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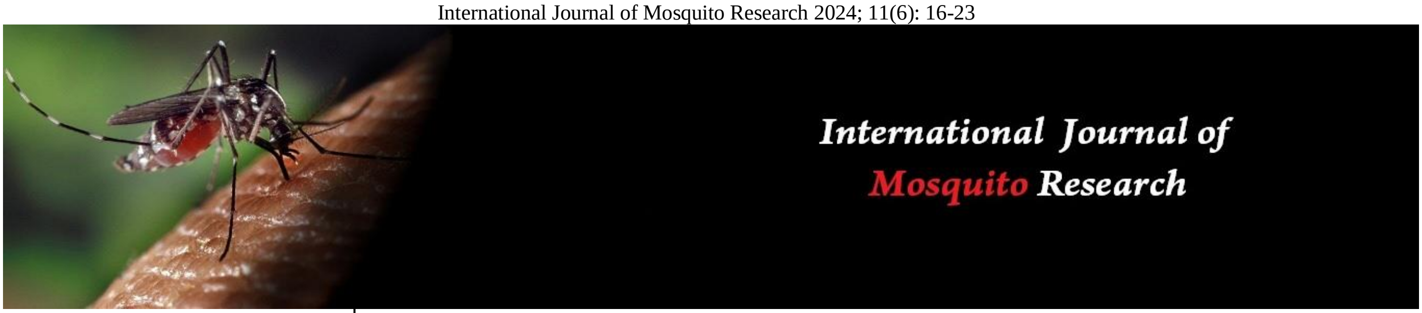

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Characterization of mosquito larval habitats and spatio-temporal variation of culicidae fauna in seaport environment of Cotonou, Benin

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Abstract

Mosquitoes are major vectors of several diseases such as dengue, malaria, and others. Their presence and diversity directly influence epidemic risks. Climate change and urbanization have promoted the expansion of mosquitoes and their adaptation to new habitats. The objective of this study is to characterize the larval habitats of three mosquito species (Aedes, Culex, and Anopheles) in the Port of Cotonou and aims to provide information for mosquito control in a highly anthropized environment. The study was conducted at the Port of Cotonou between June 2023 and May 2024. Monthly captures of adult mosquitoes were carried out using BG sentinel and BG Pro traps at nine sites to measure specific density variation. Additionally, larvae were sampled in natural habitats (puddles, marshes) and artificial habitats (tires, gutters, flower saucers). The larvae were then reared in an insectarium and morphologically identified using specific identification keys. In total, 4,773 mosquitoes belonging to five genera and eight species were captured, with *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles gambiae* being the dominant species. Specific diversity was low, and the most common larval habitats were used tires for *Aedes*, puddles for *Anopheles*, and gutters for *Culex*. A significant ecological plasticity was observed in these species, adapting to unconventional habitats in the absence of their preferred breeding sites. The study reveals a low specific diversity of mosquitoes in the Port of Cotonou, dominated by three species. Effective mosquito management, particularly of larval habitats, is crucial to reducing the epidemic risks of the diseases they vector. Environmental and behavioral measures are recommended to limit breeding habitats, particularly the management of used tires and stagnant water containers.

Keywords: Mosquito larval habitats, Spatio-temporal variation, Culicidae fauna

Introduction

Mosquitoes are arthropod insects, with several species serving as significant vectors of parasites, bacteria, and viruses that cause diseases in humans and animals. Three main mosquito genera are involved in most infections. According to the WHO, mosquito-borne diseases kill 1 million people worldwide and infect nearly 700 million. Dengue, one of the infectious diseases transmitted by mosquito species of the *Aedes* genus and the fastest-growing in the world, affects 100 to 400 million new people each (Brady & Hay, 2020) [3], with approximately 3.83 billion people living in areas conducive to its transmission (Messina *et al*., 2019) [15]. As for mosquitoes of the *Anopheles* genus, certain species are primarily known to be the major vectors of the parasite responsible for malaria, the world's leading vector-borne (Delcus, 2017) [5]. However, in the absence of the primary vectors, *Anopheles* mosquitoes can also transmit other diseases to a lesser extent, such as lymphatic filariasis and Rift Valley fever. Thus, the presence of the vector in an environment is a necessary condition for the transmission of a vector-borne disease, and the resulting epidemic risk largely depends on this presence (Fontenille *et al*., 2009) [11]. Certain species of the *Culex* genus are known to be vectors of West Nile fever by the West Nile virus, Rift Valley fever which primarily affects

