Anthropogenic impacts on *Aedes* mosquito community dynamics in Côte d'Ivoire

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List of Abbreviations

Aaa	*Aedes aegypti aegypti*
Aaf	*Aedes aegypti formosus*
Ae.	*Aedes*
a.m.	Ante meridiem
An.	*Anopheles*
ANOVA	Analysis of variance
AOM	Agriculturally-Occurring Microhabitat
Arbovirus	Arthropod borne virus
BG-Sentinel	Biogents' mosquito trap
BI	Breteau Index
CDC	Centers for disease control and prevention
CI	Container Index
Cq.	*Coquellittidia*
CSRS	Centre Suisse de Recherches Scientifiques in Côte d'Ivoire
Cx.	*Culex*
DALY	Disability-Adjusted Life Years
DENV	Dengue Virus
df	degree of freedom
DZ	Domestic Zone
e.g.	exempli gratia
Er.	*Eretmapodites*
ESKAS	Swiss Government Excellence Scholarships for Foreign Scholars and Artists
etc.	et cetera
FCS	Federal Commission for Scholarships for Foreign Students
FCFA	Franc de la Communauté Financière Africaine
Fi	*Filcabia*
FP	Frequency of *Aedes*-Positive breeding site
f/p/d	female/person/day
GLM	Generalized Linear Model
GPS	Global Positioning System
H.	*Haemagogus*

HI	House Index
i.e.	id est
LMIC	low- and middle-income countries
LSTM	Liverpool School of Tropical Medicine
Ma.	*Mansonia*
MMM	Man-Made Microhabitat
MO	Mean number of specimens per Ovitrap
Na or NA	non-applicable or Non-Applicable
NOM	Naturally-Occurring Microhabitat
p or P	p-value or P-value
PALMCI	Palm of Côte d'Ivoire
p.m.	post meridiem
PP	Proportion of *Aedes*-Positive breeding site
PPM	Proportion of *Aedes*-Positive microhabitat among Macrohabitat
PPSA	Proportion of *Aedes*-Positive microhabitat in the Study Area
OMS	Organisation Mondiale de la Santé
PhD	Philosophiae Doctor (Doctor of Philosophy)
PZ	Peridomestic Zone
RH	Relative Humidity
S	*Sabethes*
SE	Standard Error
sp.	species (singular)
spp.	species (plural)
Swiss TPH	Swiss Tropical and Public Health Institute
SZ	Sylvatic Zone
Tx.	*Toxorhynchites*
UFHB	Université Félix Houphouët-Boigny of Côte d'Ivoire
Ur.	*Uranotenia*
USA	United States of America
WHO	World Health Organization
χ^2	Chi-square

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Summary

Background: The recent emergence, re-emergence and spread of arboviral diseases (e.g. yellow fever, dengue, chikungunya and Zika) that are transmitted by *Aedes* mosquitoes have raised concerns worldwide, and especially in tropical and subtropical regions of the world. In the past several years, Côte d'Ivoire has experienced sporadic, single and dual outbreaks of yellow fever and dengue in the southeastern part of the country, partially explained by high rates of urbanization and intensified agriculture. However, the impacts of these anthropogenic changes (urbanization and transformation of rainforests to vast agricultural areas) on the ecology of *Aedes* arbovirus vectors remain unexplored. Understanding of the impacts of these anthropogenic factors on the ecology of *Aedes* mosquitoes is crucial for predicting and preventing arboviral outbreaks, and developing, optimizing and evaluating existing and novel vector control measures and tools aimed at reducing disease incidence.

Objectives: This PhD thesis aimed to assess the anthropogenic impacts, including effects of urbanization and agricultural land use changes, on *Aedes* mosquito community dynamics in yellow fever and dengue foci in southeastern Côte d'Ivoire. The thesis specifically sought to: (i) explore the oviposition ecology of *Aedes* mosquitoes and *Aedes aegypti* dynamics in variously urbanized settings; (ii) assess the larval ecology of *Aedes* alongside a rural-to-urban gradient; and (iii) evaluate the ecology of *Aedes* mosquitoes along an anthropogenic disturbance gradient in oil palm-dominated landscapes.

Research partnerships: This PhD thesis was carried out within the frame of an existing and productive partnership between the Swiss Tropical and Public Health Institute (Swiss TPH) and the University of Basel, Basel, Switzerland, the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS) and the Université Félix Houphouët-Boigny (UFHB), Abidjan, Côte d'Ivoire and the Liverpool School of Tropical Medicine (LSTM), Liverpool, United Kingdom. The work was funded by Swiss TPH, CSRS and a Scholarship for Foreign Students program (FCS), Bern, Switzerland.

Methods: *Aedes* mosquito eggs, larvae, pupae and adults were collected along a gradient of urbanization (rural, suburban and urban) and different agricultural land uses, including an oil palm-dominated landscape (rainforest, polyculture, oil palm monoculture and rural housing area). Ovitraps were employed, alongside larval surveys and human-baited double-net trap methods from January 2013 to December 2014. *Aedes* immatures were reared in the laboratory until adult stage emerged for subsequent species identification. Socio-ecological data were conjointly sampled.

Results: *Aedes* mosquito ecology significantly varied from rural-to-urban areas and among human-disturbed ecosystems in oil palm-dominated landscapes. A total of 51,439 specimens of *Aedes* mosquitoes belonging to 20 species (*Ae. aegypti*, *Ae. africanus*, *Ae. albopictus*, *Ae. angustus*, *Ae. apicoargenteus*, *Ae. argenteopunctatus*, *Ae. dendrophilus*, *Ae. fraseri*, *Ae. furcifer*, *Ae. haworthi*, *Ae. lilii*, *Ae. longipalpis*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. opok*, *Ae. palpalis*, *Ae. stokesi*, *Ae. unilineatus*, *Ae. usambara* and *Ae. vittatus*) in rural, suburban and urban areas. The highest *Aedes* species richness (18 species) was observed in rural areas. A considerably lower *Aedes* species richness was noted in suburban (seven species) and urban areas (three species). Conversely, the highest *Aedes* abundance was found in urban (n = 26,072 specimens), followed by suburban (n = 16,787 specimens) and rural (n = 8,580 specimens). *Ae. aegypti* was the predominant species in all three types of study areas, with the highest abundance in urban areas (n = 26,072; 99.4%).

Aedes mosquito breeding site positivity rate was higher in urban (2,136/3,374; 63.3%), followed by suburban (1,428/3,069; 46.5%) and rural (738/2,423; 30.5%) areas. Rural areas exhibited a larger array of *Aedes* breeding sites ranging from natural containers (tree holes, coconuts, etc.) to traditional containers (clay pots, calabashes, etc.), and industrial containers (cans, tires, etc.) that hosted several wild species. In contrast, the highest proportions of artificial breeding sites (cans, tires, vehicle bodies, building tools and water storage containers) were found in urban areas where human activities (water storage practices, tire selling and environment management) were favourable to the creation of the breeding sites and proliferation of *Aedes* mosquitoes, mainly *Ae. aegypti*. The predatory larvae of *Eretmapodites*, *Toxorhynchites* and *Culex tigripes* were frequently found associated with the larvae of *Aedes* mosquitoes in rural areas. In all areas, the diversity and proportion of *Aedes* breeding sites, specimens and species were higher in the peridomestic zones and during the rainy seasons.

Aedes mosquito diversity and distribution were strongly associated with agricultural land-use changes. For example, no *Aedes* were found in oil palm monocultures. Conversely, the highest *Aedes* species richness (11 species) was observed in the rainforests, while the highest *Aedes* abundance (n = 28,276; 60.9%) was found in the polycultures. *Aedes* females displayed higher anthropophagy tendency in the polycultures (21.5 females/person/day) and the rural housing areas (4.5 females/person/day), and poor anthropophagy (0.6 females/person/day) in the rainforest. *Aedes* females' host-seeking activities showed bimodal feeding cycles, with interruption from 11:00 to 14:00 hours in the rural housing areas, and a continuous pattern in the polycultures.

Conclusions: The findings revealed that anthropogenic changes influence significantly the ecology of *Aedes* mosquitoes by shaping the breeding sites, and altering the species diversity and abundance towards a predominance of *Ae. aegypti* in urban areas, lack of species in oil palm monocultures, high prevalence of species in polycultures and restriction of wild species in rural areas and rainforests that may serve as bridge vectors. Such *Aedes* species segregation thus suggests a coexistence of several arbovirus transmission cycles: enzootic, epizootic and epidemic. Moreover, the identification of new *Aedes* species in rural and forested areas supports the existence of still unidentified enzootic sylvatic transmission cycles of arboviruses. The high abundance of natural breeding sites (e.g. tree holes) of *Aedes* mosquitoes in the rainforests and rural areas can strongly limit the effectiveness of the removals of discarded containers, and calls for integrated vector management strategies. The evidence generated by this PhD work provides an important contribution to the comprehension of the emergence of arboviral diseases (yellow fever and dengue), *Aedes* vector surveillance and control in the contexts of urbanization and transformation of rainforests into large industrial oil palm monocultures.

Résumé

Impacts anthropogéniques sur la dynamique des populations de moustiques du genre *Aedes* en Côte d'Ivoire

Introduction: Les récentes émergence, réémergence et propagation des maladies arbovirales (fièvre jaune, dengue, chikungunya, Zika, etc.) transmises par les moustiques du genre *Aedes* constituent un problème majeur de santé publique à travers le monde, principalement dans les régions tropicales et subtropicales incluant les pays africains à revenues faibles et intermédiaires. Ces dernières années, la Côte d'Ivoire a été confrontée à de récurrentes épidémies sporadiques, isolées ou doubles de fièvre jaune et de dengue, notamment dans la région sud-est soumise à une urbanisation galopante et vouée à une agriculture intensive. Cependant, les impacts de ces modifications anthropogéniques (urbanisation et la conversion des forêts tropicales en de vastes périmètres agricoles) sur l'écologie des *Aedes*, vecteurs d'arboviroses, demeurent encore inexplorés. La compréhension des effets de ces facteurs anthropogéniques sur l'écologie des *Aedes* est cruciale pour la prédiction et la prévention des épidémies, et le développement, l'optimisation et l'évaluation des mesures et outils existants et nouveaux de lutte antivectorielle afin de réduire l'incidence des arboviroses.

Objectifs: Cette thèse de doctorat visait à évaluer les impacts des transformations anthropogéniques telles que l'urbanisation et les practices agricoles sur la dynamique des populations d'*Aedes* dans les foyers épidémiques de fièvre jaune et de dengue au sud-est de Côte d'Ivoire. Les travaux visaient plus spécifiquement à : (i) explorer l'écologie de l'oviposition des *Aedes* et la dynamique d'*Aedes aegypti* le long d'un gradient d'urbanisation, (ii) évaluer l'écologie larvaire des *Aedes* suivant le même gradient d'urbanisation, et (iii) évaluer l'écologie des *Aedes* suivant un gradient de perturbations anthropogéniques des écosystèmes en zones de palmeraie.

Partenariats de recherche: Cette thèse de doctorat a été réalisée en partenariat avec l'Institut Suisse de Santé Publique et Tropicale (Swiss TPH), Bâle, Suisse ; le Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS), Abidjan, Côte d'Ivoire ; l'Université de Bâle, Bâle, Suisse ; l'Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire ; et L'Ecole de Médecine Tropicale de Liverpool (LSTM), Liverpool, Royaume-Uni. Les travaux ont été conjointement financés par le Swiss TPH, le CSRS et la Commission Fédérale de Bourses pour les Etudiants Etrangers (FCS), Berne, Suisse.

Méthodes: Les œufs, larves, nymphes et adultes des *Aedes* ont été collectés selon des gradients d'urbanisation (milieux ruraux, suburbains et urbains) et de perturbations des écosystèmes en zones de palmeraie industrielle (forêt, polyculture, monoculture de palmeraie, et village) à l'aide de pondoirs-pièges, prospections larvaires et doubles moustiquaires-pièges à appâts humains de Janvier 2013 à Décembre 2014. Les formes immatures des moustiques collectées ont élevées jusqu'au stade adulte au laboratoire pour l'identification des espèces. Les données socio-écologiques ont été également récoltées.

Résultats: L'écologie des *Aedes* a significativement varié des milieux ruraux aux milieux urbains, et entre les écosystèmes anthropogéniques en zones de palmeraie. Au total, 51 439 spécimens d'*Aedes* appartenant à 20 espèces (*Ae. aegypti*, *Aedes africanus*, *Ae. albopictus*, *Ae. angustus*, *Ae. apicoargenteus*, *Ae. argenteopunctatus*, *Ae. dendrophilus*, *Ae. fraseri*, *Ae. furcifer*, *Ae. haworthi*, *Ae. lilii*, *Ae. longipalpis*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. opok*, *Ae. palpalis*, *Ae. stokesi*, *Ae. unilineatus*, *Ae. usambara* et *Ae. vittatus*) ont été collectés en milieux ruraux, suburbains et urbains. La plus forte richesse spécifique des *Aedes* observée en milieux ruraux (18 espèces). La richesse spécifique d'*Aedes* a substantiellement diminué en milieux suburbains (sept espèces) et a été plus faible en milieux urbains (trois espèces). En revanche, la plus forte abondance d'*Aedes* a été observé en milieux urbains (n = 26 072 spécimens), suivis par les milieux suburbains (n = 16 787 spécimens) et ruraux (n = 8580 spécimens). *Ae. aegypti* a été l'espèce prédominante dans les trois types de sites d'étude, avec une plus forte abondance en milieux urbains (n = 26 072 ; 99,4%).

Le plus fort taux de positivité des gîtes larvaires d'*Aedes* a été détecté en milieux urbains (2136/3374; 63,3%), suivis par les milieux suburbains (1428/3069; 46,5%) et ruraux (738/2423; 30,5%). Les milieux ruraux ont présenté la plus grande diversité des gîtes larvaires d'*Aedes* allant des gîtes naturels (creux d'arbres, noix de cocos, etc.) aux gîtes traditionnels (canaris, calebasses, etc.) et industriels (boites de conserves, pneus, etc.) qui hébergeaient diverses espèces sauvages. En revanche, les plus fortes proportions de gîtes artificiels (boites de conserves, pneus, carcasses de voitures, matériaux de construction, et récipients de stockage d'eau) ont été rencontrées en milieux urbains où les activités humaines telles que le stockage d'eau, la vente de pneus, et la gestion de l'environnement étaient favorables à la création des gîtes larvaires et à la prolifération des *Aedes*, particulièrement d'*Ae. aegypti*. Les larves prédatrices of *Eretmapodites*, *Toxorhynchites* et *Culex tigripes* ont été fréquemment associées aux larves d'*Aedes* en milieux ruraux. Dans tous les sites d'étude, la diversité and proportions des gîtes larvaires, spécimens et espèces des *Aedes* ont été plus elevées dans les zones peri-domestiques et durant les saisons pluvieuses.

Les espèces et leurs gîtes larvaires d'*Aedes* étaient quasiment absents dans la monoculture de palmeraie. A l'inverse, la plus forte richesse spécifique d'*Aedes* a été observée dans la forêt (11 espèces), tandis que la plus forte abondance d'*Aedes* a été détectée dans la polyculture (n = 28 276 ; 60,9%). Les femelles d'*Aedes* ont présenté une forte tendance à l'anthropophagie dans la polyculture (21,48 femelles/homme/jour) et les villages (4,48 femelles/homme/jour), et une faible anthropophagie dans la forêt (0,62 femelles/homme/jour). Les cycles d'agressivité horaire ont présenté une allure bimodale, avec une interruption entre 11 heures du matin et 2 heures du soir dans les villages, et une continuité dans la polyculture.

Conclusions: Ces résultats révèlent que les modifications anthropogéniques impactent significativement l'écologie des *Aedes* en modulant leurs gîtes larvaires, et en altérant la diversité et l'abondance des espèces conduisant ainsi à une forte prédominance d'*Ae. aegypti* en milieux urbains, et un confinement des espèces sauvages aux milieux ruraux et forêts qui pourrait servir de vecteurs « ponts ». Une telle ségrégation des espèces d'*Aedes* suggère une coexistence de plusieurs cycles arboviraux: enzootiques, épizootiques et épidémiques. De plus, l'identification de nouvelles espèces d'*Aedes* en zones rurales et forestières augure une probable existence des cycles enzootiques ou sylvatiques d'arbovirus non encore identifiés. L'abondance des gîtes larvaires naturels (creux d'arbres) d'*Aedes* dans les forêts et milieux ruraux peut fortement limiter l'efficacité de l'élimination des gîtes larvaires artificiels et requiert une stratégie de lutte antivectorielle intégrée. Les nouvelles évidences générées par cette étude apportent une contribution significative à la compréhension des épidémies d'arboviroses (fièvre jaune et dengue), et à la surveillance des *Aedes* vecteurs et à la lutte antivectorielle dans des contextes d'urbanisation et de transformation des forêts en de vastes zones agricoles vouées à la monoculture industrielle de palmeraie en Côte d'Ivoire.

1. Thesis outline and research questions

This PhD thesis aims to generate new evidence on the effects of anthropogenic land use changes, particularly urbanization and changing patterns of agriculture as drivers of *Aedes* mosquito community dynamics in yellow fever and dengue foci in Côte d'Ivoire. It is divided into three main research questions:

1. How does urbanization affect the oviposition ecology and the species composition of *Aedes* mosquitoes, and how does it influence *Ae. aegypti* dynamics?
2. How does urbanization influence the breeding sites, the larval ecology and the species dynamics of *Aedes* mosquitoes?
3. How do agricultural land use changes influence the ecology of *Aedes* mosquitoes in industrial oil palm-dominated landscapes?

The thesis commences with an introduction (chapter 2), including a literature review to provide an overview of *Aedes* mosquitoes and the diseases transmitted by these mosquitoes, and the effects of anthropogenic land use changes such as urbanization and changing partterns of agriculture on the ecology of *Aedes* mosquitoes.

The third chapter outlines the background of the thesis, highlighting the identified research needs and the specific objectives. Chapter 4 focuses on the methodology used, including the description of the study area, and the materials used and the methods applied.

From the fifth to seventh chapter, three manuscripts which are published (one paper), revised and re-submitted (one paper), and prepared for submission for the peer-reviewed literature, highlights the finding on the oviposition ecology and the species composition of *Aedes* mosquitoes in variously urbanized settings (chapter 5); larval ecology of *Aedes* mosquitoes alongside a rural-to-urban gradient (chapter 6); the abundance, distribution, host-seeking behaviours of *Aedes* mosquitoes along anthropogenic disturbance gradient in oil palm-dominated landscapes (chapter 7).

Chapter 8 presents the overview of the main findings and shows how the results fit into the Swiss Tropical and Public Health Institute (Swiss TPH) nexus of innovation, validation and application. Moreover, this chapter will discuss the main results in accordance with the specific objectives outlined in chapter 3.

Chapter 9 is the concluding chapter, in which the implications of the findings of this PhD study are discussed. This last chapter also provides specific recommendations to the national and broader international communities. Finally, the topics for future study relative to the open research needs that will extend our knowledge are outlined.

2. Introduction

2.1. *Aedes* mosquitoes and arboviruses

2.1.1. Global situation and in Africa

Several *Aedes* mosquito species are involved in the transmission of multiple arthropod-borne viruses (arboviruses), including dengue (DENV1-4) and yellow fever, responsible for major health burdens worldwide [1, 2]. More than 100 arboviruses are known to cause disease in humans [3]. The disability-adjusted life years (DALYs) attributable to yellow fever, Japanese encephalitis, chikungunya, and Rift Valley fever are estimated to range between 300,000 and 5,000,000 [3]. Dengue causes 390 million of cases and 20,000 deaths per year [4]. The economic burden of dengue in 2013 was estimated at US$ 8.9 billion [5]. Despite an effective vaccine, yellow fever causes 200,000 cases and 30,000 deaths annually [6]. In recent years, there has been a dramatic resurgence of dengue fever worldwide [4, 7-9] and re-emergence of yellow fever in some parts of Africa [10]. Yellow fever outbreaks occurred in Angola from December 2015 to October 2016, causing 4,300 suspected cases and 376 deaths, while in Brazil there were 555 suspected cases and 107 deaths in January 2017 [11]. In addition, other arboviruses vectored by *Aedes* mosquitoes, such as dengue [12], chikungunya [13], Rift valley [14] and Zika virus [15] are emerging or re-emerging in Africa, particularly in West Africa [16-19]. More than 90% of the yellow fever cases occurred in sub-Saharan Africa (33 countries), and most of the outbreaks were reported from in West Africa [20]. The yellow fever burden in Africa was estimated for the year 2013 as 130,000 cases with fever and jaundice or hemorrhage, including 78,000 deaths [20]. Between 2011 and 2015, US$ 330 million was used to purchase yellow fever vaccines in endemic countries, including parts of Africa [21]. Studies indicate that mortality and morbidity attributable to yellow fever are underestimated by a factor of 10-500 [22]. Only 78 million of 656 million cases of fevers occurring among the African children are infected with *Plasmodium falciparum*, and the etiologies of other enormous numbers of accurate non-malaria febrile illnesses are poorly defined [23].

2.1.2. Situation in Côte d'Ivoire

In Côte d'Ivoire, yellow fever and dengue occur on epidemic patterns with variable intervals, ranging from a few months to decades between the sporadic outbreaks making predictions difficult. Single and dual epidemics of dengue and yellow fever involving several wild *Aedes* species and the major urban vector, *Ae. aegypti*, have been reported in both rural and urban areas [24]. Sylvatic dengue virus circulation, without human infections, was documented by isolation of DENV-2 serotype from wild *Aedes* vectors, including *Ae. africanus*, *Ae. furcifer*, *Ae. luteocephalus*, *Ae. opok* and *Ae. vittatus* in rural areas in the 1980s [25]. Since then, several sporadic outbreaks of dengue DENV-1, DENV-2 and DENV-3 serotypes and yellow fever sometimes resulting in fatal outcomes have occurred [26-28]. The outbreaks were mostly concentrated within and in surrounding villages and periurban areas of Abidjan, the economic capital and the most densely populated city of Côte d'Ivoire [26, 27], where the number and abundance of *Aedes* species are high [28]. The main vector, *Ae. aegypti*, is resistant to common insecticides [29, 30].

