (12.50) | 0 (0.0) | 1 (2.50) |
| Overall total | | 110 | 102 | 9 (8.82) | 5 (4.90) | 55 (53.92) | 19 (18.63) | 2 (1.96) | 12 (11.76) |

PBS: Positives Breeding Sites; PBS: *Aedes* Breeding Sites; WSC: Water Storage Containers; AC: Abandoned Containers; NBS: Natural Breeding Site; OBS: Other breeding sites; AA: Activity Area; RA: Residential Area; FP: Fishing Port; SE: SEPBA; SUC: Space Under Customs; HC: Health Center; DG: DG Quarters; RC: Rade Quarters

**Table 4:** Productivity of deposits according to their nature in the port of San Pedro from March to July 2018

| Breeding sites | Breedings positives | Breedings to *Aedes* | pupae | Productivity |
|---|---|---|---|---|
| Water storage containers <15L | 12 | 9 | 37 | 11.56 |
| Water storage containers >15L | 5 | 5 | 19 | 5.93 |
| Abandoned containers | 58 | 55 | 235 | 73.43 |
| Tires | 19 | 19 | 20 | 6.25 |
| Other breeding sites | 14 | 12 | 7 | 2.18 |
| Natural breeding sites | 2 | 2 | 2 | 0.62 |
| Total | 110 | 102 | 320 | 100 |

### Factors influencing the presence of *Aedes* larval

Bivariate analysis showed that water quality the presence of organic debris and vegetation around the breeding sites positively influenced the presence of *Aedes aegypti* larvae. *Aedes aegypti* larvae preferred breeding sites with turbid water three times more than those with clear water. While in breeding sites with organic debris and those surrounded by vegetation were respectively 2.45 and 2.01 times more preferred by *Aedes aegypti* for breeding than those without organic debris and vegetation around (Table 5).

**Table 5:** Environmental factors influencing *Aedes aegypti* colonization of roost sites at San Pedro Port from March to July 2018

| Factors | Value | IC 95% |
|---|---|---|
| Sampling area | 0.95 | 0.55-1.62 |
| Water quality | 3.01 | 1.57-5.76 |
| Presence of organic débris | 2.45 | 1.42-4.20 |
| Vegetation around | 2.01 | 1.05-3.84 |
| Sunny | 0.78 | 0.45-1.37 |

### Larvae index risk

The container index was 44.96% in the port of San Pedro. This index was respectively 45.59% and 43.96% in the activity and residential areas. The housing index (HI) and the Breteau index (BI) were estimated only in a residential area. They were respectively 20.80% and 27.20% (Table 6).

**Table 6:** Stegomyian risk index at San Pedro port sites from March to July 2018

| Area | Sites | PBS | BA | HV | UH | HI | CI | BI | WHO Scale |
|---|---|---|---|---|---|---|---|---|---|
| Activity Area | Fishing Port | 57 | 27 | 0 | -- | -- | 47.37 | -- | 9 |
| | SEPBA | 46 | 16 | 0 | -- | -- | 34.78 | -- | 8 |
| | Space Under customs | 20 | 10 | 0 | -- | -- | 50 | -- | 9 |
| | Health Centre | 13 | 9 | 0 | -- | -- | 69.23 | -- | 9 |
| | Total | 136 | 62 | 0 | -- | -- | 45.59 | -- | 9 |
| Residentials Area | Rade quarters | 67 | 25 | 100 | 385 | 17 | 37.31 | 20 | 3-8 |
| | DG Quarters | 24 | 15 | 25 | 85 | 36 | 62.50 | 56 | 5-9 |
| | Total | 91 | 40 | 125 | 470 | 20.80 | 43.96 | 27.20 | 4-9 |
| Overall total | | 227 | 102 | 125 | 470 | 20.80 | 44.93 | 27.20 | 4-9 |

PBS: Potential breeding sites; BA: Breeding *Aedes*; HV: Houses visited; HU: Housing Unit; HI: Housing Index; CI: Containers Index; BI: Breteau Index;

## Discussion

The development of maritime transport, port cities, and international trade, combined with the adaptability of *Aedes* mosquitoes, has increased the risk of their introduction and establishment in countries [3, 4, 27]. This spread of vectors poses a health threat against arboviruses [17]. To know the level of infestation of mosquitoes of the genera *Aedes* and the entomological risk of arboviruses through the description of their larval ecology, this study has been undertaken, at the port of the city of San-Pedro, the second largest port in Côte d'Ivoire.