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animals but can also infect humans, yellow fever, a potentially deadly viral disease, lymphatic filariasis, a disease affecting the lymphatic system, and to a lesser extent, Zika fever (Zika Virus). Moreover, climate change, the intensification of international trade, and the rapid urbanization observed in recent decades have accelerated the destruction of natural habitats for several species and their expansion into new areas. These species, in their new distribution zones where they are now classified as exotic, can establish themselves, proliferate, and become invasive, leading to the resurgence or emergence of several epidemic outbreaks. However, they represent not only a health threat but also an economic burden for all countries, especially those already financially vulnerable. To address this issue, the International Health Regulations (IHR) (2005), 2006 [17], strongly recommend monitoring exotic species for early detection of invasion hotspots at countries' entry points, particularly in seaports.

Mosquitoes represent the foremost family of invasive species worldwide, with Aedes albopictus being the most prominent (Diagne *et al*., 2021) [6]. Studies conducted across West Africa suggest that this mosquito species may be more anthropophilic than previously assumed (Paupy *et al*., 2009; Kamgang *et al*., 2012) [16, 13]. However, effective control of adult mosquitoes requires an understanding of several factors, such as their diversity, spatiotemporal distribution, and larval breeding habitats (Rejmánková *et al*., 2013) [18]. A thorough understanding of the ecology and larval breeding habitats is essential for developing effective control tools and strategies in a rapidly changing and heavily anthropized environment such as the port. This study aims to characterize the breeding habitats of three major mosquito species in the Port of Cotonou. The various determinants of larval habitats, such as temperature, light, water salinity, hydrology, vegetation, and species interactions, will not be considered in this study, nor will the abundance of larvae and pupae; instead, only the types of larval sites (locations where eggs are laid, where larvae hatch, molt, pupate, and emerge as adults) will be investigated.