Yellow fever is historically well known as a key factor having forced the transfer of the colonial capital of Côte d'Ivoire from Grand-Bassam to Abidjan in 1899 [31, 32]. Between 1899 and 1903, Grand-Bassam had faced recurrent and severe epidemics of yellow fever that had killed at least half of the European populations, totaling around 100 people, and many native people [31, 32]. Despite this historical background, the resurgence of yellow fever and dengue outbreaks is not presently resolved and their sporadic occurrence creates major public health concerns [32]. Between 2001 and 2007, 1,468 suspected, 41 confirmed and 26 fatal cases of yellow fever were reported. In 2001, the operational cost of the vaccination campaign to combat yellow fever in Abidjan was estimated at FCFA 1.4 billion (approximatively US$ 2.5 million) [33]. During the period of 2007–2001, 111 suspected with 31 confirmed and 43 deadly cases of yellow fever were notified. The incidence of yellow fever gradually increased and peaked in 2011 with 79 cases and 35 deaths. In 2008, 9 cases of yellow fever and 2 cases of dengue DENV-3 were recorded. In 2010, 13 confirmed and 2 fatal cases of yellow fever, and 1 deadly case of DENV-1 were reported. Incomplete vaccination coverage (estimated at 67%), non-immunized people movements and changes in land-use patterns constitute a risk factor [22]. In recent years, the circulation of dengue viruses (DENV-3) among the population through febrile illnesses monitoring was reported in Abidjan in 2008, 2010 and 2011-2012 [34, 35]. A recent outbreak of dengue fever occurred in urban areas within Abidjan in May 2017.

2.2. Transmission cycles of arboviruses

The transmission of arboviruses is supported by three transmission cycles: enzootic, epizootic and epidemic cycles Arboviruses originated from enzootic (sylvatic/jungle) cycles, associated with wild *Aedes* vectors in rural areas (Figure 2.1) [36-38]. Enzootic cycles are linked to urban transmission cycles by a major domestic vector, *Ae. aegypti* [36-38]. Both yellow fever and dengue viruses are medically important flaviviruses transmitted in an urban cycle between humans by *Ae. aegypti*. Yellow fever occurs in enzootic cycles in Africa and the Americas, and dengue occurs in enzootic cycles in Africa and Asia with often epizootic outbreaks in rural settings. The enzootic cycles are maintained in the sylvan reservoir hosts (non-human primates and birds), although several other mosquito genera or species such as *Anopheles* spp. (*An. coustani* and *An. gambiae*), *Culex* spp. (*Cx. perfuscus*, *Cx. pipiens fatigans* and *Cx. thalassius*), *Eretmopidites* spp. (*Er. chrysogaster*, *Er. inornatus* and *Er. quinquevittatus*), *Coquellitidia* spp. (*Cq. fuscopennata*), *Mansonia* spp. (*Ma. africana* and *Ma. uniformis*) have shown their ability to transmit, or have natural infection and/or laboratory competence for arboviruses through the sylvatic cycles in Africa, the majority of the infections is transmitted by *Aedes* species belonging to *Stegomyia* subgenus followed by *Diceromyia* and *Aedimorphus* subgenera [10, 36, 37]. Though all *Aedes* species are potential vectors of arboviruses, the vectors reported are *Ae. africanus*, *Ae. albopictus*, *Ae. aegypti*, *Ae. furcifer*, *Ae. luteocephalus*, *Ae. opok*, *Ae. vittatus*, *Ae. bromeliae*, *Ae. keniensis*, *Ae. neoafricanus*, *Ae. simpsoni*, *Ae. metallicus*, *Ae. lilii*, *Ae. pseudoafricanus*, *Ae. taylori*, *Ae. stokesi*, *Ae. tarsalis*, and *Ae. dentatus* [10, 36, 37].

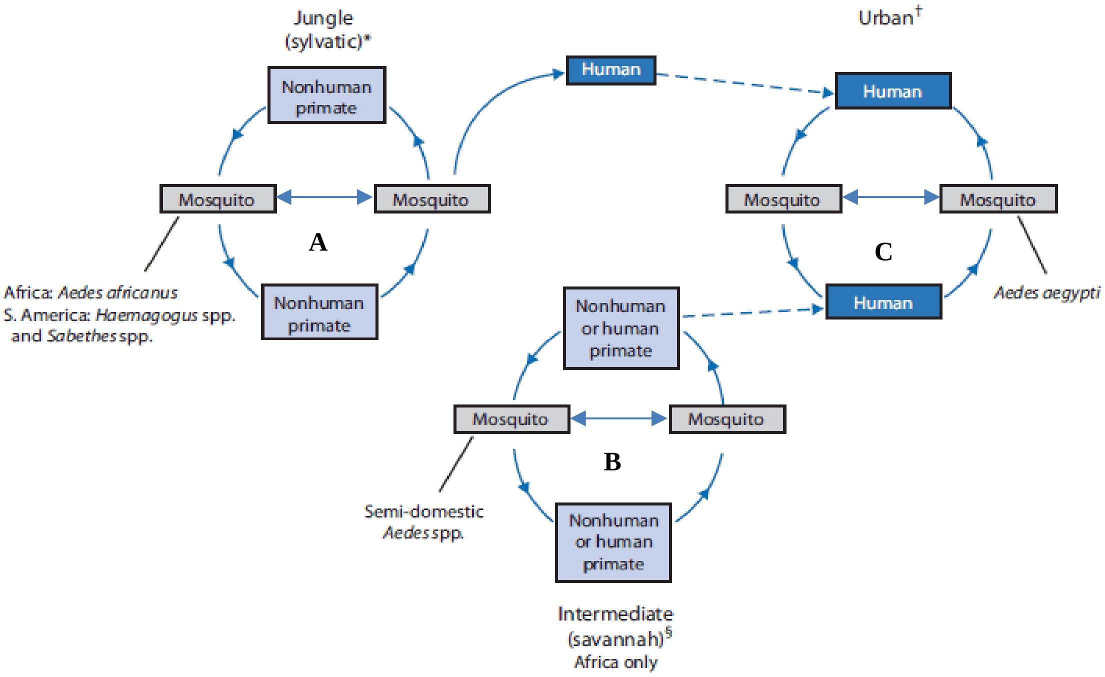

Figure 2.1: Transmission cycles of yellow fever virus in Africa and South America.

The transmission of yellow fever virus is supported by three transmission cycles in Africa (A, B and C) and two transmission cycles in South America (A and C) [24]. In the jungle/sylvatic cycle (A), *Ae. africanus* is responsible for the transmission among non-human primates in Africa, while *H. janthinomys* and *S. chloropterus* feed on infected non-human primates. In the intermediate cycle (B), human activities result in the biting of intermediate cycle *Aedes* spp. vectors. In the urban transmission cycle (C), *Ae. aegypti* transmits yellow fever virus by feeding on viremic humans.

However, in the Americas, le jungle cycle is supported by *Haemagogus janthinomys* and *Sabethes chloropterus* mosquitoes, while *Ae. aegypti* is responsible for the urban cycle [10, 36, 37]. *Ae. aegypti* is well-known to be globally the primary vector of arboviruses [36, 37]. *Aedes* mosquitoes are able to ensure dual horizontal (oral) and vertical (transovarial) transmission patterns for arboviruses [36] and ensure their co-circulation during outbreaks [37].

2.3. *Aede*s mosquitoes and anthropogenic changes

Aedes mosquito ecology has been affected by far-reaching impacts of anthropogenic changes including human-mediated land use changes such as urbanization and changing patterns of agriculture and insecticide uses [39, 40]. Anthropogenic changes can modify at least one of the four components of the arbovirus transmission tetrahedron: host-agent-vector-environment (Figure 2.2). The four components of the transmission tetrahedron are interrelated and changes in individual or combination of elements by anthropogenic activities such as urbanization, changing patterns of agriculture and insecticide uses may enable or disable the arboviral disease transmission [2, 40-42].

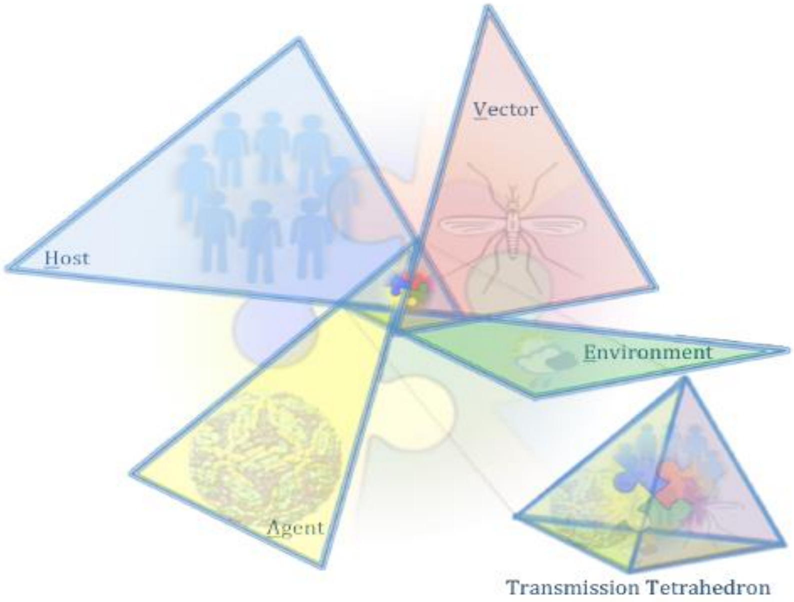

Figure 2.2: Host-agent-vector-environment tetrahedron showing the multidimensional nature of arbovirus transmission [39].

Host-agent-vector-environment tetrahedron has four elements interacting simultaneously. The green base of the tetrahedron represents a suitable environment. The red right side of the tetrahedron illustrates the necessity for an appropriate mosquito vector population to be present and support within the environment. The yellow left side of the tetrahedron displays the agent, arbovirus, which acts as the disease causing pathogens in disease transmission. The blue back side of the tetrahedron representing the host population allows for the completion of arbovirus transmission cycles, assuming temporal and spatial interaction with the other three elements aforementioned.

2.3.1. Urbanization

Urbanization, driven by human population growth and movement, will continue across the globe [40]. Urbanization alters *Aedes* mosquito habitats, hosts and predators, and can increase the permeability of the landscape for urban and anthropophilic mosquitoes and decrease movement for species that are particularly dependent on natural habitats and hosts [40, 41]. Urbanization can be defined as the conversion of forest-to-human habitats, including peri-domestic and domestic landscapes, rural, suburban and urban settings [41], all of which shape and might increase the number of breeding sites, accelerate the development and survivorship and enhance the population size of *Aedes* mosquito [42]. *Aedes* mosquito-transmitted yellow fever, dengue and many other arboviruses have emerged from their sylvatic reservoirs and dispersed globally due to the evolving factors that include anthropological behaviours, trade, transportation land-remediation, and changes in habitat and host availability [43]. The patterns of arboviral disease transmission and its geographic expansion are likely a result of intensive urbanization [2, 9, 37, 44]. The management of human waste and water storage practices in highly populated urban areas is a challenging issue that often leads to an increased availability of discarded containers, water storage receptacles and hosts for the proliferation of *Aedes* mosquitoes [45, 46].

2.3.2. Agricultural land-use changes

Agricultural land-use changes result in the deforestation, forest-degradation and forest-fragmentation that lead to arbovirus emergence or re-emergence [36, 43, 47]. The tropical rainforests are rich in biodiversity of *Aedes* mosquitoes due to their potentials to provide several trees with holes that breed larvae and diverse plant species that foliage can supply for microbial food sources for the larvae [41, 48]. Native forested-landscapes provide the hosts with multiple wild animal species that serve as blood-food sources for adult *Aedes* mosquito females that thus ensure the maintenance of arbovirus circulation among non-human primates (sylvatic cycle) [36, 47]. The rainforest also supply for wide range of opportunities of resting and mating places, shade and nectar sources for *Aedes* mosquitoes [48, 49]. Alterations of the natural land-covers for agricultural purposes, for example oil palm cultivation, result in the losses of *Aedes* mosquito habitats, hosts and predators, and affect the dynamics, abundance, oviposition patterns and host-seeking behaviours of vectors searching for alternative habitats and new blood-feeding sources [41, 50]. Such changes in *Aedes* mosquito behaviours raise the arbovirus outbreak risks in human-inhabited areas [51]. Other cultivations such as rubber plantations and plants with sheathing leaf axils (e.g. bromeliads, banana and taro), and fruit husks (e.g. coconuts) can be important sources of *Aedes* mosquitoes [48, 52]. In addition, containers used to supply water to animal and plant husbandry support *Aedes* mosquito larval growth [53].

2.3.3. Insecticide uses

One of the large-scale environmental impacts that affect the compositional and the distributional patterns of insect communities is the application of organochlorine pesticides (mostly DDT) in agriculture, forestry and public health [40]. The cessation of the widespread use of DDT for agriculture in most parts of the world is believed to be the driving force that facilitated the invasion of *Aedes* mosquitoes and the transmission of arboviruses, such as West Nile, Zika, dengue and chikungunya [40]. Intensive insecticide application alters ecological processes, structuring mosquito communities by its devastating effects on the predators and prey of mosquitoes [40] and induces insecticide-resistance in vectors [54]. *Ae. albopictus* was observed to spread into urban niches vacated by insecticide-induced populations reductions, due to the interspecific competitor *Ae. aegypti* [55]. The spread of DDT, pyrethroids, carbamates and organophosphates over large geographical areas to control onchocerciasis and human and animal trypanosomiasis vectors from 1966 to 1983 in Côte d'Ivoire have induced multiple-resistances of malaria vectors to insecticides [54].

2.3.4. Other abiotic factors

Climate change is expected to increase the geographical distribution of *Aedes* vectors and the transmission of arboviral diseases worldwide [56]. The topographical diversity and distribution of *Aedes* mosquitoes is influenced by meteorological variables, including the climate or the weather components, such as temperature, relative humidity and precipitation [41, 57], shade and sunlight [42] and altitude [58]. The seasonal variations in *Ae. aegypti* and *Ae. albopictus* abundance are strongly associated with rainfall patterns, history, variability and intensity that govern the fluctuations of seasonal flooding-drying cycles [59]. *Aedes* mosquito eggs enter into a dormant stage or diapause to withstand desiccation or wintering periods during the dry or cold seasons, while precipitations flood the breeding sites and increase the abundance of *Aedes* mosquitoes [41]. The levels of temperature and humidity favour or disfavour the egg fertility, hatching rate and desiccation-resistance, larval and adult survival or mortality, and the fecundity, gonotrophic cycles and oviposition, blood-feeding behaviours of *Aedes* females [41, 60]. Heavy precipitation flushes eggs, larval and pupae of *Aedes* mosquitoes from their breeding sites [61, 62]. The distribution of *Aedes* mosquitoes is also governed by the physical and chemical characteristics of the breeding sites, including the material type, color, location, water depth, water clearance, substrate type, shade, detrital and microbial foods, canopy coverage and microclimate (water temperature and relative humidity) [42]. All these factors that influence the distribution of *Aedes* mosquito vectors also shift the transmission patterns of the yellow fever, dengue, chikungunya and other arboviruses [41, 61].

2.3.5. Other biotic factors

Competition, predation, parasitism, sympathy, coexistence, satyrization and invasion play important roles in the *Aedes* mosquito diversity, abundance and distribution and arbovirus transmission [41, 55, 60, 63-65]. Interspecific and intraspecific competitions for food and habitat resources can lead to the decline, displacement (competitive displacement) or exclusion (competitive exclusion) of the inferior competitor and invasion of the superior competitor [41, 63]. Interphyletic competition involving the tadpoles of toad and arachnids also alters the distribution of *Aedes* vectors [55, 63]. Intraguid predation in which the later-instar larvae of *Aedes* mosquitoes prey on the newly-hatched conspecifics and the top-down predation that imply the predators such as *Culex tigripes*, *Corethrella*, *Eretmopodites*, *Toxorhynchites* mosquitoes, can also exert biocontrol and structure communities [41, 63, 66]. *Aedes* females prefer to oviposit in breeding sites containing predator-killed conspecifics because of the microbial byproducts provided by the predation [63]. The parasitism by gregarines affects the biological interactions among *Aedes* mosquito larvae [41]. All these biotic interactions influence the life-history traits and biodiversity of *Aedes* mosquitoes by the biotic facilitation or resistance to invasion, resilience, co-existence and exclusion of communities, and alter the transmission of arboviral diseases [41].

2.4. *Aedes* mosquito life-cycle

Ae. aegypti and other mosquitoes have a complex life-cycle with dramatic changes in shape, function and habitat (Figure 2.3). There is an aquatic phase (larvae, pupae) and a terrestrial phase (eggs, adults) in the life-cycle [67]. After taking a blood-meal, *Ae. aegypti* gravid females lay their around 100-200 eggs per batch on the inner, wet walls of containers with water such as natural tree holes, or artificial containers like barrels, tires, discarded items, etc. The eggs (picture 1) are smooth, long, ovoid shaped and roughly 1 mm long. The eggs can develop within two days or remain at dry or dormant state over one year and immediately hatch one submerged in water. Larvae (picture 2) hatch when water inundates the eggs as a result of rains or the addition of water by people. In the following days, the larvae will feed on microorganisms and particulate organic matter, shedding their skins three times to be able to grow from first to fourth instars. When the larva has acquired enough energy and size and is in the fourth instar, metamorphosis is triggered, changing the larva into a pupa (picture 3). Pupae do not feed; they just change in form until the body of the adult, flying mosquito is formed. Then, the newly formed adult (picture 4) emerges from the water after breaking the pupal skin. The entire life cycle lasts 8-10 days at room temperature, depending on the level of feeding.

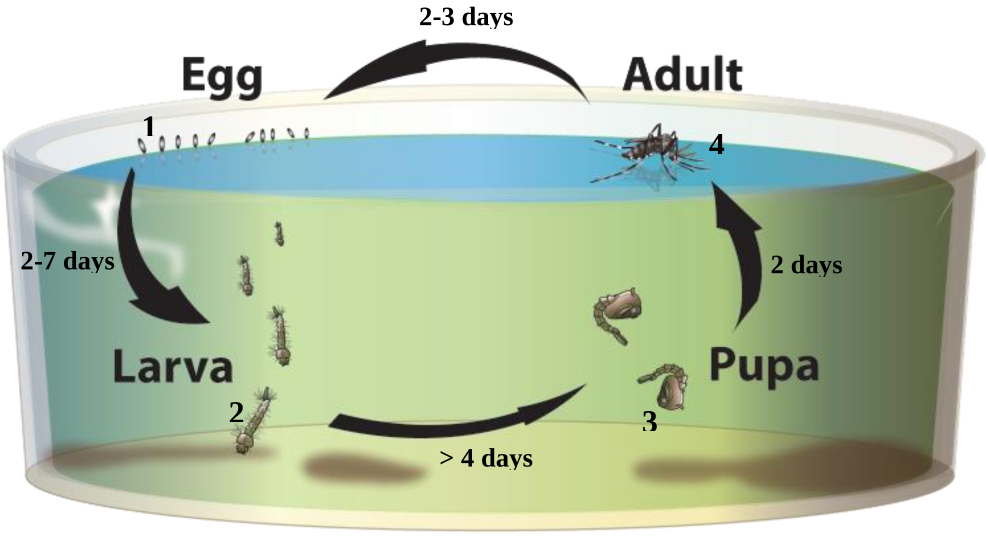

Figure 2.3: *Aedes aegypti* mosquito life-cycle (source: CDC)

2.5. *Aedes* mosquito sampling methods

2.5.1. Egg sampling

As *Aedes* mosquito females oviposit on substrates subjected to flooding, ovitraps are a commonly used method for sampling their eggs [10, 68, 69]. An ovitrap consists of a dark filled container and a thin paddle of wood serving as oviposition substrates [70]. Different materials can be used as a container, including tin cans, glasses, plastic, ceramic and bamboo cups [68]. *Ae. aegypti* and *Ae. albopictus* gravid female prefers ovitrap painted black, red, blue, checkered over white, orange, green, striped or ovicups [71, 72]. Oviposition rough substrates are preferred over smooth [72]. Adding 10% hay or herbal infusions to the water can increase the ovitrap attractiveness [73].

Ovitraps are easy to construct and use, low-cost, low-tech, and highly sensitive in detecting the presence of *Ae. aegypti* in the environment and thus allow to predict the risk of yellow fever and dengue emergence [74]. Contaminated-ovitraps against multiple stages [75], or gravid females [76] of *Ae. aegypti* have been suggested as vector control strategies.

2.5.2. Larval sampling

The larvae and pupae of *Aedes* mosquitoes can be sampled using dippers with different sizes or capacities (50-500 ml), the aquatic nets and flexible collection tube connected to a manual suction pump according to the aperture and the depth of the containers [68, 69]. The larvae are collected by skimming the sampling device through the water at an angle or lowering the device slowly into the water [69]. The collected larvae are cleaned with tap water and the eventual associated predacious larvae such *Cx. tigripes*, *Eretmapodites* and *Toxorhynchites* mosquitoes and other amphibians like tadpoles of toads are removed. The samples are transferred into small plastic tubs and transported to the laboratory [69]. Larval sampling also allows measuring of the yellow fever and dengue outbreak risks and assessing the biological interactions (competition, predation) among *Aedes* mosquito larvae [42, 48].

2.5.3. Adult sampling

Aedes mosquito can be collected by trapping or aspirating the emerging, flying, biting and resting adults using attractant or non-attractant devices such as floating traps, sweep nets, human-landing catches, human or animal-baited double-net traps, indoor insecticide spray, mouth aspirator, BG-Sentinel traps, Backpack aspirator, Prokopack aspirator, light or carbon dioxide traps [68, 69, 74]. These trapping methods show different performances, mosquito species, sex and physiological status of the females (unfed, fed, gravid, parious and unparous) according to the attractant (light, carbon dioxide, animal-bait and human-bait), and the location (indoor, outdoor) and collection period (night, daytime) [68, 69, 74].

2.6. Arboviral disease and *Aedes* vector controls

The absence of specific treatment (the therapy is mainly symptomatic and supportive), and the existence of wildlife reservoir hosts for the arboviruses render elimination difficult or impossible, and then the prevention and the control must focus on vaccination and vector control programmes [36]. Only yellow fever 17D vaccine, considered to be the safest and most efficacious vaccines in use today, and the Japanese encephalitis virus inactivated vaccine licensed for human use are available. Despite considerable efforts are being made for developing and testing vaccines for dengue and West Nile viruses, none have been licensed for human use. Hence the primary means of control and prevention involve vector control using insecticide applications and environment management based on the removals of discarded containers. In the mid-1900s, yellow fever was controlled in Francophone Africa by vaccination, while both yellow fever and dengue were eliminated in the Americas by effective control of the major vector, *Ae. aegypti* [77]. After this great success, vector control programmes failed to maintain control efforts resulting in the return of vectors and viruses and thus the resurgence of yellow fever and dengue [77]. Moreover, the principal vector, *Ae. aegypti*, exhibits resistance to several classes of insecticides [78, 79, 80].