After larval rearing, we have obtained 1453 mosquitoes belonging to three genera (*Aedes, Anopheles,* and *Culex*) and six species (*Ae. aegypti, An. gambiae, Cx. fraseri, Cx. nebulosus, Cx. quinquefasciatus,* and *Cx. rima*). *Aedes aegypti* accounted for 67.38% of the emergences. This abundance of *Aedes aegypti* would link to urbanization associated with human activities around the port. These have contributed to the reduction of forest galleries in the port area. Our results had in agreement with those of Konan *et al.* [28] and Fafana *et al.* [14] in their studies conducted in Abidjan. In these studies, 78.70% and 96.71% of *Ae. aegypti* obtained after rearing of larvae. Cornet *et al.* [29] found that urbanization changes the species composition of *Aedes* mosquitoes toward dominance of *Aedes aegypti* in urban areas.

The results revealed six categories of sites colonized by *Aedes aegypti*. Abandoned containers and tires constituted 53.92 and 18.63% of *Aedes* breeding sites, respectively. These observed abundances could explain the poor management of solid waste in the port area. In addition, their continual exposure to rainwater could also explain their abundance. Our results corroborate those of Shamal *et al.* [30], who found that in the port area of Goa, *Aedes aegypti* uses all types of anthropogenic breeding sites for its development. Tia *et al.* [31] showed that populations create favorable conditions for mosquito maintenance. Our results corroborate those of Wilson-Bahun *et al.* [32] who found that in Brazzaville, abandoned breeding sites (domestic waste) were more colonized by *Aedes aegypti* and *Aedes albopictus*. These authors mentioned that this proliferation of peri-domestic sites is due to their poor management and unplanned urbanization. At the port of Mumbai, India, Kumar *et al.* [27] found that the high presence of *Aedes aegypti* in fire extinguisher buckets was related to their poor management. The low proportion of water storage containers containing *Aedes aegypti* larvae in the residential area could be related to their proper management in households.

Abandoned containers were 73.43% productive witnessing the strong preference of *Aedes* mosquitoes towards the (CA) in the study area. Alami *et al.* [33] mentioned that the hatching of mosquito species is conditioned by the nature of the larval breeding site. This confirms the preference of *Aedes* mosquitoes to colonize domestic water sources and small water containers exposed to rainwater. Our results corroborate those of Akono *et al.* [34] who showed that in Cameroon, mosquitoes colonize abandoned containers more.

The preference of *Aedes aegypti* for sites containing organic debris, sites with surrounding vegetation, and sites with turbid water is thought to be related to the availability of nutrients at these breeding sites. Leaves from the vegetation surrounding the sites typically fall back into the breeding sites and provide food for the developing larvae. In addition, this organic debris could serve as a hiding place for larvae to escape predators [34]. In Yaoundé, Tedjou *et al.* [36] and in Brazzaville, Wilson

Bahun *et al*. [32] found that *Aedes aegypti* and *Aedes albopictus* larvae colonize more sites associated with organic debris and surrounding vegetation. Barrera *et al*. [37], and Overgaard *et al*. [38] have shown that the high presence of *Aedes* in sites associated with turbid water and the presence of organic debris are related to the amount of nutrients produced by the organic debris for the larvae. was 45.59% and 43.93 respectively in the business and residential areas.
The House Index (HI) and the Breteau Index (BI) were 20.80% and 27.20%. According to the WHO [26] estimate, the risk of an epidemic is moderate in the port of San Pedro. The recorded index values indicate the existence of a sufficient density of *Aedes* to cause an outbreak of arbovirus. Our results corroborate those of Fofana *et al*. [14] who observed a moderate risk level between 4-9 on the WHO density scale with CI = 40.26; HI = 70.9 and BI = 21.3. However, our results differ from those of Bhadauriya *et al*. [4]. At Kandla Port, India, they recorded BI = 11.11 and 4.4, CI = 8.10 and 1.17, and HI = 7.4 and 4.44, respectively, in residential and commercial areas. In Côte d'Ivoire, studies have revealed indices that differ from ours. Konan *et al*. [28] obtained indices between 2 and 5 on the WHO density scale.

## Conclusion
Entomological investigations carried at the port San Pedro have identified six (6) categories of breeding sites, all colonized by *Aedes aegypti*, vector of arboviruses in Côte d'Ivoire. These vectors preferentially use sites consisting of abandoned containers. Breeding sites with turbid water, those with organic debris, and those with surrounding vegetation were more preferable by *Aedes* for breeding. The indices larvae of risk obtained show that the risk of arbovirus epidemics was moderate on the WHO density scale. These findings indicate a need for entomological surveillance and vector control in the port area of San Pedro. We recommend that the port community properly recycle and manage receptacles that contain water after use. Vector control (mosquito control) with specialized teams could significantly reduce the density of culicid populations.

## Declaration of interest
The authors declare that they have no competing interest in relation to this article. All the authors read and approved the final version.

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