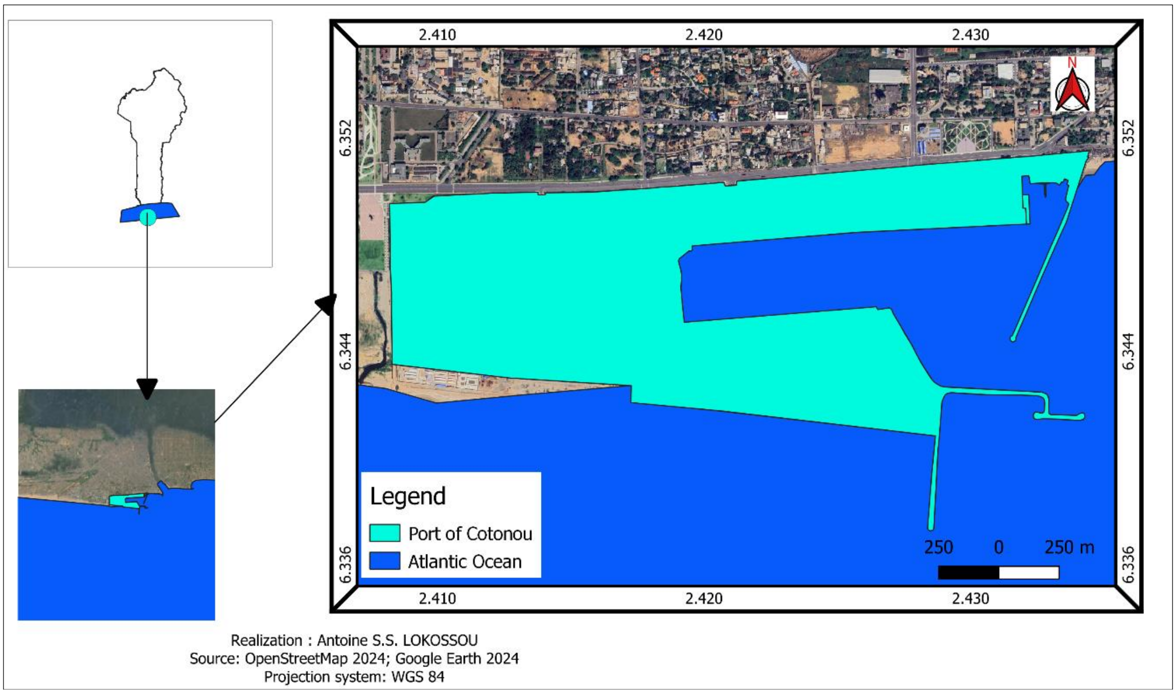
Materials and Methods

Study Area

Our study was conducted in the Port of Cotonou from June 2023 to May 2024. The Port of Cotonou is located in the economic capital (Cotonou) of Benin. The area experiences a humid tropical climate with two rainy seasons and two dry seasons. The major rainy season lasts from March to July, and a minor rainy season from September to October. There is also a minor dry season in August, and finally, the major dry season extends from November to February. Humidity is high throughout the year, often exceeding 80%.

The average annual precipitation is approximately 1,300 mm to 1,500 mm, with an average annual temperature of around 27°C. The Port of Cotonou is the primary entry and exit point for goods in import/export activities in Benin. It also serves as a port for landlocked neighboring countries such as Niger, Burkina Faso, and Mali for their import and export needs. As a result, the Port of Cotonou is a major source of tax revenue for the Beninese government through customs duties on imported goods, contributing to over 60% of the GDP.

To enhance its competitiveness, significant investments are made to modernize port infrastructure, aiming to increase handling capacity and improve the efficiency of port operations. The area, initially covering approximately 400 hectares, is currently undergoing expansion, which includes the construction of new docks and the implementation of computerized cargo management systems.



Realization : Antoine S.S. LOKOSSOU
Source: OpenStreetMap 2024; Google Earth 2024
Projection system: WGS 84

Fig 1: Map of the Port of Cotonou

Mosquito capture using biogent traps

Monthly adult mosquito captures are conducted using BG Sentinel and BG Pro traps equipped with CO_2 production devices at 9 selected sites, which are maintained throughout the study. Four sites include four food storage warehouses near the dock where boats unload, and four other sites located in a vehicle park with diverse origins, plus one site situated between the warehouses and the vehicle park. To achieve high species diversity, captures are conducted from 3:00 PM to 10:00 AM the following morning. This time frame allows for the capture of both diurnal and nocturnal species. Captured mosquitoes are immediately identified using a magnifying glass.

Characterization of Larval Habitats

To characterize the different larval habitats surveyed, we classified these habitats into two categories: natural larval habitats and artificial larval habitats. Natural habitats include: water puddles and swamps, while artificial habitats include tires, tire tracks from vehicles, ditches, gutters, barrels, flower pots, excavator buckets, drainage trenches, and oviposition traps.

Larval Sampling

Larval sampling was conducted at all potential breeding sites during the rainy season. This involved searching for and identifying all potential mosquito larval habitats, followed by collecting larvae and pupae from positive sites using the dipping method. This method entails collecting water from the habitat using ladles with handles appropriate to the type of habitat (large ladles for more accessible sites and smaller ladles for smaller or more restrictive habitats). The collected water is then filtered, poured into jars according to each site, and subsequently georeferenced. It should be noted that habitats with at least one larva or pupa are considered positive. In parallel, oviposition traps were set up from December 2023 to July 2024 to examine the egg-laying behavior of species during the seasons. These traps are checked weekly for the collection of larvae and pupae.