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3. Background of the PhD thesis

3.1. Identified research needs

The recent emergence, re-emergence and the spread of *Aedes* mosquito-transmitted arboviruses such as yellow fever, dengue, Zika and chikungunya, paralleled with anthropogenic changes, have raised concerns worldwide [1-4]. Anthropogenic land use changes due to urbanization and intensified agriculture, and the application of insecticides as public health measure, and in agriculture present, huge challenges on the environment, food security and people's well-being [5]. Anthropogenic impacts, driven by human population growth and movement, are projected to increase substantially in the future across the globe. As a result, the distribution of *Aedes* vectors and the arboviruses they transmit will alter [5].

Since the 1900s, Côte d'Ivoire has been repeatedly facing yellow fever and dengue outbreaks [6, 7]. Several vectors have been involved, namely, *Ae. aegypti*, *Ae. africanus*, *Ae. furcifer*, *Ae. luteocephalus*, *Ae. opok* and *Ae. vittatus* in the rural areas devoted to industrial oil palm agriculture, and *Ae. aegypti* in urban areas [8-14]. A recent outbreak of dengue fever occurred in Abidjan in May 2017. At present, 51.9% of the population in Côte d'Ivoire live in urban areas [15]. The agrarian land is estimated at 64.8% [16]. The effects of anthropogenic changes resulting from land use changes, such as urbanization and intensified agriculture in oil palm plantation areas, on the ecology of *Aedes* mosquitoes are poorly explored. A deeper understanding of these factors is essential for developing, optimizing and applying at scale novel control strategies and tools aimed at reducing arboviral disease transmission.

3.2. Goals and objectives of the PhD thesis

This PhD thesis aims to improve our understanding of how anthropogenic factors, such as land use changes due to urbanization and intensified agriculture in industrial oil palm cultivation areas, influence *Aedes* mosquito community dynamics in yellow fever and dengue co-endemic areas of Côte d'Ivoire. To these ends, the thesis has three main goals:

Goal 1: To improve our understanding of how urbanization influences the oviposition ecology and species composition of *Aedes* mosquitoes in arbovirus foci.

There are four specific objectives:

1. to assess the species composition and dynamics of *Aedes* mosquitoes in a typical rural, suburban and urban areas;

2. to explore the oviposition ecology of *Aedes* mosquitoes in rural, suburban and urban areas;
3. to determine the geographic variations in the oviposition ecology and species composition of *Aedes* mosquites in rural, suburban and urban areas;
4. to determine the seasonal variations in the oviposition ecology and species composition of *Aedes* mosquitoes in rural, suburban and urban areas.
5. to assess the geographic and seasonal dynamics of *Ae. aegypti* species in rural, suburban and urban areas.

Goal 2: To assess how urbanization affects the breeding sites, larval ecology and species composition of *Aedes* mosquitoes in arbovirus endemic areas.

There are five specific objectives:

1. to assess the species composition and dynamics of immature *Aedes* mosquitoes in rural, suburban and urban areas;
2. to characterize the breeding sites of *Aedes* mosquitoes in rural, suburban and urban areas;
3. to explore the biological associations at play among immature *Aedes* mosquitoes in rural, suburban and urban areas;
4. to determine the geographic variations in the breeding sites and species composition of immature *Aedes* mosquites in rural, suburban and urban areas;
5. to determine the seasonal variations in the breeding sites and species composition of immature *Aedes* mosquites in rural, suburban and urban areas.

Goal 3: To deepen our understanding of the impacts of agricultural land use changes on the abundance, distribution and host-seeking behaviours of *Aedes* mosquitoes in oil palm-dominated landscapes.

There are three pecific objectives:

1. to assess the abundance of *Aedes* mosquito species among four macrohabitats: rainforest, polyculture, oil palm monoculture and rural-housing areas;
2. to determine the distribution and the biodiversity of *Aedes* mosquito species among the aforementioned macrohabitats; and
3. to explore adult *Aedes* females' host-seeking behaviours among rainforest, polyculture, oil palm monoculture and rural-housing areas.

3.3. Research partnerships

This PhD thesis is carried out within the framework of a partnership between the Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland; Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS), Abidjan, Côte d'Ivoire; the University of Basel Basel, Switzerland; the Université Félix Houphouët-Boigny of Côte d'Ivoire (UFHB), Abidjan, Côte d'Ivoire; and the Liverpool School of Tropical Medicine, Liverpool (LSTM), United Kingdom. The work was funded by Swiss TPH, CSRS, and a Scholarship for Foreign Students program (FCS), Bern, Switzerland.

3.4. References

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4. Methodology

4.1. Study area

The study was conducted between January 2013 and December 2014 in southeastern Côte d'Ivoire, specifically in Abidjan and Aboisso regions, where several *Aedes* mosquito-transmitted yellow fever and dengue outbreaks have been reported in the last decades [1-8]. The population of Côte d'Ivoire in 2014 was 22,671,331, with the majority living in urban areas (51.8%) [9]. The annual population growth rate is estimated at 5.3%. The economy is mainly based on agriculture, dominated by the production of cocoa, coffee, rubber and oil palm. Abidjan is the economic capital and the most populated town with 4,707,404 inhabitants, while the population of Aboisso is estimated at 307,852 people. The climate is warm and humid and, overall, transitional from equatorial to tropical with four seasons. The seasons are more clearly distinguished by rainfall than temperature. The two rainy seasons are separated by a dry season. The main rainy season extends from May to July, while the shorter occurs in October and November. The main dry season extends from December to April. The average annual precipitation ranges from 1,200 to 2,400 mm. The annual temperature and relative humidity range around 26.5 °C and 78-90%, respectively.

The purpose of the PhD thesis was to assess the effects of anthropogenic land use changes, particularly urbanization and industrialized oil palm agricultural land-use changes on *Aedes* mosquito community dynamics and insecticide-susceptibility in *Ae. aegypti* in yellow fever and dengue foci in Côte d'Ivoire. We therefore selected settings with different levels of urbanization (rural, suburban, and urban). In rural areas devoluted to industrial oil palm plantations, we compared *Aedes* species composition, breeding sites and behaviours in natural rainforests, polyculture crops, oil palm monoculture and housing areas.

4.1.1. Urbanization

For assessing the effects of urbanization *Aedes* mosquito ecology, the study was carried out in three areas located within the traditional arbovirus foci in southeastern Côte d'Ivoire: the villages of Ehania-V1 (geographical coordinates 5° 18 N latitude, 3° 04 W longitude), Blockhauss (5° 19 N, 4° 00 W) and Treichville (5° 18 N, 4° 00 W), representing rural, suburban and urban settings, respectively (Figures 5.1 and 6.1). The selection of the study areas as rural, suburban and urban was mainly based on the population density, state of road, land use, business, public services and vegetation cover.

Ehania-V1 is a rural area with a population density of approximately 48 people/km^2 and unpaved roads. The village of Ehania-V belongs to the district of Aboisso some 140 km east of Abidjan. The residencies are composed of traditional and ordinarily modern houses. This area is surrounded by industrial oil palm plantation (*Elaesis guineensis*) of 11,444 ha and 100 ha of preserved primary rainforest. The rainforest provides vegetation with dense canopy cover and trees, and hosts non-human primates.

Blockhauss is a suburban area with approximatively 750 people/km^2 and paved roads. This setting is located in the periphery of Abidjan bordered in its northern part by Banco National Park with over 3,750 ha of rainforest. The land use comprises a mixture of residential buildings, hospitals and schools. The residencies are ordinarily modern houses and some blocks with flats. Urbanization is underway at high pace.

Treichville is an urban and highly populated area with more than 1,800 people/km^2 and paved roads. This setting is the oldest part of Abidjan and is situated in central of thecity. The density of the population greatly increases during the daytime due to the convergence of people from other municipalities of Abidjan for trading, businesses and sports. The land use is essentially residential, commercial, cultural and sportive buildings, seaport and public services, such as schools and hospitals, filled with green spaces set apart. The residencies are mostly composed of blocks of flats and some ordinarily modern houses. Urbanization is very much advanced and there is hardly any additional space for construction of new houses and other insfractructure.

4.1.2. Agricultural land-use changes

The study was carried out in areas of Ehania-V1 comprising the villages of Ehania-V1, Cité-cadre, Akakro and small villages belonging to the Sud-Comoé region located in southeastern Côte d'Ivoire (Figure 7.1). The villages are situated at the interface between the industrial and traditional agricultural exploitations. The industrial exploitations are devoted to the monoculture of oil palm covering 11,444 ha managed by an agro-industrial unit PALMCI. The industrial part also has a preserved rainforest of 100 ha. The traditional exploitations are the systems of polyculture comprising oil palm trees, rubber trees, banana, taro, bromeliads and cocoa. Several small villages averaging 20 people are dispersed in the traditional lands. The preserved rainforest and traditional explorations host threes, bamboo, and diverse animal species (primates and birds). The population is composed of people working for agro-industrial unit PALMCI and on their own lands for subsistence farming.

4.2. Methods

4.2.1. Study design

For the study focusing on the effects on urbanization on *Aedes* oviposition and larval ecology, the rural area was divided into three ecozones: domestic, peridomestic and sylvatic **(paragraphs 5.3.2 and 6.3.3).** The suburban and urban areas were divided into domestic and peridomestic zones because there were no sylvatic zones. According to Cordellier et al. [10], domestic zone refers to the human-inhabited space, the peridomestic zone covers the vegetated environment surrounding the domestic zone in which humans usually discard artificial items that serve as breeding sites for *Aedes* mosquitoes, the sylvatic zone is primarily a wild environment free of discarded containers that host forests with natural containers (e.g. tree holes) and wild animals.

In the study assessing the effects of agricultural land uses on *Aedes* mosquito ecology, the study area was divided into four macrohabitats according to the landscape cover **(paragraph 7.3.3)**: rainforest, polyculture, oil palm monoculture and rural housing area (Figures S7.1 and Table 7.1). The rainforest referred to the area covered with dense forest showing natural ecosystem with strong canopy coverage and comprising big threes, creepers, fixed masses of bamboo (*Bambusae*), and wild vertebrate animals such as non-human primates, birds and reptiles. The polyculture was defined as an area covered with mosaic of multiple crops growing in the same place. As oil palm monoculture, we considered the area covered uniquely with oil palm trees. The rural housing areas covered human-inhabited space comprising buildings such as houses and and other social buildings.

Aedes mosquitoes (Figures 11.1 and 11.2), predatory species (Figure 11.3) and other non-*Aedes* mosquitoes (Figure 11.4) were collected as eggs, larvae, pupae or adults. Entomological and socioecological data were sampled and processed using WHO standard sensitive sampling methods (Figure 4.1).

4.2.2. Aedes *egg sampling*

Aedes spp. eggs were sampled using the standard WHO ovitrap method [10-13]. Ovitraps were made of bamboo cut (bamboo-ovitraps) or metallic can cut (metallic-ovitraps) to imitate natural and artificial breeding sites of *Aedes* mosquitoes, respectively (Figures 4.1). Metallic-ovitraps were covered with black paint, while bamboo-ovitraps kept their original colors. Each ovitrap type had 400 cm^3 volume, and was filled at ¾ full with distilled water mixed with rainwater and 10% infusions of herbs (*Panicum maximum*) to increase the attractiveness of the ovitraps [10, 14-16]. A 5 x 7 x 0.3 cm paddle made of hardboard, rough on one side

Bamboo-ovitrap **Metallic-ovitrap** **Larval survey** **Human-baited double trap**

***Aedes* egg hatching** ***Aedes* larvae rearing** ***Aedes* adult rearing**

***Aedes* egg counting** ***Aedes* larvae counting** ***Aedes* adult identification**

A: Entomological methods

Questionnaire interview **Household inspection** ***Aedes* larvae sampling**

B: socioecological methods

Figure 4.1: Entomological and socioecological methods

and serving as oviposition substrate, was plunged in each box and left for one week during each survey. All mosquito samples were stored separately in plastic boxes and transferred in a cool box to a field laboratory.

4.2.3. Aedes *larval survey*

Readily visible and accessible containers in the selected households and surrounding premises were examined for the presence of water and mosquito larvae. In a preliminary survey, existing larval breeding sites, such as natural and artificial cavities or containers with a potential to contain water were kept in an inventory, characterized and assigned a unique label (Table 6.1 and Figure S6.1, and Table 7.1 and Figure S7.1). Microhabitats were examined for the presence of water and immature stages of mosquitoes. If mosquito larvae and/or pupae were present, the content of microhabitat was completely removed using the WHO standard equipment adapted to the aperture and the depth of microhabitats. A flexible collection rubber tube connected to a manual suction pump was used to sample water from bromeliads and bamboo holes. Ladles of 350 ml capacity were used to collect immature mosquitoes from larger breeding sites (e.g. tree holes, recipients, tires and puddles). Non-*Aedes* mosquito larvae such as *Anopheles* spp., *Coquelitidia* spp., *Culex* spp., *Eretmapodites* spp., *Filcabia* spp., *Toxorhynchites* spp. and *Uranotenia* spp. were also recorded. The predacious larvae of mosquitoes, such as *Cx. tigripes*, *Eretmapodites* spp. and *Toxorhynchites* spp. were removed from the samples to avoid predation on the other species and preserved separately. The microhabitats sampled were refilled to their initial volume with the original water, and completed with tap water. The presence of shade, predators, plant leaves, water, and other microhabitat characteristics were recorded. All mosquito samples were stored separately in plastic boxes and transported in an icebox to a field laboratory.

4.2.4. Aedes *adult sampling*

Adult mosquitoes were sampled using human-baited double-net traps for three consecutive days from 04:00 to 20:00 hours (Figure 4.1). A double-net trap was a combination of two nets: the internal and small which protected the bait and the external and big net with openings in places which allowed the entry of mosquitoes and precluded their exit. For each double-net trap, there was a pair of persons: one person inside the small net who served as bait to attract mosquitoes, while the other outside the double-net device collected hourly mosquitoes trapped within the external net. Each human-baited double-net device was monitored by two teams of two persons who took turns at 12:00 a.m. Volunteers were vaccinated against yellow fever and also protected by malaria prophylaxy.

4.2.5. Laboratory treatment procedures

In the laboratory (Figure 4.1), the paddles were dried during a period of 5 days at 25 ± 1 °C room temperature, relative humidity of 80–90% and a 12:12 hours light:dark photoperiod. They were screened with white, insect-proof, nylon netting to prevent eventual oviposition from other mosquitoes and potential predators. The paddles were then separately immersed in plastic cups (6 × 9 × 15 cm) 3/4 filled with distilled water for attached egg hatching. The process was repeated three times after flooding of 5 days to maximize egg hatching. Emerging larvae were counted and recorded. The larvae were reared until adult stage under identical conditions as paddle drying. To avoid overcrowding and limit mortality, at most 20 emerging larvae were placed per 200 ml plastic cup filled to 3/4 with distilled water. Each plastic cup was labeled with a unique number of the sample, the study area and the date of collection. Larvae were fed each morning (07:00-08:00 hours) with Tetra-Min Baby Fish Food®. Emerging pupae were transferred to new plastic cups using plastic pipettes for adult emergence. The cups containing the pupae were netted to avoid draining the newly hatched adults. *Culex* egg rafts were not dried but were stored at 15 ± 1 °C to avoid desiccation [17]. In addition, the larvae hatched from *Culex* egg rafts and the larvae of *Aedes*, *Culex* and *Eretmapodites* found in the collected water from the ovitraps were also separately reared until adult stage, under the same conditions as described above. The larval sampled from the field were also reared in the same manner. Predacious larvae of *Toxorhynchites* spp. and *Cx. tigripes* were fed with larvae from colonies that were sampled from the study areas. During rearing, emerging adult *Aedes* spp., *Anopheles* spp., *Coquelitidia* spp., *Culex* spp., *Eretmapodites* spp., *Filcabia* spp., *Toxorhynchites* spp. and *Uranotenia* spp. specimens were identified to the species level using morphological criteria [10, 18, 19]. The mosquito individuals were stored at subgenus, species and sex levels and the data were recorded in an entomology collection database.

4.2.6. Statistical analysis

All statistical analyses were conducted using Stata version 14.0 (Stata Corporation; College Station, TX, USA). A significance level of 5% was set for statistical testing.

Aedes species proportions were calculated as the percentage of specimens among the total mosquito fauna.

Aedes species richness was expressed as the number of collected species in each study area. *Aedes* species abundance was assessed as the mean number or the proportion (%) of specimens. The frequency of *Aedes*-positive breeding sites or trap was calculated as the percentage of water holding containers or trap with at least one larva or pupa or adult.

Aedes mosquito biodiversity was assessed by the species diversity, dominance and community similarity in the study and among the macrohabitats that were estimated by Shannon Index (H) [20], Simpson Index (D) [21] and Sorenson's Coefficient (CC) [22], using the following formula:

$$\text{Shannon Index (H)} = -\sum_{i=1}^{s} p_i \ln p_i$$

The Shannon Index (H) is an information statistic index, which means it assumes all species are represented in a sample and that they are randomly sampled.

p is the proportion *(n/N)* of specimens of one particular species found *(n)* divided by the total number of specimens found *(N)*. *s* is the number of species.

$$\text{Simpson Index (D)} = \frac{1}{\sum_{i=1}^{s} p_i^{2}}$$

The Simpson Index (D) is a dominance index as it gives more weight to common or dominant species.

p is the proportion *(n/N)* of specimens of one particular species found *(n)* divided by the total number of specimens found *(N)*. *s* is the number of species.

$$\text{Sorenson's Coefficient (CC)} = \frac{2C}{S1 + S2}$$

Sorenson's Coefficient (CC) helps to assess the community similarities.

C is the number of species the two communities have in common. *S*1 is the total number of species found in community 1. *S*2 is the total number of species found in community 2.

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5. ARTICLE 1: *Aedes* mosquito oviposition ecology, species composition and *Aedes aegypti* dynamics in variously urbanized settings in arbovirus foci, southeastern Côte d'Ivoire

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5.1. Abstract

Background: *Aedes* mosquito-transmitted outbreaks of dengue and yellow fever have been reported from rural and urban parts of Côte d'Ivoire. The present study aimed at assessing *Aedes* spp. oviposition ecology in variously urbanized settings within arbovirus foci in southeastern Côte d'Ivoire.

Methods: *Aedes* spp. eggs were sampled using a standard ovitrap method from January 2013 to April 2014 in different ecosystems of rural, suburban and urban areas. Emerged larvae were reared until the adult stage for species identification.

Results: *Aedes* spp. oviposition ecology significantly varied from rural-to-urban areas and according to the ecozones and the seasons. Species richness of *Aedes* spp. gradually decreased from rural (eight species) to suburban (three species) and urban (one species) areas. Conversely, emerged adult *Aedes* spp. mean numbers were higher in the urban (1.97 *Aedes*/ovitrap/week), followed by the suburban (1.44 *Aedes*/ovitrap/week) and rural (0.89 *Aedes*/ovitrap/week) areas. *Aedes aegypti* was the only species in the urban setting (100%), and was also the predominant species in suburban (85.5 %) and rural (63.3 %) areas. The highest *Ae. aegypti* mean number was observed in the urban (1.97 *Ae. aegypti*/ovitrap/week), followed by the suburban (1.20 *Ae. aegypti*/ovitrap/week) and rural (0.57 *Ae. aegypti*/ovitrap/week) areas. *Aedes africanus* (9.4 %), *Ae. dendrophilus* (8.0 %), *Ae. metallicus* (1.3 %) in the rural, and *Ae. vittatus* (6.5 %) and *Ae. metallicus* (1.2 %) in the suburban areas each represented more than 1 % of the total *Aedes* fauna. In all areas, *Aedes* species richness and abundance were higher in the peridomestic zones and during the rainy season, with stronger variations in species richness in the rural and in abundance in the urban areas. Besides, the highest *Culex quinquefasciatus* abundance was found in the urban areas, while *Eretmapodites chrysogaster* was restricted to the rural areas.

Conclusions: Urbanization correlates with a substantially higher abundance in *Aedes* mosquitoes and a regression of the *Aedes* wild species towards a unique presence of *Ae. aegypti* in urban areas. *Aedes* wild species serve as bridge vectors of arboviruses in rural areas, while *Ae. aegypti* amplifies arbovirus transmission in urban areas. Our results have important ramifications for dengue and yellow fever vector control and surveillance strategies in arbovirus foci in southeastern Côte d'Ivoire.

Keywords: Arboviruses, *Aedes*, Oviposition ecology, *Culex*, *Eretmapodites*, Ovitrap, Socio-ecological survey, Urbanization, Côte d'Ivoire

5.2. Background

Several *Aedes* mosquito species are involved in the transmission of arboviral diseases, including dengue and yellow fever, responsible for major health burdens worldwide [1-3]. In the mid-1990s, yellow fever was controlled in Francophone Africa by vaccination while both yellow fever and dengue were eliminated in the Americas by effective control of *Aedes aegypti* [4]. However, in recent years, there has been a dramatic resurgence of dengue fever worldwide [5-8] and re-emergence of yellow fever in some parts of Africa [9]. In addition, other arboviruses vectored by *Aedes* mosquitoes, such as chikungunya [10], Rift valley [11] and Zika virus [12] are emerging or re-emerging in Africa, particularly in West Africa [13-15]. The patterns of arboviral disease transmission and its geographic expansion are likely a result of intensive urbanization [1, 2, 6, 16]. However, dengue and yellow fever originated in enzootic (sylvatic) cycles associated with wild *Aedes* vectors in rural areas. Enzootic cycles are linked to urban transmission cycles by a major domestic vector, *Ae. aegypti* [17].

In Côte d'Ivoire, single and dual epidemics of dengue and yellow fever involving several wild *Aedes* species and the major urban vector, *Ae. aegypti*, have been reported in both rural and urban areas [18]. Sylvatic dengue virus circulation, without human infections, was documented by isolation of DENV-2 serotype from wild *Aedes* vectors, including *Aedes africanus*, *Aedes furcifer*, *Aedes luteocephalus*, *Aedes opok* and *Aedes vittatus* in rural areas in the 1980s [19]. Since then, several sporadic outbreaks of dengue DENV-1, DENV-2 and DENV-3 serotypes and yellow fever sometimes resulting in fatal outcomes have occurred [20-22]. The outbreaks were mostly concentrated in surrounding villages and periurban areas of Abidjan, the economic capital and the most densely populated city of Côte d'Ivoire [21, 22].

The comprehension of the shaping patterns of immature *Aedes* mosquito ecology along the urbanization gradient is of paramount importance in determining their role in maintenance of epidemic arboviral diseases transmission [1, 2]. Knowledge of such patterns may therefore enable a more effective deployment of vector control measures for the benefit of public health. *Aedes* mosquitoes are readily adapted to a broad range of ecological settings and are expected to vary according to natural and urbanized environment [2, 9]. Certain *Aedes* mosquito species are confined and limited to sylvatic, rural or urban areas, whereas others have a large distribution and colonize almost every environment, such as the wild, rural and urbanized settings, the domestic and peridomestic premises, the types of landscapes and the microhabitats [2, 9]. Those species of *Aedes* occurring in transitional environments may serve as bridge vectors between enzootic diseases and humans in rural areas. Moreover, *Aedes* mosquitoes are the main reservoirs of arboviruses and the longest link of the transmission chain since they host the viruses during longer duration compared to humans and wild animals [23]. These *Aedes* vector species show both oral and transovarial infection [23, 24]. The extent to which eggs are resistant against desiccation varies between species and strains, and depends on climatic conditions [25, 26]. Otherwise, *Aedes* mosquito species can be associated with other mosquito species for different interaction purposes such as predation, competition and symbiosis [9]. *Eretmapodites chrysogaster* is a predaceous mosquito and lays its eggs in *Aedes* species breeding sites [27]. *Aedes* and *Culex* species, mainly *Cx. quinquefascistus* and *Ae. aegypti*, are sympatric and co-occur in the same containers [28].

Aedes aegypti is an urban species and a major vector of dengue and yellow fever by amplifying epidemics among the urban populations [9, 17]. This species consists of two subspecies, *Ae. aegypti aegypti* and *Ae. aegypti formosus* that are morphologically [29], behaviorally and genetically distinct [30-32]. However, there are ambiguities resulting in confusion over morphological distinction between the two subspecies of *Ae. aegypti* in West Africa [13, 33].

Urbanization could potentially modify *Aedes* mosquito ecology by changing the composition and dynamics of species, and increasing the abundance of their breeding sites due to environmental changes, and thus contribute to arbovirus outbreaks [2]. However, *Aedes* mosquito egg laying ecology is unknown in arbovirus foci located in variously urbanized settings of southeastern Côte d'Ivoire. To fill this gap, our study explored *Aedes* mosquito egg laying patterns, species composition and dynamics in Ehania-V1, Blockhauss and Treichville representing rural, suburban and urban settings of southeastern Côte d'Ivoire. Because immature mosquitoes are sensitive to environmental changes [2, 25, 26], we hypothesized that *Aedes* mosquito oviposition ecology and species composition, and the dynamics of *Ae. aegypti* change from rural to suburban and urban settings. Field surveys of *Aedes* mosquito egg were performed using highly sensitive sampling method such as standardized World Health Organization (WHO) ovitraps [23, 24], larval rearing in the laboratory and adult stage identification were conducted to test this hypothesis. The findings provide valuable information on *Aedes* mosquito egg laying patterns, species composition and *Ae. aegypti* dynamics in different urbanized ecosystems. The key results open new perspectives for improving current vector control and surveillance strategies for dengue and yellow fever that are tailored for specific settings of southeastern Côte d'Ivoire.

5.3. Methods

5.3.1. Study area

The study was conducted in three settings in southeastern Côte d'Ivoire: Ehania-V1, Blockhauss and Treichville, representing rural, suburban and urban areas, respectively (Figure 5.1).

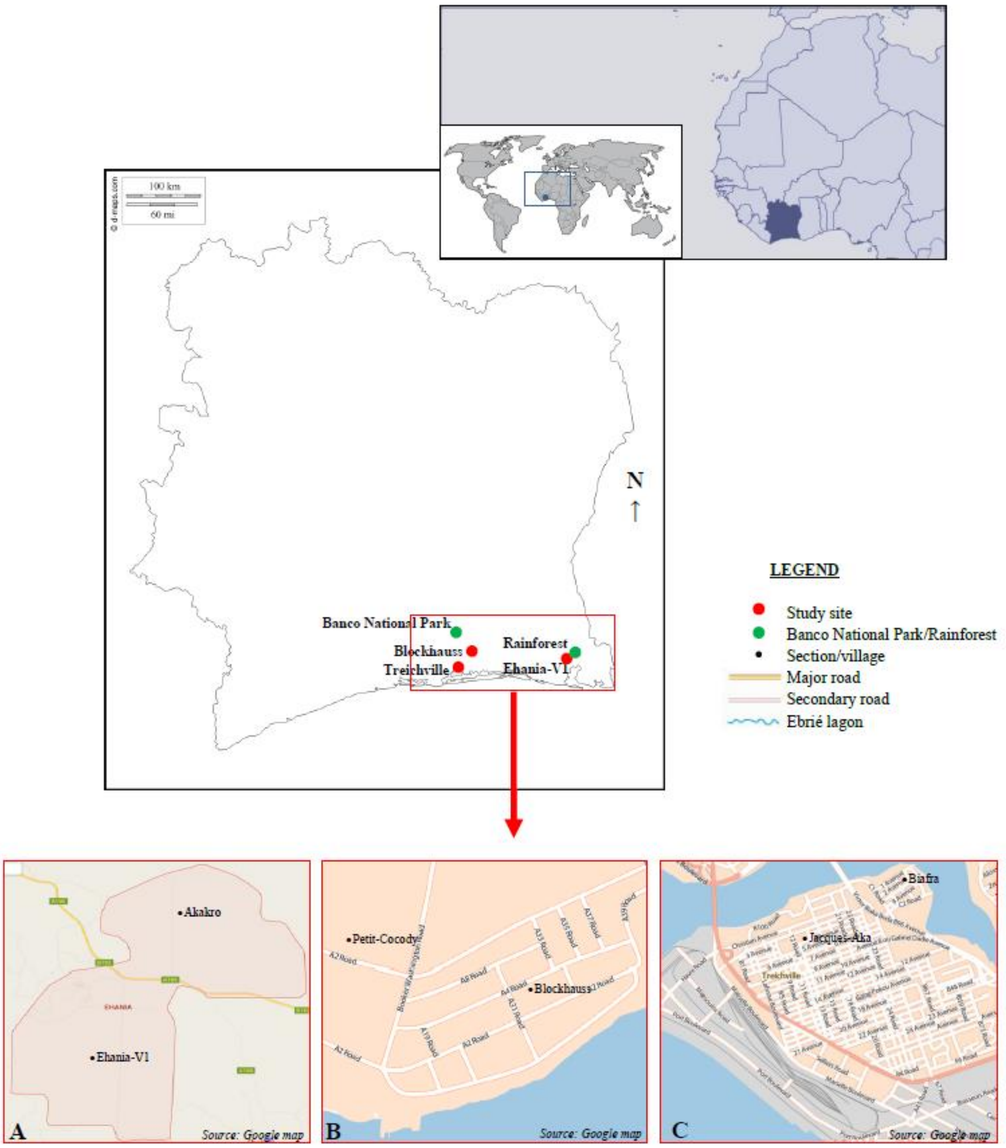


Figure 5.1: Map of study areas located in southeastern Côte d'Ivoire. The ecological study of *Aedes* mosquitoes was carried out in three areas: **a** Ehania-V1 (covers the villages of Ehania-V1 and Akakro and represents the rural area without major and secondary paved roads. The site is close to the primary rainforest reserve). **b** Blockhauss (comprises the villages of Blockhauss and Petit-Cocody and represents the suburban area with only secondary paved roads. It is about 5 km away from the rainforest of Banco National Park). **c** Treichville (includes the sections of Jacques-Aka and Biafra and is the urban area with numerous major and secondary paved roads. It is located in the centre of Abidjan and is separated from Blockhauss by Ebrié lagoon).

The village of Ehania-V1 (5°18'N, 3°04'W) belongs to the district of Aboisso some 140 km east of Abidjan.Ehania-V1 is a rural area with a population density of approximately 48 people/km^2 and unpaved roads. The residencies are composed of traditional and ordinarily modern houses. This area is surrounded by industrial oil palm plantation (*Elaesis guineensis*) of 11,444 ha and 100 ha of preserved primary rainforest. The rainforest provides strong vegetation with dense canopy cover, trees with holes and bamboos and hosts non-human primates and birds.Blockhauss (5°19'N, 4°00'W) is located within Abidjan bordered in its northern part by Banco National Park with over 3,750 ha of rainforest. This setting is a suburban area with *c*.750 people/km^2 and paved roads. The land use comprises a mixture of residential buildings, hospitals and schools. The residencies are ordinarily modern houses and some blocks with flats. Urbanization is underway in untapped spaces. Treichville (5°18'N, 4°00'W) is situated in central Abidjan and separated from Blockhauss by the Ebrié Lagoon that has a width of approximately 4 km. This setting is an urban area with more than 1,800 people/km^2 and paved roads. The density of the population greatly increases during the daytime due to the convergence of people from other municipalities of Abidjan for trading, businesses and sports. The land use is essentially residential, commercial, cultural and sportive buildings, seaport, and public services such as schools and hospitals, filled with green spaces set apart. The residencies are mostly composed of blocks of flats and some ordinarily modern houses. Urbanization is completed due to the lack of availability of additional space for the construction of new houses.

In southeastern Côte d’Ivoire, the climate is warm and humid and overall, transitional from equatorial to tropical with four seasons. The seasons are more clearly distinguished by rainfall than temperature. The two rainy seasons are separated by a dry season. The main rainy season extends from May to July, while a short rainy season occurs in October and November. The main dry season extends from December to April. This classic sequence of seasonality has been disrupted due to strong climate variability [34]. The average annual precipitation ranges from 1,200 to 2,400 mm. The annual temperature is around 26.5 °C and the annual relative humidity (RH) ranges between 78 % and 90 %.

5.3.2. Design of sample collection

Aedes spp. eggs were sampled using the standard WHO ovitrap method [23, 24]. Ovitraps were metallic boxes cut with 400 cm^3 volume and covered with black paint to attract gravid female *Aedes* mosquitoes in search of egg laying grounds. They were filled (75 % full) with distilled water mixed with rainwater and 10 % infusions of herbs (*Panicum maximum*) to increase the attractiveness of the ovitraps [23]. A 5 × 7 × 0.3 cm paddle made of hardboard, rough on one side and serving as oviposition substrate, was plunged in each box and left for one-week period during each of six surveys. The rural area was divided into three ecozones: domestic, peridomestic and sylvatic. The suburban and urban areas were divided into domestic and peridomestic zones because there were no sylvatic zones. According to Cordellier et al. [23], domestic zone refers to the human-inhabited space, the peridomestic zone covers the vegetated environment surrounding the domestic zone in which humans usually discard artificial items that serve as breeding sites for *Aedes* mosquitoes, the sylvatic zone is primarily a wild environment free of discarded containers that host forests with natural containers (e.g., tree holes) and wild animals. In our study, the peridomestic zone extended from the edge of the domestic zone to 300 m while the sylvatic zone was located from 300 to 800 m around the village. During each of the 6 surveys, 50 ovitraps were repeatedly placed in the same location in each defined ecozone. In the human-inhabited zone, the ovitraps were suspended at 1.5 m above the ground to secure and protect them. In total, 900, 600 and 600 ovitraps were deployed in the rural, suburban and urban areas, respectively, from January 2013 to April 2014. In addition, from April to July 2013, we conducted socio-ecological investigations on the 50 households representative of each study area in which ovitraps were placed to identify their sociogeographic status.

5.3.3. Key socio-geographic characteristics

The socio-ecological investigations showed that the surveyed households varied highly along the increasing urbanization gradient. The mean number (mean ± standard error) of people per household was 5.9 ± 2.8 in the rural, 8.6 ± 2.1 in the suburban and 11.9 ± 3.2 in the urban areas. The potential resident containers were mainly discarded items (cans, pots, barrels, tyres) (n = 50; 58.0 %) and natural containers (coconut, tree hole, bamboo, snail shell) (46.0 %) in the rural area. The containers were mostly artificial and discarded receptacles with 78.0 % and 92.0 % in the suburban and urban areas, respectively. The households stored water in the proportions of 88.0 % (n = 50) in the rural, 98.0 % in the suburban and 100 % in the urban areas.

5.3.4. Sample laboratory treatment

In the field, the paddles, *Culex* egg rafts and remaining water from the ovitraps were separately stored in plastic boxes and then transported in a cold box to the laboratory. The paddles were dried during a period of 5 days at 25 ± 1 °C room temperature, RH of 80–90 % and a 12:12 h light:dark photoperiod. They were screened with white, insect-proof, nylon netting to prevent eventual egg laying from other mosquitoes and potential predators. The paddles were then separately immersed in plastic cups (6 × 9 × 15 cm) 75 % filled with distilled water for attached egg hatching. The process was repeated three times after flooding of 5 days to maximize egg hatching. Emerging larvae were counted and recorded. As there was no reliable larval identification key, the larvae were reared until adult stage under identical conditions as paddle drying. To avoid overcrowding and limit mortality, at most 20 emerging larvae were placed per 200 ml plastic cup filled to 75 % with distilled water. Each plastic cup was labeled with a unique number of the sample, the study area and the date of collection. Larvae were fed each morning (7:00–8:00 h) with Tetra-Min Baby Fish Food®. Emerging pupae were transferred to new plastic cups using plastic pipettes for adult emergence. The cups containing the pupae were netted to avoid draining the newly hatched adults. *Culex* egg rafts were not dried but were stored at 15 ± 1 °C to avoid desiccation [36]. In addition, the larvae hatched from *Culex* egg rafts and the larvae of *Aedes*, *Culex* and *Eretmapodites* found in the collected water from the ovitraps were also separately reared until adult stage, under the same conditions as described above. During rearing, emerging adult *Aedes, Culex* and *Eretmapodites* specimens were identified to the species level using morphological criteria [23, 24, 37]. The mosquito individuals were stored at subgenus, species and sex levels and the data were recorded in an entomology collection database.

5.3.5. Statistical analysis

The measures of *Aedes* species proportions were expressed as the percentage of specimens among *Aedes* fauna for each study area and analysed using Fisher's exact test to look at the relationship between the species composition and the study area and ecozone, and followed by the Proportion-test. Fisher's exact test was used because expected numbers of specimens were equal or less than five. *Aedes* species richness was assessed as the number of collected species in each study area and compared using a one-way analysis of variance (ANOVA), followed by Bonferroni's correction. The species diversity and dominance of *Aedes* spp. were estimated by Shannon-Weaver's index [38] and Simpson's index [39] and analyzed by

Kruskal-Wallis test because the log-transformed data exhibited significant deviations from normality. The abundance of *Aedes* spp. and *Ae. aegypti* was expressed as the mean number of specimens per ovitrap and analysed using repeated measures approaches in a Generalized Linear Mixed Model (GLMM) framework in order to take into account the possible interactions between the variables "month", "study site" and "ecozone" [35]. To account for overdispersion due to excessive numbers of zeroes, the data were log-transformed [log (number of specimens + 1)] [36]. The log-transformed data were subjected to GLMM procedures and analysed as follows [35]. We compared the mean numbers of *Aedes* mosquito specimens per ovitrap between the study areas, the ecozones and the months using mixed-effects regression (*xtmixed* command), performed the joint tests of the interactions and the main effects of the study sites, the ecozones and months (*contrast* command) to understand the significant interactions, followed up the simple effects of each study area and ecozone over the months by pairwise comparisons (*margins* and *pwcompare* commands) and the *post-hoc* test of the trends (*contrast p.* operator) and the *post-hoc* test of the partial interaction (*contrast a.* operator). The mortality of the larvae during rearing was compared using negative binomial error. The extra sub-site, sylvatic zone, was excluded from the analysis when performing the comparisons between the study areas, and only included when the comparisons were conducted among the ecozones in the rural area. A significance level of 5 % was set for statistical testing. All data were analysed using Stata version 14.0 (Stata Corporation; College Station, TX, USA).

5.4. Results

5.4.1. Species composition of emerged adult mosquitoes

The mortality of the larvae hatched from *Aedes* spp. eggs during the rearing to adult stage was not statistically significant (all $P > 0.478$) thus making the comparison of emerged adults possible. Table 5.1 shows the species composition of adult *Aedes* spp. emerged from eggs collected from the different study areas. Totals of 2,441, 2,440 and 3,098 adult *Aedes* spp. emerged from the eggs collected in the rural, suburban and urban areas, respectively. *Aedes* species belonged to three subgenera (*Stegomyia*, *Aedimorphus* and *Diceromyia*) in the rural areas, two subgenera (*Stegomiya* and *Aedimorphus*) in the suburban areas and a single subgenus (*Stegomyia*) in the urban areas. The species richness of *Aedes* spp. gradually decreased from the rural (eight species) to the suburban (three species) and urban (one species) areas. Fisher's exact test indicated that *Aedes* species richness significantly varied

Table 5.1: Species composition of emerged adult *Aedes* spp. collected in the rural, suburban and urban areas of southeastern Côte d'Ivoire

Subgenus	Species	Rural					Suburban					Urban				
		Female	**Male**	**Total**	**%**	**MO ± SE**	**Female**	**Male**	**Total**	**%**	**MO ± SE**	**Female**	**Male**	**Total**	**%**	**MO ± SE**
Aedes (*Stegomyia*)	*Aedes aegypti*	913	841	1,754	63.3^{a}	0.57 ± 0.05	1,124	1,035	2,159	85.5^{a}	1.20 ± 0.09	1,521	1,577	3,098	100	1.97 ± 0.10
	Aedes africanus	137	139	276	9.4^{b}	0.08 ± 0.02	0	0	0	0	0	0	0	0	0	0
	Aedes dendrophilus	122	139	261	8.0^{b}	0.07 ± 0.02	0	0	0	0	0	0	0	0	0	0
	Aedes metallicus	22	14	36	1.3^{c}	0.01 ± 0.01	20	12	32	1.2^{c}	0.01 ± 0.01	0	0	0	0	0
	Aedes usambara	20	12	32	0.5^{c}	0.01 ± 0.00	0	0	0	0	0	0	0	0	0	0
	Aedes fraseri	6	11	17	0.3^{c}	0.01 ± 0.00	0	0	0	0	0	0	0	0	0	0
	Aedes luteocephalus	8	3	11	0.3^{c}	0.00 ± 0.00	0	0	0	0	0	0	0	0	0	0
Aedes (*Aedimorphus*)	*Aedes vittatus*	0	0	0	0	0	130	119	249	6.5^{b}	0.09 ± 0.02	0	0	0	0	0
Aedes (*Diceromyia*)	*Aedes furcifer*	20	14	34	0.7^{c}	0.01 ± 0.01	0	0	0	0	0	0	0	0	0	0
Total	**Abundance**	**1,248**	**1,173**	**2,421**	**100**	**0.89 ± 0.06**	**1,274**	**1,166**	**2,440**	**100**	**1.44 ± 0.09**	**1,521**	**1,577**	**3,098**	**100**	**1.97 ± 0.10**
	Richness (no. of spp.)	**8**					**3**					**1**				

Letters indicate the results of the Proportion-test. Groups that do not share the same letter for the same study area are significantly different ($P < 0.05$)
Abbreviations: MO, mean number per ovitrap; SE, standard error of the mean number per ovitrap

from one study area to another (all $P < 0.001$). Proportion-testing indicated that there was a significant difference in *Aedes* species proportions in the rural ($\chi^2 = 9411.15$, $df = 7$, $P < 0.0001$) and the suburban ($\chi^2 = 5052.86$, $df = 2$, $P < 0.0001$) areas. *Aedes aegypti* was the predominant species with significantly higher proportions among *Aedes* fauna collected in the rural ($Z = 18.91$, $P < 0.001$) and suburban areas ($Z = 7.83$, $P < 0.001$), and the sole *Aedes* species in the urban areas. *Aedes africanus* and *Ae. dendrophilus* in the rural areas and *Ae. vittatus* in the suburban areas were found in significantly higher proportions. *Aedes metallicus* represented more than 1 % of the total *Aedes* fauna in the rural and the suburban areas whereas *Ae. furcifer*, *Ae. fraseri* and *Ae. luteocpehalus* were collected in lower proportions in the rural areas.

Non-*Aedes* mosquito species were also sampled in the all study areas. Totals of 277, 108 and 67 specimens of *Culex* spp. were sampled from the rural, suburban and urban areas, respectively. In the rural area, *Culex* spp. was composed of three species, *Cx. nebulosus* (n = 277; 49.4 %), followed by *Cx. quinquefasciatus* (28.2 %) and *Cx. poicilipes* (22.4 %). The diversity of *Culex* spp. then decreased to a single species, *Cx. quinquefasciatus*, in the suburban (n = 108) and urban (n = 133) areas. *Eretmapodites* spp. was restricted to the rural area and composed of only one species, *Er. chrysogaster*, with 274 specimens.

5.4.2. Richness, diversity and dominance of Aedes *spp.*

Table 5.2 presents the species richness, diversity and dominance of *Aedes* spp. in all of the study areas and different ecozones. *Aedes* spp. species richness was significantly different among the study areas ($F = 18.60$, $df = 2$, $P = 0.0001$) and ecozones ($F = 9.24$, $df = 6$, $P < 0.0001$), with higher numbers of species in the rural area and the peridomestic zone of the same area. The species diversity of *Aedes* spp. was statistically different among the study areas ($\chi^2 = 14.00$, $df = 2$, $P = 0.0009$) and ecozones ($\chi^2 = 27.65$, $df = 6$, $P = 0.0001$), with higher values for both diversity indices in the rural area and the sylvatic zone of the rural area. Moreover, *Aedes* spp. species dominance was significantly different among the study areas ($\chi^2 = 13.86$, $df = 2$, $P = 0.0011$) and ecozones ($\chi^2 = 28.00$, $df = 6$, $P = 0.0001$), with higher Simpson's index values in the urban area and both peridomestic and domestic zones of the urban area.