Breeding and Morphological Identification

The larvae and pupae collected from infected habitats are then reared in an insectarium under the required conditions until adult emergence. Morphological identification of the different species is performed using Coetzee *et al*. (2020) for the genus *Anopheles* and Robert *et al*. (2020) for the genera *Culex* and *Aedes*.

Data Analysis

GPS coordinates of the various larval habitats and adult capture sites are georeferenced using the Kobo Collect application. Once data collection is complete, the data is stored in Microsoft Excel for further analysis. The relative abundance of each species is calculated according to (Ali *et al*., 2013) [1], and the results are categorized according to Attaullah *et al*., (2023) [2] as follows: Satellite (RA < 1%), Sub-dominant (RA < 5%), and Dominant Species (RA > 5%). Species biodiversity is assessed using the Shannon diversity index, H’, calculated using the following formula: $H' = -\sum(p_i \cdot \ln p_i)$; p_i is the proportion of individuals of species i relative to the total number of individuals

Results

Temporal Variation in Mosquito Density

A total of 4,773 mosquitoes were captured, representing five (05) genera and eight (08) species (Table 1). June is noted as the month with the highest mosquito density. The Shannon diversity index indicates low species diversity (H’ = 0.36)

Table 1: Seasonal Variation in Mosquito Density at the Port of Cotonou

Species names	Jun-23	Jul-23	Aug-23	Sep-23	Oct-23	Nov-23	Dec-23	Jan-24	Feb-24	Mar-24	Apr-24	May-24
Aedes aegypti	265	89	36	66	172	51	32	57	98	254	103	212
Anopheles gambiae	62	45	7	32	43	39	23	10	15	58	41	51
Anopheles pharoensis	4	6	0	0	9	0	4	5	0	2	3	4
Culex quinquefasciatus	429	351	91	126	179	106	89	191	295	170	436	271
Culex nebulosus	0	0	0	0	5	0	0	0	0	1	0	0
Lutzia tigripes	13	15	1	7	6	2	0	9	5	9	9	8
Mansonia uniformis	5	0	0	3	1	0	0		0	2	0	
Mansonia africana	3	0	0	0	3	0	0	12	0	8	0	14
Total	781	506	135	234	418	198	148	284	413	504	592	560

In terms of relative abundance, three species are dominant: *Culex quinquefasciatus* is the most abundant, followed by Aedes aegypti, and finally *Anopheles gambiae. Lutzia tigripes* is the only species classified as sub-dominant. *Anopheles pharoensis*, *Culex nebulosus*, *Mansonia africana*, and *Mansonia uniformis* are considered satellite species.

Table 2: Relative Abundance and Status of Mosquito Species at the Port

Species names	Number of specimens	Relative Abundance	Status
Aedes aegypti	1435	30,0649	Dominant
Anopheles gambiae	426	8,9252	Dominant
Anopheles pharoensis	37	0,77519	Satellite
Culex quinquefasciatus	2734	57,2805	Dominant
Culex nebulosus	6	0,12571	Satellite
Lutzia tigripes	84	1,7599	Sub-dominant
Mansonia uniformis	11	0,23046	Satellite
Mansonia africana	40	0,83805	Satellite

Quantitative Characterization and Variation of Larval Habitats: Several types of larval habitats were identified, including tires, tire tracks, drainage ditches, water puddles, cans, sumps, jerrycans, flower pots, and excavator buckets. At the Port of Cotonou, the habitat of a species varies according to availability and season. Tires were the most commonly infected habitats with Aedes larvae. Additionally, 100% of the oviposition traps used were positive.