Table 5.2: Species richness, diversity and dominance of *Aedes* spp. in the rural, suburban and urban areas and ecozones in southeastern Côte d'Ivoire

Area / Ecozone	Richness	Shannon's diversity index	Simpson's dominance index
Area			
Rural	8^{a}	1.39^{a}	0.55^{b}
Suburban	3^{b}	$0.57^{a,b}$	$0.79^{a,b}$
Urban	1^{b}	0^{b}	1^{a}
Ecozone			
Sylvatic[1]	$5^{a,b}$	1.90^{a}	0.28^{c}
Peridomestic[1]	$7^{a,b}$	$1.23^{a,b}$	$0.58^{b,c}$
Domestic[1]	$5^{b,c}$	$0.75^{a,b,c}$	$0.77^{a,b,c}$
Peridomestic[2]	$3^{b,c}$	$0.67^{a,b,c}$	$0.74^{a,b,c}$
Domestic[2]	$3^{b,c}$	$0.35^{a,b,c}$	$0.89^{a,b,c}$
Peridomestic[3]	1^{c}	0^{c}	1^{a}
Domestic[3]	1^{c}	0^{c}	1^{a}

Letters indicate the results of one-way ANOVA test followed by Bonferroni correction (richness) and Kruskal-Wallis test (Shannon diversity index, Simpson dominance index). Groups that do not share the same letter are significantly different ($P < 0.05$)
[1] Ecozone in the rural area
[2] Ecozone in the suburban area
[3] Ecozone in the urban area

5.4.3. *Dynamics of* Aedes *spp. numbers*

The highest mean numbers of emerged adult *Aedes* spp. were found in the urban setting (1.97 ± 0.10 *Aedes*/ovitrap/week), followed by the suburban (1.44 ± 0.09 *Aedes*/ovitrap/week) and rural (0.89 ± 0.06 *Aedes*/ovitrap/week) areas. The mean numbers of emerged adult *Aedes* spp. were significantly different between the rural and urban areas ($Z = 5.01$, $P < 0.001$). The effects and the interactions among the study areas, the ecozones and months, and the trends of *Aedes* spp. numbers over the months were statistically significant (Table 5.3).

Table 5.4 summarizes the geographical variation of adult *Aedes* species collected in each of the three study areas. In the rural areas, specimens of *Ae. africanus*, *Aedes dendrophilus*, *Aedes metallicus* and *Aedes fraseri* were collected in the domestic zone, while significant numbers of *Ae. aegypti* were sampled in the sylvatic zone. Emerged adult *Aedes* spp. mean numbers were significantly higher in the peridomestic zone with 1.36 ± 0.14 *Aedes*/ovitrap/week in the rural (Contrast = 0.50, $Z = 5.16$, $P < 0.001$), suburban (2.10 ± 0.15 *Aedes*/ovitrap/week; Contrast = -4.89, $Z = -4.81$, $P < 0.001$) and urban (2.80 ± 0.21 *Aedes*/ovitrap/week; Contrast = -0.49; $Z = -4.85$, $P < 0.001$) areas.

Table 5.3: Effects, interactions and trends of *Aedes* spp. and *Ae. aegypti* numbers in the rural, suburban and urban areas in southeastern Côte d'Ivoire. The results are the outputs of the Generalized Linear Mixed Model (GLMM) procedures. The extra sub-site, sylvatic zone, was excluded from the data

		***Aedes* spp.**			***Aedes aegypti***		
		χ^2	***df***	***P***	χ^2	***df***	***P***
1. Main effect & interaction							
1.1. Main effect							
Study area		20.16	2	< 0.00001*	50.37	2	< 0.00001*
Ecozone		43.76	1	< 0.00001*	26.32	1	< 0.00001*
Month		112.78	5	< 0.00001*	82.67	5	< 0.00001*
1.2. Interaction							
Study area × ecozone		7.09	2	0.0288*	13.25	2	0.0013*
Study area × month		15.90	10	0.1027	26.52	10	0.0031*
Ecozone × month		12.26	5	0.0314*	8.69	5	0.1221
Study area × ecozone × month		14.96	10	0.1335	8.29	10	0.6003
2. *Post-hoc* test of trends							
2.1. Study area	Trend						
Rural	Linear	2.55	1	0.1102	0.43	1	0.5109
	Quadratic	1.19	1	0.2752	2.81	1	0.0935
	Cubic	9.36	1	0.0022*	5.20	1	0.0225*
	Quartic	6.08	1	0.0136*	4.70	1	0.0302*
	Quintic	1.03	1	0.3099	0.06	1	0.7999
Suburban	Linear	7.31	1	0.0068*	4.86	1	0.275
	Quadratic	2.91	1	0.0880	0.92	1	0.3377
	Cubic	10.09	1	0.0015*	5.67	1	0.0173*
	Quartic	16.54	1	< 0.00001*	7.05	1	0.0079*
	Quintic	0.46	1	0.4969	3.58	1	0.0584
Urban	Linear	26.45	1	< 0.00001*	27.16	1	< 0.00001*
	Quadratic	0.02	1	0.8767	0.02	1	0.8798
	Cubic	15.22	1	0.0001*	15.64	1	0.0001*
	Quartic	28.58	1	< 0.00001*	29.42	1	< 0.00001*
	Quintic	2.67	1	0.1020	2.74	1	0.0981
Joint		128.68	15	< 0.00001*	109.47	15	< 0.00001*
2.2. Ecozone							
Peridomestic	Linear	23.32	1	< 0.00001*	21.11	1	< 0.00001*
	Quadratic	1.24	1	0.2658	0.53	1	0.4679
	Cubic	17.00	1	< 0.00001*	9.87	1	0.0017*
	Quartic	47.09	1	< 0.00001*	31.48	1	< 0.00001*
	Quintic	2.87	1	0.0900	0.21	1	0.6487
Domestic	Linear	8.28	1	0.0040*	3.96	1	0.0465*
	Quadratic	1.08	1	0.2978	1.71	1	0.1915
	Cubic	17.26	1	< 0.00001*	15.23	1	0.0001*
	Quartic	7.85	1	0.0051*	7.46	1	0.0063*
	Quintic	0.02	1	0.8846	0.23	1	0.6330
Joint		122.97	10	< 0.00001*	90.52	10	< 0.00001*

*Significant effects ($P < 0.05$)

Abbreviations: χ^2, chi-square; *df*, degrees of freedom; *P*, *P*-value

Table 5.4: Geographical variations in the number of emerged adult species of *Aedes* spp. in the rural, suburban and urban areas in southeastern Côte d'Ivoire

Species	**Rural**						**Suburban**				**Urban**			
	Sylvatic zone		**Peridomestic zone**		**Domestic zone**		**Peridomestic zone**		**Domestic zone**		**Peridomestic zone**		**Domestic zone**	
	n	**MO ± SE**	***n***	**MO ± SE**	***n***	**MO ± SE**	***n***	**MO ± SE**	***n***	**MO ± SE**	***n***	**MO ± SE**	***n***	**MO ± SE**
Aedes aegypti	132	0.15 ± 0.04	901	0,85 ± 0.12	721	0.84 ± 0.10	1,353	1.64 ± 0.14	806	0.86 ± 0.11	1,938	2.80 ± 0.21	1,160	1.34 ± 0.16
Aedes africanus	106	0.10 ± 0.03	161	0.14 ± 0.04	9	0.01 ± 0.01	0	0	0	0	0	0	0	0
Aedes dendrophilus	121	0.11 ± 0.03	91	0.07 ± 0.03	49	0.05 ± 0.02	0	0	0	0	0	0	0	0
Aedes metallicus	2	0.00 ± 0.00	7	0.01 ± 0.00	27	0.02 ± 0.01	28	0.02 ± 0.01	4	0.01 ± 0.01	0	0	0	0
Aedes usambara	0	0	32	0.02 ± 0.02	0	0	0	0	0	0	0	0	0	0
Aedes fraseri	0	0	1	0.00 ± 0.00	16	0.01 ± 0.01	0	0	0	0	0	0	0	0
Aedes luteocephalus	0	0	11	0.01 ± 0.01	0	0	0	0	0	0	0	0	0	0
Aedes vittatus	0	0	0	0	0	0	201	0.15 ± 0.05	48	0.30 ± 0.00	0	0	0	0
Aedes furcifer	34	0.02 ± 0.02	0	0	0	0	0	0	0	0	0	0	0	0
Abundance	**395**	**0.44 ± 0.03**	**1,204**	**1.36 ± 0.14**	**822**	**1.01 ± 0.01**	**1,582**	**2.10 ± 0.15**	**858**	**0.94 ± 0.11**	**1,938**	**2.80 ± 0.21**	**1,160**	**1.34 ± 0.16**

Abbreviations: *n*, total number of specimens; MO, mean number of specimens per ovitrap per week; SE, standard error of the mean

Additional files 1 and 2: S5.1 Figure and Table S5.1 indicate the seasonal variation of emerged adult *Aedes* spp. in all of the different study areas. *Aedes metallicus*, *Aedes usambara*, *Ae. fraseri*, *Ae. luteocephalus* and *Ae. furcifer* were not collected in January 2014 and April 2014. However, *Ae. aegypti* was sampled in all surveys in each study area. In all of the study areas, higher numbers of emerged adult *Aedes* spp. were found in July 2013 with 1.47 ± 0.18 *Aedes*/ovitrap/week in the rural, 2.31 ± 0.29 *Aedes*/ovitrap/week in the suburban and 4.06 ± 0.28 in the urban areas (Figure 5.2). Conversely, the significantly respective lowest numbers of *Aedes* spp. were recorded in January 2014 with 0.47 ± 0.13 (all $P < 0.05$), 0.43 ± 0.17 (all $P < 0.05$) and 0.47 ± 0.11 (all $P < 0.001$) *Aedes*/ovitrap/week.

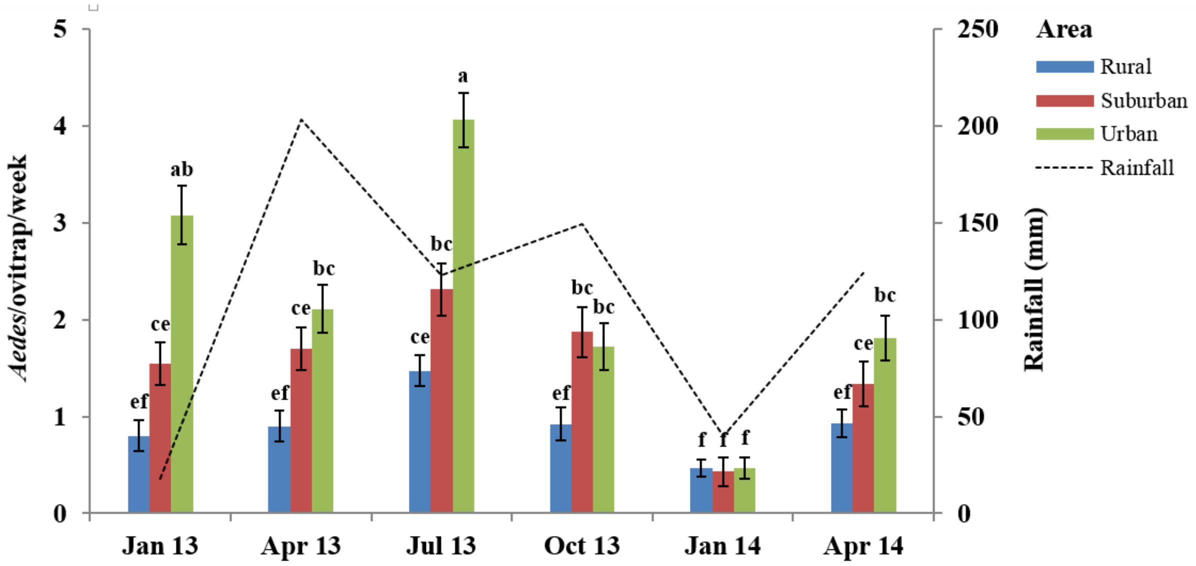


Figure 5.2: Monthly variations in mean numbers of emerged adult *Aedes* spp. as a function of the rainfall period. Rainfall was unexpectedly high in April 2013. The highest precipitations (374 mm) occurred in June 2013. Error bars show the standard error (SE) of the mean number of *Aedes* spp. per ovitrap. The letters indicate the results of the data analysed as repeated measures by Generalized Linear Mixed Model (GLMM) procedures. Groups that do not share a same letter are significantly different ($P < 0.05$).

5.4.4. Dynamics of Ae. aegypti

A total of 1,754 (n = 2,421; 63.3 ± 1.2%) adult *Ae. aegypti* emerged from the eggs collected from the rural areas, 2,159 (n = 2,440; 85.5 ± 0.8%) from the suburban and 3,098 (n = 3,098; 100 %) from the urban areas (Table 5.1). The highest mean numbers of *Ae. aegypti* were found in the urban setting, with 1.97 ± 0.10 *Ae. aegypti*/ovitrap/week. Considerably lower mean numbers were recorded in the rural and suburban settings, with 0.57 ± 0.05 and 1.20 ± 0.09 *Ae. aegypti*/ovitrap/week, respectively. *Aedes aegypti* mean numbers were significantly different between the urban and rural ($Z = 6.23$, $P < 0.001$), and the suburban and rural ($Z = 2.15$, $P < 0.05$) areas. The effects of and interactions among the study areas, ecozones and months, and the trends of *Ae. aegypti* numbers over the months were statistically significant (Table 5.3).

Figure 5.3 shows the geographical variations of adult *Ae. aegypti* mean numbers and frequencies. Significantly higher mean numbers per ovitrap of *Ae. aegypti* were found in the peridomestic zones with 0.85 ± 0.12 *Ae. aegypti*/ovitrap/week in the rural (Contrast = 0.48, $Z = 5.68$, $P < 0.001$); 1.64 ± 0.14 *Ae. aegypti*/ovitrap/week in the suburban (Contrast = -0.36, $Z = -3.65$, $P < 0.001$); and 2.80 ± 0.21 *Ae. aegypti*/ovitrap/week in the urban (Contrast = -0.49, $Z = -5.04$, $P < 0.001$) settings. *Aedes aegypti* was collected in all of the ecozones of each study area. Its frequencies gradually increased from the sylvatic zone of the rural area ($n = 395$; 33.4 %) to the domestic zone of the urban area ($n = 1,160$; 100 %) ($Z = 31.43$, $P < 0.001$).

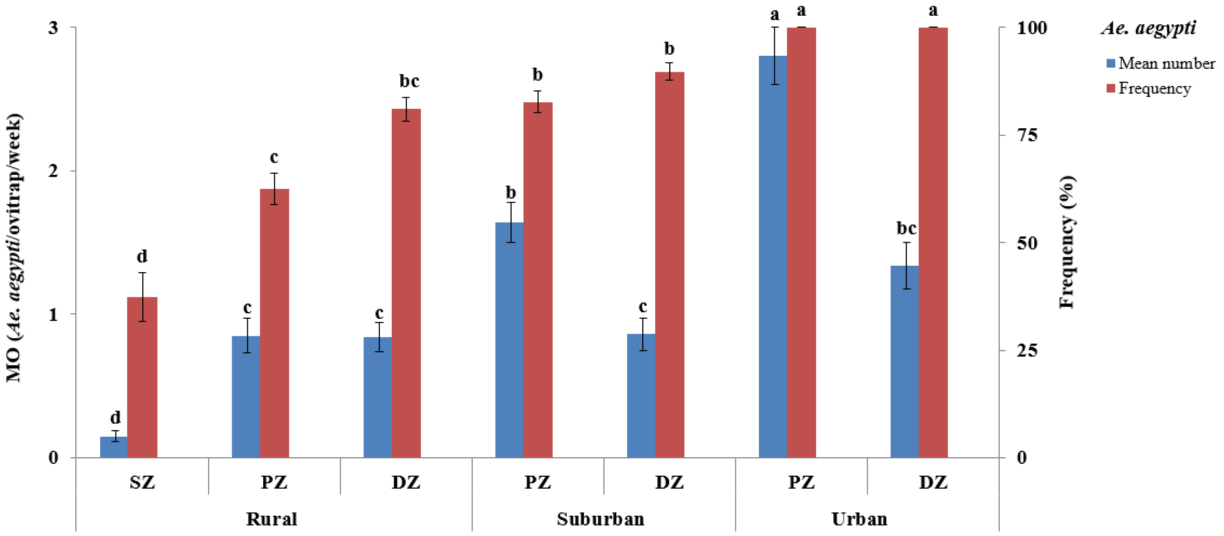


Figure 5.3: Geographical variations in mean numbers and frequencies of emerged adult *Ae. aegypti*. Error bars show the standard error (SE) of the mean number per ovitrap (MO) and the frequency. The letters indicate the results of the data analysed as repeated measures by Generalized Linear Mixed Model (GLMM) procedures for the mean number and Proportion-test for the frequency. Groups that do not share a same letter are significantly different ($P < 0.05$). *Abbreviations*: MO, mean numbers per ovitrap; SZ, sylvatic zone; PZ, peridomestic zone; DZ, domestic zone

Figure 5.4 shows the monthly variations of emerged adult *Ae. aegypti* mean numbers in relation to the rainfall. Emerged adult *Ae. aegypti* mean numbers significantly varied as a function of rainfall fluctuation in the all study areas. The highest mean numbers were found during the rainy season in July 2013 with 0.96 ± 0.14 *Ae. aegypti*/ovitrap/week in the rural and 4.06 ± 0.28 *Ae. aegypti*/ovitrap/week in the urban areas, and in October 2013 with 1.65 ± 0.25 *Ae. aegypti*/ovitrap/week in the suburban areas. In urban areas, *Ae. aegypti* mean numbers dramatically declined in January 2014 (0.47 ± 0.11 *Ae. aegypti*/ovitrap/week) compared to July 2013 (Contrast = -1.25, Z = -7.88, $P < 0.001$). In the same study area, *Ae. aegypti* numbers were significantly higher in January 2013 (3.08 ± 0.11 *Ae. aegypti*/ovitrap/week) compared to January 2014 (Contrast = -1.02, Z = -6.57, $P < 0.001$).

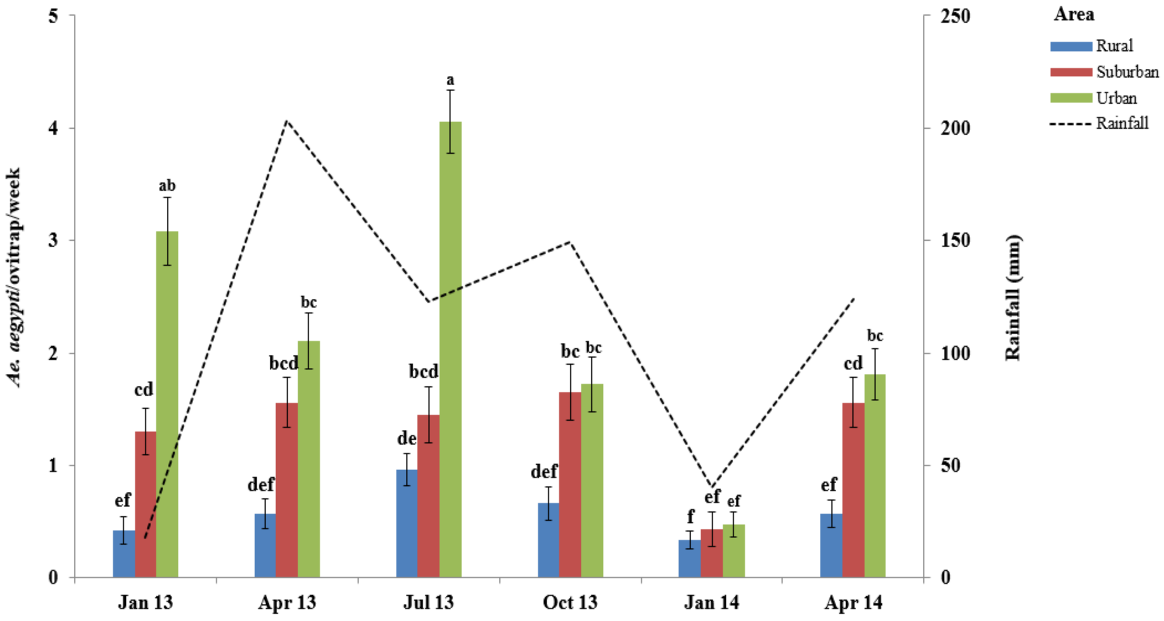


Figure 5.4: Monthly variations in mean numbers of emerged adult *Ae. aegypti* as a function of the rainfall period. Rainfall was unexpectedly high in April 2013. The highest precipitations (374 mm) occurred in June 2013. Error bars show the standard error (SE) of the mean number of *Ae. aegypti* per ovitrap. The letters indicate the results of the data analysed as repeated measures by Generalized Linear Mixed Model (GLMM) procedures. Groups that do not share a same letter are significantly different ($P < 0.05$).

5.5. Discussion

To our knowledge, this is the first study exploring oviposition ecology of *Aedes* mosquitoes in variously urbanized settings of Côte d'Ivoire. Importantly, several species of *Aedes* were involved in previous dengue and yellow fever outbreaks in Côte d'Ivoire [18-22]. The outbreaks might be explained by the paucity of vector control strategies. A deeper understanding of the modifications induced by urbanization in the ecology of *Aedes* mosquitoes is crucial. Our data suggest that *Aedes* oviposition ecology and species composition, and *Ae. aegypti* dynamics differ from rural to suburban and urban areas in southeastern Côte d'Ivoire.

Our data highlighted that the mean numbers of emerged adult *Aedes* spp. increase from rural to urban areas. An increase in *Aedes* species prevalence and abundance by urbanization was indicated by Li et al. [2]. This phenomenon is probably due to elevated numbers of *Aedes* breeding sites such as tyres, discarded cans or water storage containers, provided by urbanizing environment [2]. In addition, an urbanized environment accelerates *Aedes* mosquito development and survivorship [2]. By increasing *Aedes* spp. abundance, urbanization could potentially aggravate epidemic risk factors for arbovirus.

Our results showed that urbanization alters *Aedes* mosquito species composition towards dominance of *Ae. aegypti* in the urban areas, while rural and suburban areas favour other wild *Aedes* species, including *Ae. vittatus, Ae. dendrophilus, Ae. africanus, Ae. luteocephalus, Ae. furcifer, Ae. metallicus, Ae. usambara* and *Ae. fraseri*. *Aedes aegypti* eggs are expected to be more desiccation-resistant [23, 24]; this might raise their ability to survive in a deforested environment such as the urban areas exposed to direct sunlight and thus increase the species geographical invasion. Conversely, the wild *Aedes* species collected only in the rural and suburban settings probably originated from a natural environment such as the preserved rainforest and the Banco National Park forest, respectively. The disappearance of wild *Aedes* species in the urban settings might be explained by the destruction of the natural environment for building houses and other infrastructure. The removal of vegetation due to house constructions and other infrastructure developments results in direct exposure of *Aedes* spp. breeding sites to solar radiation. The wild *Aedes* species eggs from rural settings could be protected against solar radiation by rainforest canopy [40] since they are laid in tree holes [41] and bamboo internodes [42] filled by rainwater and maintained under low temperature. It is conceivable that wild *Aedes* species that lay more fragile and desiccation-sensitive eggs remain confined to the rural areas, mainly in the rainy forest [23, 24]. Additional field manipulations and experiments under controlled laboratory conditions testing the different *Aedes* species egg desiccation-resistance levels may be useful to better understand the segregation among the species and the population growth rates. Indeed, the forest-dwelling *Aedes* species that are still present in the rural areas may play a key role as bridge vectors between the sylvatic cycles of dengue, yellow fever and other viruses among non-human primates and humans [17]. The vector role of these *Aedes* species is subtle and difficult to trace, and often remains undetected because there are no traditional epidemiological risk indicators such as the house index, container index or Breteau index [43]. However, the exclusive existence of predators such as *Er. chrysogaster* in the rural areas might influence the abundance of *Aedes* species [27, 42]. *Eretmapodites chrysogaster* is also suspected to transmit arboviruses in tropical Africa [23]. In summary, the segregation induced by urbanization in *Aedes* species diversity is consistent with the known arbovirus transmission cycles in tropical Africa [17] and merits further consideration for dengue and yellow fever surveillance.

Our results suggested that the geographical and seasonal variations of *Aedes* spp. are associated with urbanized settings. The preference of *Aedes* spp. to lay eggs in the peridomestic vicinity confirms previous findings from urban areas in Brazil [44] and Vietnam [45]. Peridomestic premises are in close proximity to human residencies, and hence the principal blood-meal sources of adult *Aedes* mosquitoes. Furthermore, they also provide ideal ecosystems such as dense vegetation favourable for *Aedes* spp. refugia [46] and natural breeding sites such as tree holes [42, 47] and artificial containers as discarded cans and old vehicle tyres [48, 49]. Regarding the seasonal variation, *Aedes* spp. mean numbers were strongly associated with rainfall patterns, history, variability and intensity. The fluctuations in *Aedes* spp. counts could be influenced by seasonal flooding-drying cycles as reported in Côte d'Ivoire [18] and Brazil [44]. *Aedes* spp. eggs probably enter into a dormant stage to withstand desiccation periods during the dry season, while precipitations might flood the breeding sites and increase the abundance of *Aedes* spp. [50]. However, the sudden decline of *Aedes* spp. numbers in October 2013 in the urban setting might be due to heavy precipitations and exacerbated flushing of their eggs because of the lack of protective vegetation in the built-up environment [51].

Finally, our findings revealed that *Ae. aegypti* is the most common species along the increasing urbanization gradient and the unique *Aedes* species in urban settings thus suggesting particular attention on its egg laying patterns and population dynamics. *Aedes aegypti* is an urban species that preferentially feeds on humans [52] and is well adapted to live in close proximity to human habitats [53]. Such highly anthropophilic behaviour may enhance human-to-human transmission of arboviruses and trigger dengue and yellow fever outbreaks. The dominance of *Ae. aegypti* in still urbanizing and already urbanized areas of Africa is well documented [8, 9, 18] and is possibly due to its plastic oviposition behaviour allowing the colonization of natural and artificial environments [9, 42]. Otherwise, the rising occurrence of *Ae. aegypti* was also coupled with the increasing presence of another urban, anthropophilic and sympathric species, *Cx. quinquefasciatus* [54-56] and the lack of predators such as *Er. chrysogaster* [27] in the urban area. In contrast, the specimens of *Ae.*

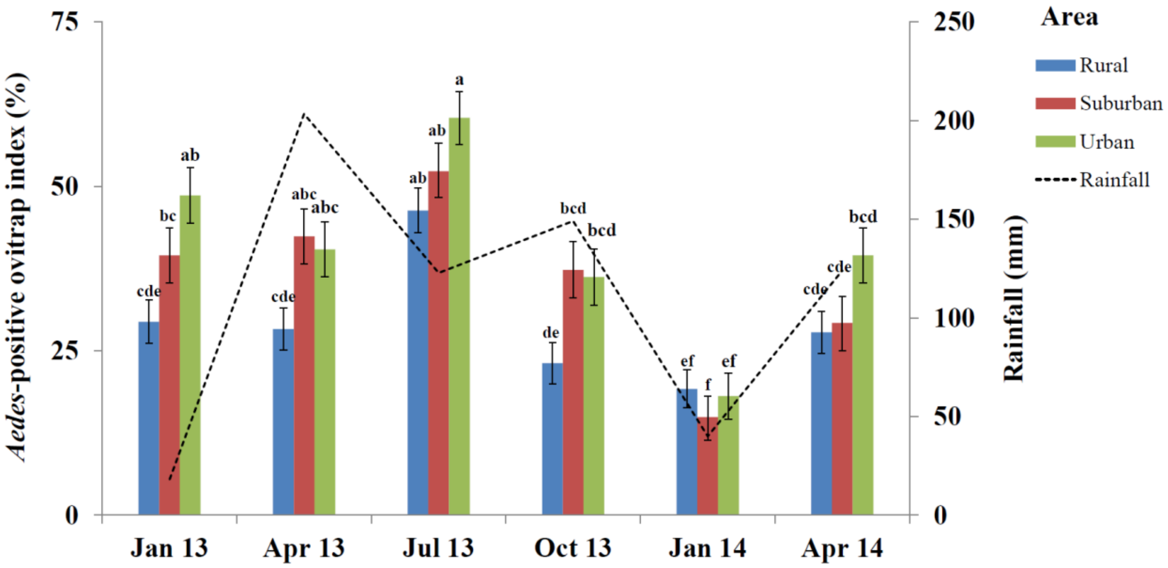
aegypti unexpectedly collected in the sylvatic zone are, perhaps, members of the *Aedes aegypti formosus*, the ancestral progenitor of *Aedes aegypti aegypti* and the only sylvan form known in West Africa [30, 31]. *Aedes aegypti formosus* has no white scales on the first abdominal tergite and a dark or black cuticle. This subspecies is exophilic, preferentially feeds on wild animals and breeds in natural containers such as tree holes [32, 57, 58], whereas, *Ae. aegypti aegypti* has scales on the first abdominal tergite and a lightly tanned cuticle and tends to be endo- and anthropophilic and breed in man-made containers [31]. However, contrary to East Africa [29, 30, 57], the scaling and behavioural patterns do not match with the discrete genetic differences in allozymes and microsatellites for *Ae. aegypti* collected in West Africa [13, 31, 33, 40, 59]. This results in confusion over morphological distinction between the two forms [13]. Due to these ambiguities, we were not able to confirm which *Ae. aegypti* form was represented among the sylvan specimens collected in the rural area. Above all, the urban and sylvan forms of *Ae. aegypti* are both competent arbovirus vectors in West Africa [13].

Urbanization continues at a rapid pace in Côte d'Ivoire, particularly in the southeastern part resulting in drastic segregation among *Aedes* species by favouring *Ae. aegypti* and restricting wild *Aedes* species to rural areas. These trends were paralleled by recurrent resurgences of yellow fever and dengue in recent years. However, yellow fever is historically well known as a key factor having forced the transfer of the colonial capital of Côte d'Ivoire from Grand-Bassam to Abidjan in 1899 [60]. Despite this historical and present background, the resurgence of yellow fever and dengue outbreaks is not resolved and their sporadic occurrence creates major public health concerns [60]. Between 2001–2007, 1,468 suspected, 41 confirmed and 26 fatal cases of yellow fever were reported. During the period of 2007–2001, 111 suspected with 31 confirmed and 43 deadly cases of yellow fever were notified. The incidence of yellow fever gradually increased and peaked in 2011 with 79 cases and 35 deaths. In 2008, 9 cases of yellow fever and 2 cases of dengue DENV-3 were recorded. In 2010, 13 confirmed and 2 fatal cases of yellow fever, and 1 deadly case of DENV-1 were reported. The strengthened warning systems and the operated vector control are usually performed in urban areas, mainly in Abidjan, the economic capital. Our study suggests that while vector control should focus on urban areas, rural areas are important as they may serve as transition zones for (re-)introduction of arboviral diseases through sylvatic bridge vectors. Because rural areas host various wild vectors, they act as a potential reservoir and originator of arboviruses from which urban areas are (re-)infected. Therefore, the rural areas also need to be considered when elaborating and applying arbovirus vector surveillance and control strategies. *Aedes* species control strategies could apply the lethal ovitrap [61] and autocidal [62] gravid ovitrap-based on mass trapping method.

5.6. Conclusions

In arbovirus foci of the southeastern Côte d'Ivoire, urbanized environment correlates with a substantially higher abundance in *Aedes* species and a regression of the *Aedes* wild species towards a unique presence of *Ae. aegypti*. *Aedes aegypti* is expected to drive arbovirus transmission in the urban areas, while other species probably serve as potential bridge vectors between sylvatic and urban cycles of human arboviral infections in the rural areas. Our findings provide valuable information on *Aedes* spp. ecology patterns in variously urbanized settings and therefore suggest that the rural areas also need to be considered when implementing arbovirus vector surveillance and control strategies.

5.7. Additional files



S5.1 Figure: Monthly variations in *Aedes* spp. positivity index in function of the rainfall period. Rainfall was unexpectedly high in April 2013. The highest precipitations (374 mm) occurred in June 2013. Error bars show the standard error (SE) of the mean of the positive ovitrap index. Groups that do not share a same letter are significantly different ($p < 0.05$).

Table S5.1: Seasonal variations in the number of emerged adult species of *Aedes* spp. in the rural, suburban and urban areas in southeastern Côte d'Ivoire

Species	**Rural**						**Suburban**						**Urban**					
	Jan-Apr 2013		**Jul-Oct 2013**		**Jan-Apr 2014**		**Jan-Apr 2013**		**Jul-Oct 2013**		**Jan-Apr 2014**		**Jan-Apr 2013**		**Jul-Oct 2013**		**Jan-Apr 2014**	
	n	**MO ± SE**	***n***	**MO ± SE**	***n***	**MO ± SE**	***n***	**MO ± SE**	***n***	**MO ± SE**	***n***	**MO ± SE**	***n***	**MO ± SE**	***n***	**MO ± SE**	***n***	**MO ± SE**
Aedes aegypti	603	0.50 ± 0.09	759	0.80 ± 0.10	392	0.45 ± 0.07	778	1.43 ± 0.15	843	1.55 ± 0.18	538	0.75 ± 0.13	1,308	2.55 ± 0.02	1,210	2.70 ± 0.19	580	1.03 ± 0.13
Aedes africanus	94	0.08 ± 0.03	105	0.09 ± 0.03	77	0.07 ± 0.03	0	0	0	0	0	0	0	0	0	0	0	0
Aedes dendrophilus	84	0.06 ± 0.03	79	0.08 ± 0.03	98	0.08 ± 0.03	0	0	0	0	0	0	0	0	0	0	0	0
Aedes metallicus	33	0.02 ± 0.02	3	0.00 ± 0.00	0	0	13	0.02 ± 0.02	0	0	19	0.02 ± 0.02	0	0	0	0	0	0
Aedes usambara	32	0.02 ± 0.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aedes fraseri	16	0.01 ± 0.01	1	0.00 ± 0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aedes luteocephalus	0	0	11	0.01 ± 0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aedes vittatus	0	0	0	0	0	0	55	0.06 ± 0.03	166	0.21 ± 0.06	28	0.03 ± 0.03	0	0	0	0	0	0
Aedes furcifer	31	0.02 ± 0.01	3	0.00 ± 0.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abundance	**893**	**0.85 ± 0.11**	**961**	**1.17 ± 0.12**	**567**	**0.69 ± 0.09**	**846**	**1.62 ± 0.15**	**1,009**	**2.08 ± 0.19**	**585**	**0.83 ± 0.14**	**1,308**	**2.55 ± 0.02**	**1,210**	**2.70 ± 0.19**	**580**	**1.03 ± 0.13**

Abbreviations: *n*, number of specimens; MO, mean number of specimens per ovitrap per week; SE, standard error of the mean

Abbreviations

GLMM, Generalized Linear Mixed Model; MO, mean number per ovitrap; DZ, domestic zone; PZ, peridomestic zone; SZ, sylvatic zone.

Ethics approval and consent to participate

The study protocol received approval from the local health and administrative authorities. In addition, all entomological surveys and sample collections carried out on private lands or private residential areas were done with the permission and written informed consent of the owners or residents. These studies did not involve endangered or protected species.

Consent for publication

Not applicable.

Availability of data and material

The datasets supporting the conclusions of this article are included within the article and its additional file.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

JBZZ, JU, MAA and BGK conceived and designed the surveys. JBZZ, MAA, YT and BGK performed the surveys. JBZZ and JU analysed the data. JU, PM, DM, YT and BGK contributed to the provision of materials, analysis and tools. JBZZ, JU, PM, DM and BGK wrote the paper. All authors read and approved the final manuscript.

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6. ARTICLE 2: Urbanization is a main driver for the larval ecology of *Aedes* mosquitoes in arbovirus-endemic settings in south-eastern Côte d'Ivoire

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Short title: Urbanization and *Aedes* mosquito larval ecology

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6.1. Abstract

Background

Failure in detecting naturally occurring breeding sites of *Aedes* mosquitoes can bias the conclusions drawn from field studies, and hence, negatively affect intervention outcomes. We characterized the habitats of immature *Aedes* mosquitoes and explored species dynamics along a rural-to-urban gradient in a West Africa setting where yellow fever and dengue co-exist.

Methodology

Between January 2013 and October 2014, we searched for immature *Aedes* mosquitoes in water containers in rural, suburban, and urban areas of south-eastern Côte d'Ivoire, using standardized sampling procedures. Immature mosquitoes were reared in the laboratory and adult specimens identified at species level.

Principal findings

We collected 6,159, 14,347, and 22,974 *Aedes* mosquitoes belonging to 17, 8, and 3 different species in rural, suburban, and urban environments, respectively. *Ae. aegypti* was the most prevalent species in all study areas, with higher abundance in urban areas (99.37%). Overall, 11 *Aedes* larval species not previously sampled in an oviposition study conducted in the same areas, were identified: *Ae. albopictus*, *Ae. angustus*, *Ae. apicoargenteus*, *Ae. argenteo-punctatus*, *Ae. haworthi*, *Ae. lilii*, *Ae. longipalpis*, *Ae. opok*, *Ae. palpalis*, *Ae. stokesi*, and *Ae. unilineatus*. *Aedes* breeding site positivity was associated with study area, container type, shade, vegetal detritus, water turbidity, geographic location, season, and the presence of predators. We found proportionally more positive breeding sites in urban (2,136/3,374; 63.3%), compared to suburban (1,428/3,069; 46.5%) and rural areas (738/2,423; 30.5%). In the urban setting, the predominant breeding sites were industrial containers, such as tires and discarded containers. In suburban areas, containers made of traditional material such as clay and wood were most frequently encountered. In rural areas, natural containers (rock holes, animal detritus, tree holes, fruit husks, and leaves) were common and represented 22.1% (163/738) of all *Aedes*-positive containers, hosting 18.7% of *Aedes* fauna. The proportion of positive containers was higher during the rainy season than during the dry season, and in peri-domestic sites compared to domestic sites. Predatory mosquitoes *Culex tigripes* were commonly sampled, while *Toxorhynchites* and *Eretmapodites* were mostly collected in rural areas.

Conclusions/significance

In south-eastern Côte d'Ivoire, urbanization is associated with high abundance of *Aedes* larvae and a predominance of artificial containers as breeding sites, mostly colonized by *Ae. aegypti* in urban areas. Nevertheless, natural containers are still common in rural areas harboring several *Aedes* species and, therefore, limiting the impact of systematic removal of discarded containers on the control of arbovirus diseases.

Author summary

Outbreaks of yellow fever and dengue caused by *Aedes* mosquitoes have been repeatedly reported in rural and urban areas in humid tropical Africa, including Côte d'Ivoire. Although controlling immature stages of *Aedes* mosquitoes in their aquatic habitats before they become adult vectors remains the best method to fight arboviral diseases, failure to identify the larval habitats can compromise intervention success. We studied the larval ecology of *Aedes* mosquitoes in different settings (rural, suburban, and urban) in Côte d'Ivoire. We found that the degree of urbanization was significantly associated with *Aedes* breeding sites. Compared with rural areas, urban and suburban areas were characterized by high numbers of *Aedes* mosquito breeding sites that were mostly artificial containers (e.g., tires and discarded containers), inhabited by the larvae of *Ae. aegypti*. In rural areas, natural containers (e.g., tree holes, bamboos, and rock holes) harbored several other *Aedes* species not found elsewhere. Our results suggest that removal of discarded containers - a common practice in arbovirus control programs - in urban areas does not suffice for controlling arboviral diseases because urban areas remain exposed to (re)infestation due to natural containers that host several *Aedes* species in rural areas. Additional vector control strategies, combining tools and methods, are required.

6.2. Introduction

Several *Aedes* species act as vectors of arboviral diseases, such as yellow fever, dengue, chikungunya, Rift valley fever, and Zika virus infections that are of public health relevance [1]. The transmission patterns of these arboviruses and their geographic expansion are expected to change due to environmental transformation, including urbanization [2, 3]. Besides yellow fever, other arboviruses are likely underestimated and underreported in Africa because of low awareness by health care providers, other prevalent non-malaria febrile illnesses, and lack of diagnostic tests and systematic surveillance [4]. Nevertheless, yellow fever, dengue (DENV1-4), chikungunya, and Zika viruses are currently circulating in West Africa through the sylvatic, rural, and epidemic cycles maintained by wild and urban vectors [5, 6]. Côte d'Ivoire has been repeatedly facing yellow fever and dengue outbreaks involving several vectors such as *Aedes africanus*, *Ae. furcifer*, *Ae. luteocephalus*, *Ae. opok*, and *Ae. vittatus* in rural, and *Ae. aegypti* in urban areas [7, 8]. These outbreaks have often occurred in foci characterized by high rates of urbanization due to economic development supported by palm oil and rubber farming, trade, and traffic [7].

Arboviral disease transmission is influenced by community-level effects of container-dwelling *Aedes* mosquito larvae by regulating the production and fitness of adult vectors [9]. *Aedes* mosquito larvae are highly sensitive to environmental changes, including urbanization [10]. Some *Aedes* species (e.g., *Ae. aegypti*) inhabit a wide variety of containers ranging from natural containers (e.g., tree and bamboo holes) to artificial containers (e.g., tires, discarded items, and other water containers) due to their ecologic plasticity [11], while others are restricted to specific breeding sites because of the higher sensibility of their offspring to environmental changes [12]. The ecologic plasticity allows *Ae. aegypti* and *Ae. albopictus* to spread worldwide by sea, air, and land transportations, and to adapt to new and changing environments [10].

The choice of breeding sites is governed by competition and predation among immature stages of *Aedes* and other mosquitoes that co-exist in the same breeding site [11, 12]. For example, intra- and interspecific competition between *Ae. aegypti* and *Ae. albopictus* [13] and among several *Aedes* species [12] has been reported. Moreover, mosquito species such as *Toxorhynchites* spp., *Eretmapodites* spp., and *Culex tigripes* predate on the larvae of *Aedes* mosquito species [12, 13]. The biotic factors may also interact with abiotic factors, such as the climate [13]. As larvae directly depend on water, precipitation is the most important physical factor. The complex patterns of flooding and drying of larval breeding sites govern arboviral transmission [14].

In Côte d'Ivoire, yellow fever has been a key factor that forced the transfer of the colonial capital from Grand-Bassam to Bingerville near Abidjan in 1899 [15]. However, more than a century later, yellow fever (and dengue) outbreaks still remain an unresolved public health issue [7, 8, 15]. During arbovirus epidemics, vector controls are mostly based on the systematic removal of artificial *Aedes* breeding sites in urban areas.

The most effective vector control strategy is the control of immature stages in their aquatic habitats [12]. Hence, effective larval control requires a deep understanding of larval ecology. Our study aimed to characterize the dynamics of *Aedes* larval breeding sites, species composition, and biological associations in terms of geographic and seasonal variations along a rural-to-urban gradient in south-eastern Côte d'Ivoire. As *Aedes* mosquito larvae are highly sensitive to environmental changes [10], we hypothesized that larval breeding sites differ in species composition between urban and rural areas.

6.3. Methods

6.3.1. Ethics statement

The study protocol received approval from the local health and other administrative authorities. In addition, all entomologic surveys and sample collections carried out on private lands or private residential areas were done with the permission and written informed consent of the residents. This study did not involve endangered or protected species.

6.3.2. Study area

The study was conducted in three areas located within a traditional arbovirus focus in south-eastern Côte d'Ivoire: Ehania-V1 (geographic coordinates 5° 18' N latitude, 3° 4' W longitude), Blockhauss (5° 19 N, 4° 0' W), and Treichville (5° 18 N, 4° 0' W), representing an increasing urbanization gradient (Figure 6.1). The degree of urbanization is characterized by land use, vegetation coverage, human population density, state of roads, and public services, as described in Zahouli et al. [16].

Natural and artificial containers such as tree holes, bamboo, fruit husks, tires, discarded items, and water storage receptacles that may serve as potential breeding sites for *Aedes* mosquitoes vary according to human habitation and activities. The rural area is surrounded by farms of palm oil trees (*Eleasis guineensis*) covering 11,444 ha and a preserved rainforest of 100 ha, while the suburban area is located about 2 km away from the Banco National Park with over 3,750 ha of rainforest. The rainforest is inhabited by a diverse fauna (e.g., primates and birds) that serve as hosts for *Aedes* mosquitoes.

The climate is characterized by high temperature and precipitation with two rainy seasons. The seasons are distinguished by rainfall rather than temperature. The main rainy season extends from May to July, while the shorter rainy season occurs from October to November, with distinct dry seasons in between. The average annual precipitation ranges from 1,200 to 2,400 mm. The annual average temperature and relative humidity are around 26.5 °C and 80-90%, respectively.