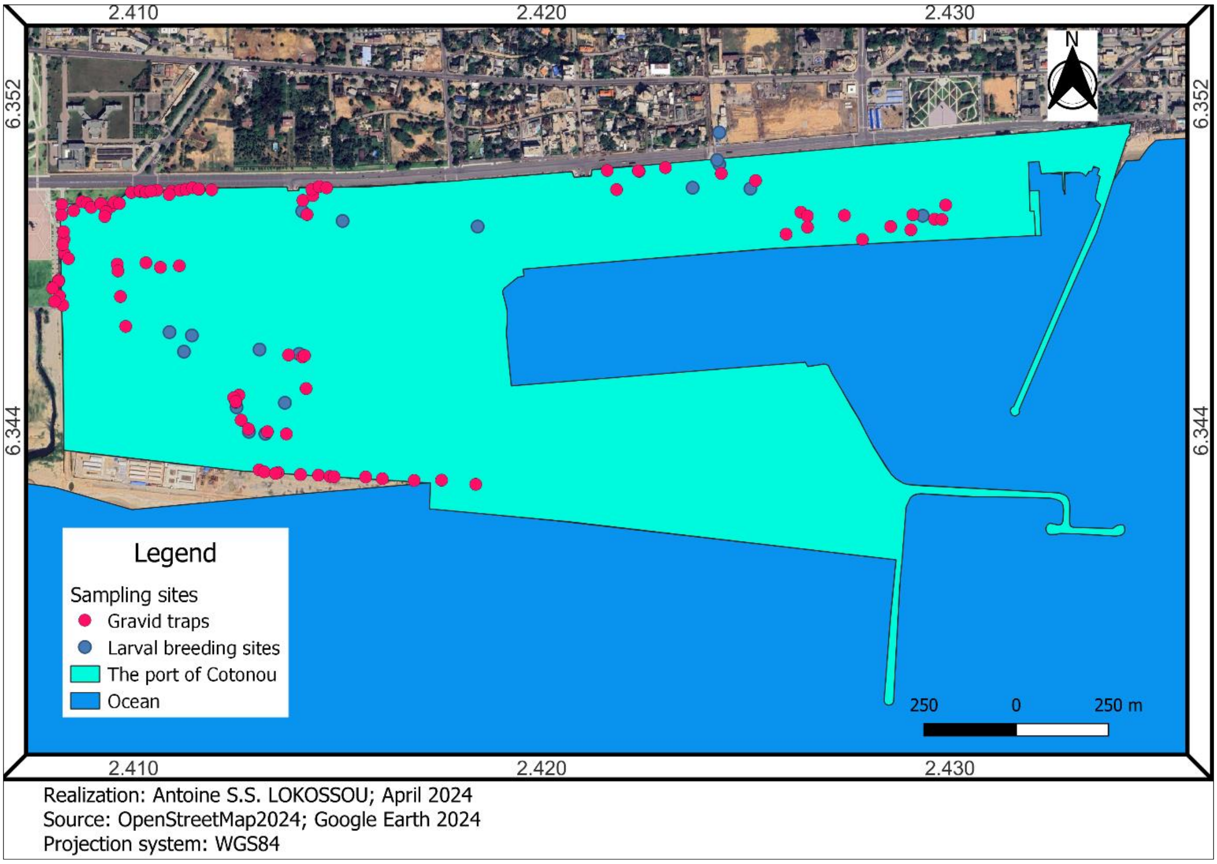


Fig 2: Map Showing the Distribution of Different Breeding Sites

Different Types of Mosquito Breeding Sites

Anopheles

During the multiple larval collections conducted in the port environment, various habitats were found to be infected with *Anopheles*: water puddles, especially prevalent during the rainy season. These waters were relatively clearer, shallow, sunny, and not covered by vegetation (Epopa *et al*., 2020; Fillinger *et al*., 2009) [8, 10]. All these breeding habitats are temporary, dependent on rainfall, and dry up during the dry season. *Anopheles* larvae were also collected in tires as well as in stagnant, polluted, or contaminated waters during the collection of *Aedes* and *Culex* (Rejmánková *et al*., 2013) [18]. It is also noteworthy that *Anopheles gambiae* s.l. larvae were present in oviposition traps during the dry season.