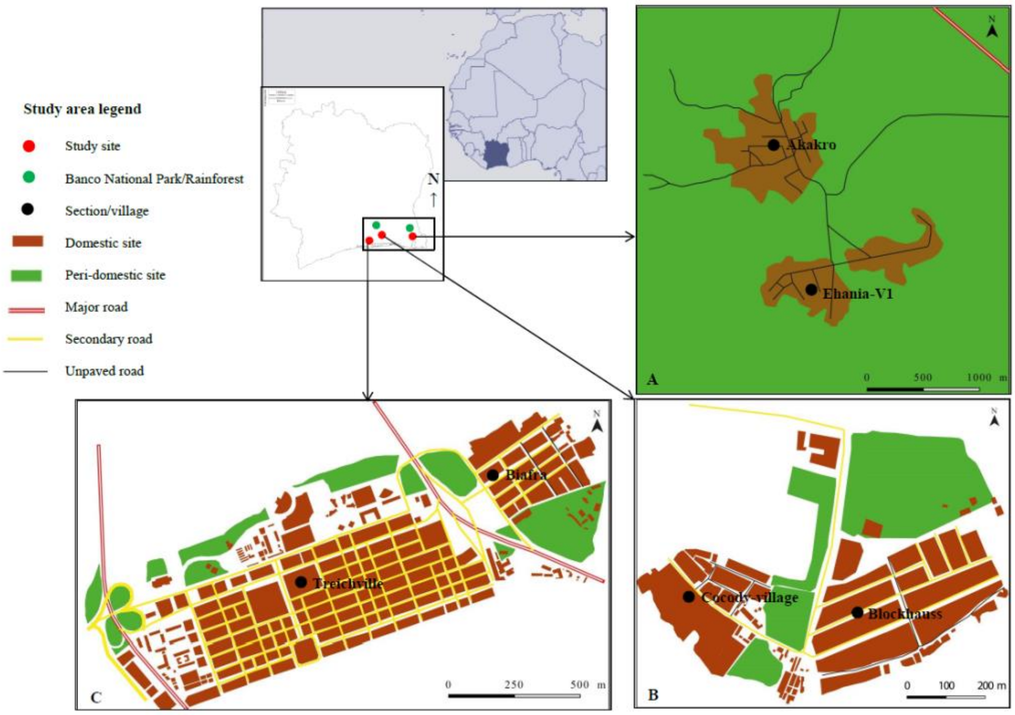


Figure 6.1: Location of the study areas in south-eastern Côte d'Ivoire. The larval breeding sites of *Aedes* mosquitoes were monitored in three areas: Ehania-V1 (A), Blockhauss (B), and Treichville (C), representing rural, suburban, and urban settings, respectively. The study site of Ehania-V1 includes the villages of Ehania-V1 and Akakro and represents the rural area without major and secondary paved roads. The site is in close proximity to a primary rainforest. The study site of Blockhauss covers the villages of Blockhauss and Petit-Cocody and represents the suburban area with only secondary paved roads. It is about 5 km away from the rainforest of the Banco National Park. The study site of Treichville comprises the sections of Jacques-Aka and Biafra and is an urban area with numerous major and secondary paved roads. It is located in the center of Abidjan and is separated from Blockhauss by the Ebrié lagoon.

6.3.3. Study design

Aedes larval breeding sites were sampled quarterly in domestic (space inhabited by humans) and peri-domestic (surrounding vegetated environment within a 600 m radius from the domestic areas) sites in rural, suburban, and urban areas from January 2013 to October 2014. While water-holding containers, tree holes, and bamboo were repeatedly sampled, other potential breeding sites were sampled for the presence of immature stages of *Aedes* mosquitoes. All accessible properties were surveyed simultaneously in the three settings. Some properties could not be sampled because the residents refused to provide access or because there were physical barriers of access.

6.3.4. Characterization of Aedes *breeding sites*

Potential larval breeding sites of *Aedes* mosquitoes were sampled in all three study sites by teams consisting of four trained mosquito collectors in each study area. Each mosquito collector team was composed of the same persons during all surveys. The number of these experienced mosquito collectors were constant on any one day in each study area, whereas the teams made rotations from one study area to another in order to ensure similar sampling efforts and efficiency in all the three study areas, and minimize potential sampling bias. The collectors worked from 8:00 to 16:00 hours, and spent proportionally equal time period searching for potential mosquito breeding sites in the study areas.

Readily visible and accessible containers in the selected households and surrounding premises were examined for the presence of water and mosquito larvae. In a preliminary survey, existing larval breeding sites, such as natural and artificial cavities or containers with a potential to contain water were kept in an inventory and assigned a unique label. Based on this preliminary survey, potential breeding sites were classified into two categories, three sub-categories and 16 types, depending on their location, origin, material, and container type (Table 6.1 and S6.1 Figure). The breeding sites were assessed for abiotic and biotic characteristics, including geographic location (domestic and peri-domestic sites), color, exposure to sunlight (full shade, no exposure to sunlight; partial shade, partial exposure to sunlight; no shade, permanent exposure to sunlight), turbidity (transparent/clear, colored, opaque), substrate type (no substrate, foliage, moss, soil), surface of water, depth, presence of mosquito larvae and predators (larvae of *Cx. tigripes*, *Eretmapodites*, spp., and *Toxorhynchites* spp., mosquitoes, and toad tadpoles, and arachnids).

6.3.5. Mosquito sampling

Larvae and pupae of *Aedes* mosquitoes were sampled using the World Health Organization (WHO) standard equipment adapted to the aperture and the depth of larval habitats. A flexible collection tube connected to a manual suction pump was used to sample water from bromeliads and bamboo holes. Scoops of 350 ml capacity were used to collect immature mosquitoes from larger breeding sites (e.g., tree holes, recipients, tires, and puddles). The collected *Aedes* mosquito were counted using a pipette and classified as young larvae (1-2 instar), old larvae (3-4 instar), and pupae. Non-*Aedes* mosquito larvae such as *Anopheles* spp., *Coquelitidia* spp., *Culex* spp., *Eretmapodites* spp., *Filcabia* spp., *Toxorhynchites* spp., and *Uranotenia* spp. were also recorded.

Table 6.1: Classification of *Aedes* mosquito breeding sites sampled in south-eastern Côte d'Ivoire from January 2013 to October 2014

N°	Breeding site	Definition
I	**Natural**[a,b]	**Containers created without or by indirect intervention of humans**
A	Rock hole[c]	Irregular and shallow shapes of massive stone of different sizes well exposed to sunlight
B	Animal detritus[c]	Debris of animal such as snail shells (carapaces of *Achatina* spp.) and animal bones
C	Tree hole[c]	Rot and pan holes of different shapes and volume located from 0 to 2 m above the ground level
D	Bamboo[c]	Cut of fixed masses of bamboos (*Bambusae*) and bars of bamboos used as fences
E	Leaf[c]	Sheathing leaf axils from plants such as bromeliads (*Ananas* spp.), bananas (*Musa* spp.) and taros (*Colocasia* spp.), and fallen sheets on the floor
F	Fruit husk[c]	Skins of the coconuts (*Cocos* spp.) and the flowers of bananas (*Musa* spp.)
II	**Artificial**[a]	**Containers created by direct intervention of humans**
II-1	**Traditional**[b]	**Handcrafted items**
G	Clay pot[c]	Ceramic containers made of clay by a potter
H	Wood-container[c]	Containers fabricated of woods such as mortars, calabashes, boats and statutes
I	Metallic pot[c]	Containers made of metals such cooking pots made by smiths
II-2	**Industrial**[b]	**Containers manufactured by factories**
J	Tarp[c]	Plastic sheets left at the ground or covering house roofs holding puddles (temporary small water collections) formed after rainfall
K	Discarded container[c]	Human wastes such as broken plastic bottles, bowls, metal boxes, used cans, vases, coolers, refrigerators, shoes and toys
L	Tire[c]	Bicycle, vehicle, and machine wheels left outdoors
M	Vehicle tank[c]	Reservoirs of abandoned cars and machines
N	Vehicle carcasses[c]	Plastic and metallic debris of abandoned cars and machines
O	Building tool[c]	Materials used to build and improve the houses such air conditioner, bricks, metal sheets, toilets and flower pots
P	Water storage container[c]	Plastic and metallic receptacles used to store potable water

[a,b,c]: The inspected containers were grouped into the 2 categories[a] , 3 sub-categories[b] and 16 types[c] as defined above. The container type often reflects the name of the container.

The predacious larvae of mosquitoes, such as *Cx. tigripes*, *Eretmapodites* spp., and *Toxorhynchites* spp. were removed from the samples to avoid predation on the other species and preserved separately. All mosquito samples were stored separately in plastic boxes and transported in an icebox to a field laboratory.

6.3.6. Laboratory procedures

In the laboratory, mosquito larvae were reared until they reached the adult stage. In order to minimize mortality, a maximum of 20 larvae were placed in 200 ml plastic cups, filled with 150 ml distilled water and covered with netting. Larvae of *Aedes* and other mosquitoes were fed each morning between 07:00 and 08:00 hours with Tetramin Baby Fish Food. Predacious larvae of *Toxorhynchites* spp. and *Cx. tigripes* were fed with larvae from colonies specially sampled from the study areas. Emerging adult mosquitoes were identified to species level using a morphological key [17]. As larval mortalities were low, the proportion of mosquito species was estimated on the basis of emerging adults. Adult specimens were stored by species and recorded in an entomology collection database.

6.3.7. Statistical analysis

The frequency of *Aedes*-positive breeding sites (FP) was calculated as the percentage of water holding containers with at least one larva or pupa (numerator) among the wet containers (denominator). The proportion of *Aedes*-positive breeding site types among the *Aedes*-positive breeding sites (PP) was expressed as the percentage of each *Aedes*-positive container type (numerator) among the total *Aedes*-positive containers (denominator) in each study area. To test whether there was a difference in the number of positive breeding sites and the number of available wet containers in each category, we used Fisher's exact and a χ^2 tests, as appropriate, to test for differences in the frequency of *Aedes*-positive breeding sites across the three study areas and between the domestic and peri-domestic sites, and between dry and rainy seasons.

Aedes species proportions were calculated as the percentage of specimens belonging to the genus *Aedes* for each study area and then compared between breeding sites as above. Larval abundances of *Aedes* mosquitoes were standardized as the mean numbers of larvae per liter of water, expressed as the geometric mean, known as Williams' mean (i.e., log[number of mosquito larvae + 1]) [18], and compared using the Kruskal-Wallis test, followed by Mann-Whitney. The Mann-Whitney U test was also performed to compare pairs of study areas when the Kruskal-Wallis H test showed a significant difference or only two habitats. *Aedes* species richness was defined as the number of collected species in each study area and compared using a one-way analysis of variance (ANOVA), followed by the Tukey post-hoc test for post-hoc pairwise comparisons [19]. *Aedes* species diversity and dominance were estimated using the Shannon-Weaver index [20] and Simpson index [21] and analyzed using a Kruskal-Wallis test. Kruskal-Wallis test was performed because a test for normality showed a significant difference in the variances after log-transforming the data. A significance level of 5% was set for statistical testing. All statistical analyses were conducted using Stata version 14.0 (Stata Corporation; College Station, TX, United States of America).

6.4. Results

6.4.1. Mosquito species composition

Table 6.2 shows the species composition of adult mosquitoes that emerged from the larvae and pupae sampled from the breeding sites along the rural-urban gradient in south-eastern Côte d'Ivoire and reared after transfer to the laboratory. In total, 7,661, 16,931, and 26,968 adult mosquitoes emerged from the collected larvae in rural, suburban, and urban areas, respectively. The rural setting had the highest mosquito species diversity (eight genera and 37 species), followed by the suburban setting (four genera and 14 species), and the urban setting (three genera and nine species). The genus *Aedes* predominated throughout, with proportions of 80.40% (n = 7,661) in rural, 84.75% (n = 16,931) in suburban, and 85.19% (n = 26,968) in urban settings. The rural setting had the largest number of *Aedes* species (17 species), followed by the suburban (eight species) and urban settings (three species).

The predacious mosquito species *Cx. tigripes* was sampled in each of the three study settings, while the predators *Eretmapodites chrysogaster*, *Eretmapodites inornatus*, and *Toxorhynchites brevipalpis* were primarily collected in rural settings. Moreover, several other vector competent mosquito species, namely *Anopheles coustani*, *An. gambiae*, *Coquelettidia fuscopennata*, *Cx. quinquefasciatus*, and *Cx. poicilipes* were sampled.

Table 6.2: Species composition of emerged adult mosquitoes collected as larvae in the rural, suburban and urban areas in arbovirus-endemic areas in south-eastern Côte d'Ivoire from January 2013 to October 2014

Genus	**Species**	**Rural**				**Suburban**				**Urban**			
		F	**M**	**T**	**%**	**F**	**M**	**T**	**%**	**F**	**M**	**T**	**%**
Aedes	*Ae. aegypti*	2331	2296	4627	60.40	6651	6827	13478	79.61	11526	11303	22829	84.65
	Ae. africanus	69	74	143	1.87	0	0	0	0.00	0	0	0	0.00
	Ae. albopictus	0	0	0	0.00	0	0	0	0.00	2	0	2	0.01
	Ae. angustus	14	19	33	0.43	23	20	43	0.25	0	0	0	0.00
	Ae. apicoargenteus	4	1	5	0.07	0	0	0	0.00	0	0	0	0.00
	Ae. argenteopunctatus	1	1	2	0.03	0	0	2	0.01	0	0	0	0.00
	Ae. dendrophilus	122	114	236	3.08	5	1	6	0.04	0	0	0	0.00
	Ae. furcifer	134	145	279	3.64	3	3	6	0.04	0	0	0	0.00
	Ae. haworthi	0	0	0	0.00	23	28	51	0.30	0	0	0	0.00
	Ae. lilii	41	33	74	0.97	0	0	0	0.00	0	0	0	0.00
	Ae. longipalpis	7	4	11	0.14	0	0	0	0.00	0	0	0	0.00
	Ae. luteocpehalus	49	43	92	1.20	0	0	0	0.00	0	0	0	0.00
	Ae. metallicus	41	38	79	1.03	7	11	18	0.11	0	0	0	0.00
	Ae. opok	19	24	43	0.56	0	0	0	0.00	0	0	0	0.00
	Ae. palpalis	126	118	244	3.18	0	0	0	0.00	0	0	0	0.00
	Ae. stokesi	0	2	2	0.03	0	0	0	0.00	0	0	0	0.00
	Ae. unilineatus	41	33	74	0.97	0	0	0	0.00	0	0	0	0.00
	Ae. usambara	18	23	41	0.53	0	0	0	0.00	0	0	0	0.00
	Ae. vittatus	91	83	174	2.27	384	359	743	4.39	65	78	143	0.53
	Total	**3108**	**3051**	**6159**	**80.40**	**7096**	**7251**	**14347**	**84.75**	**11593**	**11381**	**22974**	**85.19**
Anopheles	*An. coustani*	1	2	3	0.04	0	0	0	0.00	0	0	0	0.00
	An. gambiae	41	37	78	1.02	46	37	83	0.49	63	68	131	0.48
	An. pharoensis	10	7	17	0.22	6	2	8	0.05	1	0	1	0.01
	An. rufipes	0	1	1	0.01	0	0	0	0.00	0	0	0	0.00
	An. ziemani	6	7	13	0.17	0	0	0	0.00	0	0	0	0.00
	Total	**58**	**54**	**112**	**1.46**	**52**	**39**	**91**	**0.54**	**64**	**68**	**132**	**0.49**
Coquelitidia	*Cq. cristata*	1	3	4	0.05	0	0	0	0.00	0	0	0	0.00
	Cq. fuscopennata	3	0	3	0.04	0	0	0	0.00	0	0	0	0.00
	Total	**4**	**3**	**7**	**0.09**	**0**	**0**	**0**	**0.00**	**0**	**0**	**0**	**0.00**
Culex	*Cx. annulioris*	5	2	7	0.09	0	0	0	0.00	0	0	0	0.00
	Cx. cinereus	48	46	94	1.23	0	0	0	0.00	0	0	0	0.00
	Cx. decens	12	17	29	0.38	10	13	23	0.14	7	11	18	0.07
	Cx. nebulosus	56	42	98	1.28	39	34	73	0.42	23	18	41	0.14
	Cx. poicilipes	137	126	263	3.43	0	0	0	0.00	0	0	0	0.00
	Cx. quinquefasciatus	321	297	618	8.07	1165	1099	2264	13.37	1761	1873	3634	13.48
	Cx. tigripes	34	39	73	0.95	59	72	131	0.77	78	91	169	0.63
	Total	**613**	**569**	**1182**	**15.43**	**1273**	**1218**	**2491**	**14.70**	**1869**	**1993**	**3862**	**14.32**
Eretmapodites	*Er. chrysogaster*	57	66	123	1.61	2	0	2	0.01	0	0	0	0.00
	Er. inornatus	3	5	8	0.10	0	0	0	0.00	0	0	0	0.00
	Er. quinquevittatus	9	4	13	0.17	0	0	0	0.00	0	0	0	0.00
	Total	**69**	**75**	**144**	**1.88**	**2**	**0**	**2**	**0.01**	**0**	**0**	**0**	**0.00**
Filcabia	*Fi. circumtesta*	0	1	1	0.01	0	0	0	0.00	0	0	0	0.00
	Total	**0**	**1**	**1**	**0.01**	**0**	**0**	**0**	**0.00**	**0**	**0**	**0**	**0.00**
Toxorhynchites	*Tx. brevipalpis*	28	18	46	0.60	0	0	0	0.00	0	0	0	0.00
	Tx. lutescens	4	3	7	0.09	0	0	0	0.00	0	0	0	0.00
	Total	**32**	**21**	**53**	**0.69**	**0**	**0**	**0**	**0.00**	**0**	**0**	**0**	**0.00**
Uranotenia	*Ur. mashonensis*	2	1	3	0.04	0	0	0	0.00	0	0	0	0.00
	Total	**2**	**1**	**3**	**0.04**	**0**	**0**	**0**	**0.00**	**0**	**0**	**0**	**0.00**
Total		**3886**	**3775**	**7661**	**100**	**8423**	**8508**	**16931**	**100**	**13526**	**13442**	**26968**	**100**

F: Female, M: Male, T: total numbers of mosquitoes. %: proportion in percentage (%) of specimens of mosquitoes.

6.4.2. Ecological characterization of Aedes *species and breeding sites*

Table 6.3 summarizes the species composition of *Aedes* mosquitoes collected as larvae among different types of breeding sites in the rural, suburban and urban areas. *Ae. aegypti* and *Ae. vittatus* were commonly encountered in the three settings. *Ae. aegypti* was the most prevalent species in the all study areas, and exhibited rising abundance from rural (n = 6,159; 75.12%) to suburban (n = 14,347; 93.94%) and urban (n = 22,974; 99.37%) areas. Besides, the highest prevalence of *Ae. vittatus* (5.18%) was found in suburban areas. In rural areas, *Ae. furcifer* (4.53%), *Ae. palpalis* (3.96%), *Ae. dendrophilus* (3.83%), *Ae. vittatus* (2.83%), *Ae. africanus* (2.31%), *Ae. luteocephalus* (1.49%), *Ae. metallicus* (1.28%), *Ae. lilii* (1.22%), and *Ae. unilineatus* (1.20%) were collected at frequencies above 1%. We also found two specimens of *Ae. albopictus* (0.01%) in the urban settings.

The presence of *Aedes* mosquito larvae in breeding sites significantly varied by species (Table 6.3). For example, *Ae. aegypti* were found in all types of *Aedes*-positive breeding sites sampled in all the three study areas. Moreover, *Ae. dendrophilus*, *Ae. furcifer*, and *Ae. luteocephalus* were found in all container types in the rural areas, while *Ae. vittatus* and *Ae. metallicus* were collected from both natural and artificial containers in the suburban areas. *Ae. africanus*, *Ae. lilii*, *Ae. unilineatus*, and *Ae. usambara* were mostly present in natural containers such as tree holes, bamboo, and fruit husks in rural settings.

6.4.3. Associations among different Aedes *species*

Several species were found together in the same breeding sites. For example, *Ae. aegypti*, *Ae. dendrophilus*, *Ae. furcifer*, and *Ae. africanus* shared the same breeding sites in the rural areas, whereas *Ae. aegypti* co-existed with *Ae. vittatus* in suburban settings (n = 1,295; 12.8%). These two species co-occurred, albeit at low frequency (n = 57; 0.3%) in urban breeding sites. Additionally, *Cx. quinquefasciatus* and *An. gambiae* were often collected together with *Ae. aegypti* in tires and discarded containers in peri-domestic environments in the three study areas.

Mosquito predators, such as *Cx. tigripes*, *Er. chrysogaster*, and *Tx. brevipalpis* were found in the same breeding sites as *Ae. aegypti*, *Ae. dendrophilus*, *Ae. furcifer*, and *Ae. africanus* in rural settings. These ecologic associations were most present in tree holes, discarded containers and tires in the rural areas and in peri-domestic breeding sites in the rainy season.