Fig 3: *Anopheles* Breeding Sites. A: Water Retention; B, C: Water Puddles

Aedes

During our larval collections throughout the Port of Cotonou, *Aedes aegypti* was found in habitats consisting of tires, cans, jerrycans, and flower pots.

Table 3: Proportion of Larval Habitats for *Aedes aegypti*

Type of Habitat	Number of Containers Inspected	Number of Positive Containers	Proportion (%)
Used tires	158	136	42,23
Can	53	27	8,38
Tin	11	3	0,93
flower pot saucer	5	2	0,62
Aedes gravid traps	95	95	29,50
Total	322	263	81,67

A high abundance of the species was observed in used tires, particularly at the beginning of the rainy season, when most of the tire piles stored and surveyed were positive. Larvae were also found several weeks after the rains had stopped. In the vast majority of the habitats surveyed, *Aedes aegypti* was the predominant species, except in one site where oviposition traps were placed in an area with sparse vegetation. This finding is consistent with Kamgang *et al* (2010) [13], who observed the presence of *Aedes aegypti* in densely populated areas in Cameroon, while *Aedes albopictus* breeding sites were more surrounded by vegetation (Kamgang *et al*., 2010) [13]. Tires were the most productive breeding habitats. Aside from these well-known habitats for *Aedes* larvae, ecological deviations were observed, notably the presence of *Aedes aegypti* in polluted puddles containing rust from stored scrap metal (Kudom, 2020) [14]

Fig 4: Different Breeding Sites of *Aedes*. A: Tires, B: Flower Pot Saucer, C: Oviposition Trap

Culex

Culex larvae were found in gutters, sumps, and polluted water puddles. They were also collected in oviposition traps during the dry season, which are typically habitats for Aedes. The colonization of these various breeding sites demonstrates the high ecological plasticity of this vector.

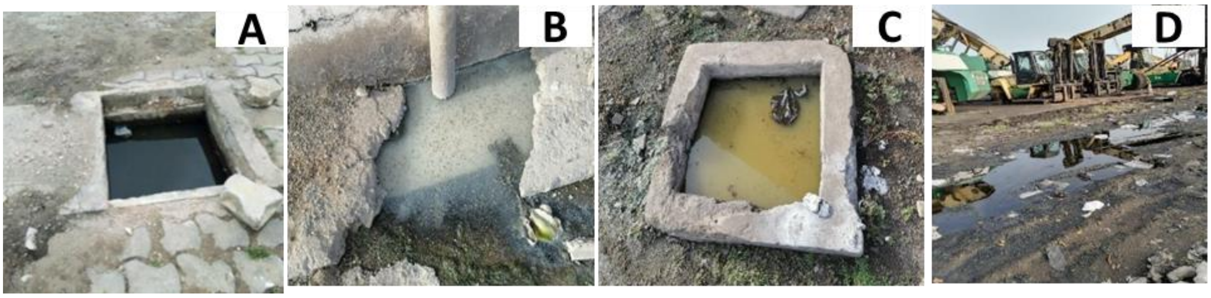


Fig 5: Different Breeding Sites of *Culex*. A: Drainage Ditch, B, C: Sump, D: Stagnant Water

Table 4: Summary of the Different Ecological Habitats of *Aedes*, *Anopheles*, and *Culex*

Species names	Breeding Habitats During the Rainy Season	Breeding Habitats During the Dry Season
Anopheles	water puddle, excavator bucket, ponds, vehicle tire tracks, ditch	Oviposition traps, tires
Aedes	Tires, flower pot saucers, cans	Oviposition traps, tin
Culex	Sumps, drainage ditches, stagnant water	Oviposition traps, sumps

Discussion

Mosquitoes are significant vectors of diseases, and their diversity, abundance, and ecology in the local environment serve as crucial indicators for monitoring the diseases they transmit. In this study, evaluating mosquito biodiversity, eight species were morphologically identified at the Port of Cotonou. According to the Shannon index, this specific diversity is low, explained by the dominance of the same three species across all sampling sites, regardless of the season and time of year. The abundance of these three dominant species (*Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles gambiae*) in this urbanized and industrial environment can also be attributed to their anthropophilic preference and the abundance of hosts. These results are consistent with those of Ferraguti *et al*., (2016) [9], which showed a link between high enthronization of an environment and its poor species richness, particularly regarding invertebrates. Tires were the most productive breeding habitat for *Aedes*. They provide, on one hand, a microclimate characterized by low light necessary for their reproduction (Dom *et al*., 2013) [7], and on the other hand, a high abundance of this habitat in the port area. Additionally, there is an almost constant availability of this habitat (tires), which, from the first rains, allows for rapid reproduction. It is therefore necessary to implement rational management of this primary breeding source for *Aedes* in the Port of Cotonou by storing all used tires in a shed or in a place protected from rainwater after puncturing them, preventing the accumulation of water in the tires (Figure 6)

Fig 6: Storage of used and punctured tires

This study demonstrates a high ecological plasticity of the three studied vectors, which adapt to other habitats in the absence of their preferred habitat. *Culex*, *Aedes*, and *Anopheles* were found in the same breeding site in more polluted water (Figure 3B). During the dry season, when most of the shallow mosquito breeding sites for *Anopheles gambiae* and *Culex quinquefasciatus* dry up, the two species were found in oviposition traps typical for *Aedes* (Figure 4C). This ecological deviation suggests that in highly anthropized environments, effective vector control against a species with a known preferred breeding habitat should also consider potential larval sites of other mosquito species in the environment. This observation aligns with what has been reported in Brazil by Cavalcanti *et al*., (2016) [4] and in some West African countries, where female *Aedes*, in the absence of their usual habitat, adapt to other sites. Under such conditions, controlling *Aedes* larvae requires managing all larval habitats by reducing breeding sources through the destruction of water receptacles and containers. Additionally, treating containers with larvicides (Yougang *et al*., 2020) [19] remains a significant chemical option for reducing larvae and, consequently, adults. In West Africa, where very few studies clearly indicate the trophic (endophagy or exophagy) and resting (endophily or exophily) behavior of *Aedes*, some studies show that *Aedes aegypti* is most commonly endophilic and will require indoor spraying in offices and stores.

Regarding the genus *Culex*, closing off wastewater receptacles and drainage conduits would help reduce both larvae and adults. However, their endophilic and endophagic behavior (Gorris *et al*., 2021) [12] allows for the implementation of indoor spraying campaigns for control. Regular filling of ditches, water puddles, and tire tracks from moving vehicles in the port could potentially reverse the vector density curve for malaria. Control of major disease vectors must be primarily an environmental and behavioral effort before chemical. Continuous awareness campaigns for port users on managing anthropogenic receptacles will reduce *Aedes* breeding habitats and the risk of dengue transmission.

Conclusion

This study on the types of breeding sites found in the Port of Cotonou, in relation to different mosquito genera present there, reveals an ecological deviation in this environment where known natural habitats are absent and species adapt according to the available breeding sites. It also highlights the continuous presence of tires and cans, which are potential breeding sites for *Aedes*. Additionally, it shows the presence of wastewater collectors that facilitate the continuous renewal of the *Culex* cycle

Conflict of Interest: None

Author’s Contributions

Conceptualization ASL, RA, RO, ET, MD, AB, HB, HA-M, YA, EA, R A-E, AD_and_GH; methodology ASL and RA; formal analysis ASL writing original draft preparation ASL; writing-review and editing ASL, RA, RO, HB, HA-M, YA, EA, R A-E, AD_and_GH supervision RA; All authors have read and agreed to the Publisher version of the manuscrit.

Data Availability Statement

The datasets used in this study are accessible upon reasonable request from the corresponding authors.

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