Table 6.3: Proportions (%) of *Aedes* mosquito species collected as larvae among different types of breeding sites in the rural, suburban and urban areas in south-eastern Côte d'Ivoire from January 2013 to October 2014

Species	**Natural[a,b]**							**Artificial[a]**												**Total**	**Total**
								Traditional[b]				**Industrial[b]**									
	Rock[c]	**Anim[c]**	**Leaf[c]**	**Husk[c]**	**Bamb[c]**	**Tree[c]**	**Total**	**Clay[c]**	**Wood[c]**	**Metal[c]**	**Total**	**Tarp[c]**	**Disca[c]**	**Tire[c]**	**Tank[c]**	**Carca[c]**	**build[c]**	**Stora[c]**	**Total**		
Rural																					
Ae. aegypti	0.41	0.47	0.54	4.58	1.27	1.23	8.49	4.34	1.64	3.41	9.38	3.88	12.97	26.04	3.52	6.10	2.24	2.48	57.25	66.63	75.12
Ae. africanus	0.00	0.00	0.00	0.50	0.70	1.12	2.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.00	2.31
Ae. angustus	0.05	0.00	0.00	0.03	0.00	0.10	0.18	0.00	0.00	0.00	0.00	0.00	0.05	0.31	0.00	0.00	0.00	0.00	0.36	0.36	0.54
Ae. apicoargenteus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.03	0.08	0.08
Ae. argenteopunctatus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.03	0.03	0.03
Ae. dendrophilus	0.00	0.13	0.26	0.18	0.15	0.29	1.01	0.05	0.26	0.11	0.42	0.15	0.62	1.56	0.00	0.08	0.00	0.00	2.40	2.83	3.83
Ae. furcifer	0.32	0.00	0.00	0.00	0.31	0.75	1.38	0.00	0.00	0.00	0.00	0.52	0.18	1.74	0.26	0.45	0.00	0.00	3.15	3.15	4.53
Ae. lilii	0.00	0.00	0.17	0.60	0.08	0.05	0.90	0.00	0.00	0.00	0.00	0.05	0.05	0.16	0.00	0.06	0.00	0.00	0.32	0.32	1.22
Ae. longipalpis	0.00	0.00	0.00	0.11	0.05	0.02	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18
Ae. luteocephalus	0.00	0.05	0.00	0.18	0.00	0.11	0.34	0.00	0.00	0.00	0.00	0.05	0.19	0.68	0.00	0.23	0.00	0.00	1.15	1.15	1.49
Ae. metallicus	0.08	0.00	0.00	0.00	0.00	0.05	0.13	0.47	0.08	0.34	0.89	0.00	0.06	0.19	0.00	0.00	0.00	0.00	0.26	1.15	1.28
Ae. opok	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.03	0.00	0.62	0.00	0.00	0.00	0.00	0.65	0.65	0.70
Ae. palpalis	0.70	0.00	0.06	0.15	0.21	0.71	1.83	0.29	0.15	0.00	0.44	0.29	0.34	0.94	0.00	0.11	0.00	0.00	1.69	2.13	3.96
Ae. stokesi	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
Ae. unilineatus	0.00	0.03	0.00	0.13	0.08	0.28	0.52	0.26	0.05	0.18	0.49	0.00	0.00	0.15	0.00	0.05	0.00	0.00	0.19	0.68	1.20
Ae. usambara	0.00	0.00	0.00	0.00	0.42	0.24	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67
Ae. vittatus	0.21	0.00	0.00	0.00	0.10	0.39	0.70	0.10	0.23	0.15	0.47	0.44	0.34	0.62	0.08	0.06	0.11	0.00	1.66	2.13	2.83
Total	**1.77**	**0.68**	**1.03**	**6.46**	**3.37**	**5.40**	**18.7**	**5.56**	**2.41**	**4.19**	**12.14**	**5.40**	**14.83**	**33.04**	**3.86**	**7.14**	**2.35**	**2.48**	**69.14**	**81.29**	**100**
Suburban																					
Ae. aegypti	na	0.06	0.00	0.47	0.24	0.00	0.77	2.47	1.15	2.52	6.14	2.83	26.21	42.70	2.10	8.21	1.92	3.06	87.03	**93.17**	93.94
Ae. angustus	na	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.06	0.15	0.00	0.05	0.10	0.00	0.00	0.00	0.00	0.15	**0.30**	0.30
Ae. argenteopunstatus	na	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	0.01
Ae. dendrophilus	na	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.03	**0.03**	0.04
Ae. furcifer	na	0.00	0.00	0.04	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	0.04
Ae. haworthi	na	0.00	0.00	0.00	0.00	0.02	0.02	0.16	0.01	0.00	0.17	0.08	0.00	0.07	0.00	0.01	0.00	0.00	0.16	**0.33**	0.36
Ae. metallicus	na	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.08	0.10	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.02	**0.13**	0.13
Ae. vittatus	na	0.08	0.00	0.04	0.15	0.00	0.27	0.53	0.27	0.78	1.58	0.62	0.47	1.81	0.03	0.00	0.30	0.09	3.32	**4.91**	5.18
Total	na	**0.14**	**0.01**	**0.55**	**0.39**	**0.03**	**1.12**	**3.25**	**1.45**	**3.44**	**8.14**	**3.53**	**26.73**	**44.73**	**2.13**	**8.22**	**2.22**	**3.15**	**90.71**	**98.87**	**100**
Urban																					
Ae. aegypti	na	0.00	0.00	0.18	0.13	na	0.32	0.45	0.41	1.11	1.97	1.97	24.49	60.14	2.67	3.83	0.67	3.32	97.08	**99.05**	99.37
Ae. albopictus	na	0.00	0.00	0.00	0.00	na	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.01	**0.01**	0.01
Ae. vittatus	na	0.00	0.00	0.03	0.05	na	0.08	0.10	0.00	0.00	0.10	0.24	0.03	0.15	0.00	0.00	0.00	0.02	0.44	**0.54**	0.62
Total	**na**	**0.00**	**0.00**	**0.21**	**0.18**	na	**0.40**	**0.55**	**0.41**	**1.11**	**2.07**	**2.21**	**24.52**	**60.30**	**2.67**	**3.83**	**0.67**	**3.34**	**97.53**	**99.60**	**100**

%: percentage; [a,b,c]: The inspected containers were grouped into the 2 categories[a] , 3 sub-categories[b] and 16 types[c] as indicated above. The container type often reflects the name of the container. The total numbers of specimens of *Aedes* mosquitoes collected were 6,159, 14,347 and 22,974 specimens in the rural, suburban and urban areas, respectively. Rock: rock holes, Anim: animal detritus, Husk: fruit husks, Bamb: bamboo holes, Tree: tree holes, Clay: clay pots, Wood: wood-containers, Metal: metallic pots, Disca: discarded containers, Tank: vehicle tanks, Carca: vehicle carcasses, Building: Building tool, Stora: water storage containers.

6.4.4. Aedes *breeding site positivity*

Among 3,569, 4,882, and 5,783 containers inspected in rural, suburban and urban settings, 2,423, 3,069, and 3,374 were wet, respectively. The urban setting had a significantly higher *Aedes*-positive breeding site rate (2,136/3,374; FP = 63.3%) as compared to suburban (1,428/3,069; FP = 46.5%) and rural settings (738/2,423; FP = 30.5%) ($\chi^2 = 478.9$, df = 2, $p < 0.05$) (S6.1 Table). The Mann-Whitney U-test indicated that the abundance of immature *Aedes* mosquitoes in one study area was significantly different compared to another. A significantly higher abundance of immature *Aedes* mosquitoes was found in urban areas with larval densities of 1.26 ± 0.01 larvae/l, followed by the suburban areas with 0.77 ± 0.01 larvae/l and rural areas with 0.42 ± 0.01 larvae/l ($\chi^2 = 663.3$, df = 2, $p < 0.001$) (Table 6.4). Urban setting show significantly higher proportions of pupae (n = 23,126; 14.9%) and 3-4 instar larvae compared to rural setting with 9.6% (n = 6,212) of pupae and 47.8% of 3-4 instar larvae ($P < 0.05$). The presence of immature *Aedes* mosquitoes was significantly associated with the sites, seasons, breeding site types and categories, substrates, color, vegetal detritus, shade, water turbidity, and predators ($P < 0.05$).

6.4.5. Dynamics of Aedes *breeding sites*

Figure 6.2 shows that the *Aedes*-positive microhabitat rate varied widely from one breeding site type to another in all three areas. The rural area showed the largest variability in *Aedes* breeding sites grouped into 16 types, followed by the suburban and urban areas presenting 15 and 12 microhabitat types, respectively. S6.1 Table indicates that immature *Aedes* mosquitoes were found in both natural (163/738; PP = 22.1%) and artificial (575/738; PP = 77.9%) breeding sites in the rural, and mostly in artificial breeding sites in the suburban (1,405/1,428; PP = 98.4%) and urban (2,129/2,136; PP = 99.7%) areas, including higher proportions of industrial containers in the urban areas (2,066/2,136; PP = 96.7%). In the rural areas, the main *Aedes*-positive breeding sites represented natural types, such as three holes (62/69; FP = 89.9%), bamboo (17/45; FP = 37.8%), and fruit husks (59/195; FP = 30.3%), traditional containers such as metallic (27/44; FP = 61.4%) and clay pots (44/101; FP = 43.6%) and wood-containers (24/69; FP = 34.8%); and industrial containers such as tarps (41/66; FP = 62.1%), tires (183/324; FP = 56.5%), vehicle tanks (41/84; FP = 48.8%), discarded containers (104/254; FP = 40.9%), and vehicle carcasses (68/171; FP = 52.0%).

Table 6.4: Dynamics of *Aedes* mosquito species collected as larvae among breeding sites in the rural, suburban and urban areas in south-eastern Côte d'Ivoire

Term	**Rural**				**Suburban**				**Urban**			
	Abundance (Mean ± SE)	**Richness**	**Shannon's diversity index**	**Simpson's dominance index**	**Abundance (Mean ± SE)**	**Richness**	**Shannon's diversity index**	**Simpson's dominance index**	**Abundance (Mean ± SE)**	**Richness**	**Shannon's diversity index**	**Simpson's dominance index**
Areas	0.42 ± 0.01	17	1.64	0.57	0.77 ± 0.01	8	0.38	0.89	1.26 ± 0.01	3	0.06	0.99
Site												
Peri-domestic	0.65 ± 0.02	16	1.69	0.55	1.39 ± 0.02	8	0.39	0.89	2.10 ± 0.01	3	0.04	0.99
Domestic	0.13 ± 0.01	14	1.20	0.70	0.23 ± 0.01	3	0.35	0.88	0.88 ± 0.37	2	0.13	0.97
Breeding site												
Rock hole	0.22 ± 0.08	6	2.18	0.26	na	na	na	na	na	na	na	na
Animal detritus	0.12 ± 0.04	4	1.41	0.52	0.78 ± 0.30	2	0.99	0.51	0.00 ± 0.00	0	na	na
Leaf axil	0.10 ± 0.03	4	1.68	0.36	0.06 ± 0.06	1	0.00	1.00	0.00 ± 0.00	0	na	na
Fruit husk	0.44 ± 0.04	9	1.62	0.52	0.71 ± 0.15	3	0.76	0.73	0.67 ± 0.29	2	0.54	0.78
Bamboo	0.34 ± 0.06	10	2.64	0.22	0.51 ± 0.21	2	0.96	0.53	0.78 ± 0.26	2	0.85	0.60
Tree hole	0.98 ± 0.05	15	3.13	0.14	0.21 ± 0.14	2	0.97	0.52	na	na	na	na
Natural	**0.32 ± 0.02**	**15**	**2.75**	**0.25**	**0.46 ± 0.18**	**6**	**1.31**	**0.52**	**0.02 ± 0.01**	**2**	**0.72**	**0.68**
Clay pot	0.58 ± 0.06	7	1.23	0.62	0.74 ± 0.07	4	1.09	0.61	0.99 ± 0.12	2	0.70	0.69
Wood	0.50 ± 0.08	6	1.04	0.67	0.63 ± 0.08	4	0.87	0.66	1.06 ± 0.19	1	0.00	1.00
Metallic pot	1.23 ± 0.10	5	1.04	0.67	0.83 ± 0.06	4	1.04	0.59	0.88 ± 0.09	1	0.00	1.00
Traditional	**0.67 ± 0.04**	**7**	**1.31**	**0.61**	**0.92 ± 0.07**	**5**	**1.07**	**0.61**	**1.02 ± 0.14**	**2**	**0.29**	**0.90**
Tarp	0.80 ± 0.08	8	1.50	0.53	0.60 ± 0.06	3	0.83	0.67	0.88 ± 0.08	2	0.49	0.81
Discarded	0.73 ± 0.04	10	0.88	0.77	0.99 ± 0.02	3	0.15	0.96	1.83 ± 0.02	2	0.01	0.99
Tire	1.20 ± 0.04	12	1.37	0.63	1.78 ± 0.02	6	0.30	0.91	2.30 ± 0.02	3	0.03	0.99
Vehicle tank	0.63 ± 0.07	3	0.50	0.84	1.45 ± 0.10	2	0.12	0.97	1.93 ± 0.06	1	0.00	1.00
Vehicle carcasses	0.51 ± 0.04	8	0.95	0.73	0.89 ± 0.04	2	0.01	0.99	1.36 ± 0.06	1	0.00	1.00
Building tools	0.70 ± 0.11	2	0.28	0.91	1.01 ± 0.08	2	0.57	0.77	0.91 ± 0.10	1	0.00	1.00
Water storage	0.03 ± 0.01	1	0.00	1.00	0.07 ± 0.01	2	0.19	0.94	0.13 ± 0.01	1	0.06	0.99
Industrial	**0.80 ± 0.02**	**13**	**1.16**	**0.69**	**1.32 ± 0.01**	**6**	**0.27**	**0.92**	**2.12 ± 0.01**	**3**	**0.04**	**0.99**
Artificial	**0.70 ± 0.02**	**13**	**1.23**	**0.68**	**1.17 ± 0.01**	**6**	**0.36**	**0.89**	**2.07 ± 0.01**	**3**	**0.06**	**0.99**

SE: Standard error of the mean number of mosquitoes per liter of water. The abundance is expressed as the mean number of *Aedes* mosquito larvae per liter of water (Larve/liter) and calculated as Williams' mean.

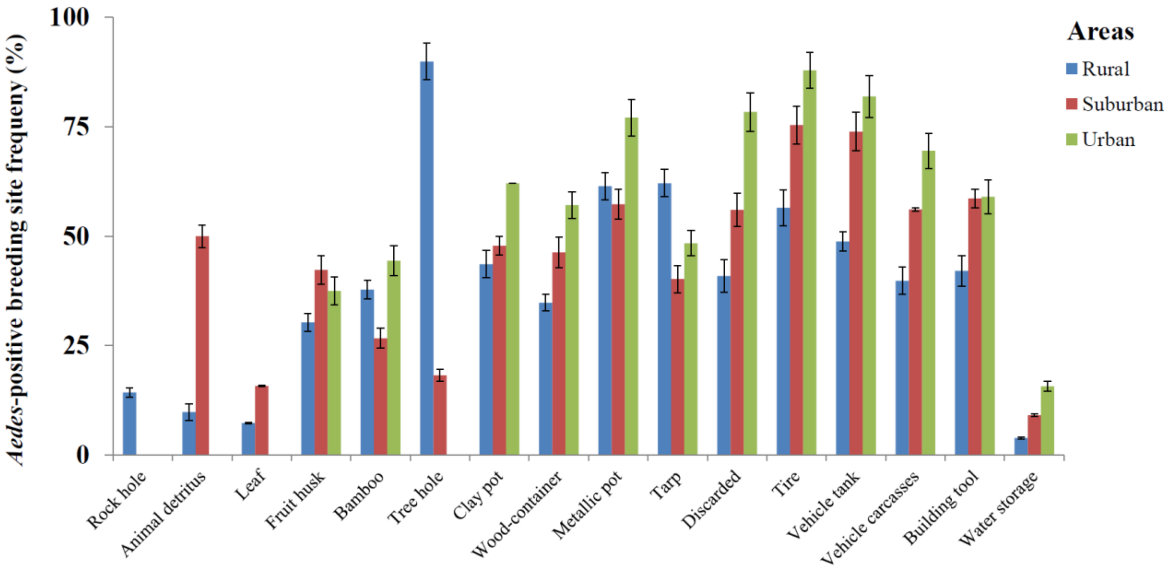


Figure 6.2: Dynamics of *Aedes* mosquito breeding sites in rural, suburban, and urban areas in south-eastern Côte d'Ivoire. Error bars show the standard error (SE).

In the urban setting, the most common *Aedes* breeding sites comprised of industrial containers such as tires (1,087/1,236; FP = 87.9%), discarded containers (601/767; FP = 78.4%), canisters (77/94; FP = 81.9%), vehicle carcasses (91/131; FP = 69.5%), and water storage containers (141/896; FP = 15.7%). Water storage containers were found to be more frequently infested with immature stages of *Aedes* mosquitoes in the urban than in the suburban (χ^2 = 17.3, df = 1, $p < 0.001$) or rural settings (χ^2 = 57.3, df = 1, $p < 0.001$). Furthermore, there was a statistically significant difference in *Aedes* mosquito positivity rate in water storage container between the suburban and rural settings (χ^2 = 15.8, df = 1, $p < 0.001$). Besides the variations in the frequency in the colonization of *Aedes* breeding sites, the most abundant *Aedes* breeding sites were tires and discarded containers in all the study areas (all $p < 0.05$) (Fig 3). Also frequently positive were natural breeding sites such as tree holes (62/738; PP = 8.4%), fruit husks (59/738; PP = 8.0%), industrial containers such as tarps (41/738; PP = 5.6%), vehicle tanks (n = 41/738; PP = 5.6%) and vehicle carcasses (68/738; PP = 9.2%) in the rural area, and water storage containers (141/2,136; PP = 6.6%) in the urban area (Figure 6.3).

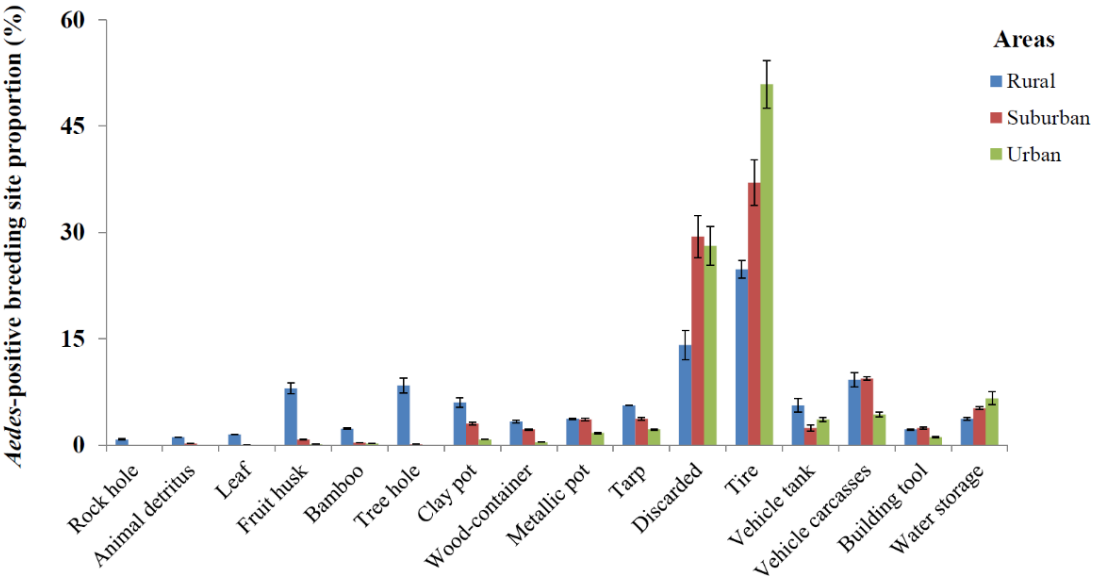


Figure 6.3: Frequency of *Aedes* mosquito breeding sites in rural, suburban, and urban areas in south-eastern Côte d'Ivoire. Error bars show the standard error (SE).

6.4.6. Ecological variations in Aedes *species*

Table 6.4 summarizes the abundance, richness, diversity, and dominance of *Aedes* mosquito species according to the breeding site types among macrohabitats and study areas. The indices highly varied between the study areas and breeding sites, with overall higher values in peri-domestic environments. The highest larval abundances of *Aedes* mosquitoes were recorded in tires in the all study areas ($p < 0.05$). In addition, tree holes and metallic pots in the rural, vehicle tanks and building tools in the suburban and discarded containers, vehicle tanks and vehicle carcasses in the urban areas were also highly productive breeding sites for *Aedes* mosquito (S6.2 Figure). *Aedes* species richness was significantly different among the microhabitats in the rural ($F = 4.3$; $df = 16$; $p < 0.001$), suburban ($F = 9.2$; $df = 7$; $p < 0.001$), and urban settings ($F = 11.1$; $df = 2$; $p < 0.001$). Significantly higher numbers of species (13 species) were found in tree holes in the rural areas. The rural areas showed the highest species diversity, as demonstrated by a Shannon's diversity index of 1.64, followed by 0.38 for the suburban and 0.06 for the urban areas. Among the breeding sites, the highest Shannon's diversity index was found in the rural areas for the tree holes with a value of 3.13. Conversely, Simpson's dominance index of *Aedes* species significantly decreased from the urban (0.99) to suburban (0.89) and rural (0.57) areas ($F = 16.2$; $df = 3$; $p < 0.001$).

6.4.7. Geographic shifts in Aedes *breeding sites*

Table 6.5 shows that the proportion of breeding sites positive for *Aedes* larvae significantly varied across the peri-domestic and domestic sites in all study areas. Overall, compared to domestic environment, peri-domestic sites showed a higher proportion of significantly *Aedes*-positive breeding sites, with FP of 84.8% (1,753/2,066) in urban (χ^2 = 1,100; df = 1; p < 0.001), 70.2% (1,176/1,676) in suburban (χ^2 = 829.2; df = 1; p < 0.001) and 42.6% (636/1,492) in rural (χ^2 = 271.5; df = 1; p < 0.001) areas (Table 6.5). In rural areas, 87.7% (143/163) of the natural breeding sites that hosted *Aedes* larvae were located in the peri-domestic sites. High numbers of tires were found infested in the domestic site, with FP of 66.5% (151/227) *Aedes*-positive breeding sites in the urban, and 35.8% (63/176) in the suburban area.

6.4.8. Seasonal shifts in Aedes *breeding sites*

In all study areas, the proportion of *Aedes*-positive breeding sites and the number of larvae varied significantly over time with more breeding sites being positive during the rainy season (Figure 6.4 and S6.3 Figure). During the rainy season, proportionally more breeding sites were positive. The frequencies of *Aedes*-positive breeding sites were 69.6% (1,650/2,369) in the urban (χ^2 = 137.7; df = 1; p < 0.001), 52.9% (1,196/2,263) in the suburban (χ^2 = 138.4; df = 1; p < 0.001), and 34.6% (642/1,857) in the rural (χ^2 = 63.5; df = 1; p < 0.001) areas (S6.2 Table). Significantly more *Aedes*-positive breeding sites were observed during the rainy season in the rural, urban, and suburban areas, with FP of 40.0% (187/468) and 72.0% (521/724) in July 2013, and 56.6% (327/578) in October 2013, respectively (S6.3 Figure). Moreover, higher densities of immature *Aedes* mosquitoes were recorded in July 2013 with 0.62 ± 0.03 and 1.70 ± 0.03 larvae/l in the rural, urban and, suburban areas, respectively, and in October 2013 with 1.02 ± 0.02 larvae/l (Figure 6.5). There were significant differences in the highest *Aedes* microhabitat rates (χ^2 = 121.2; df = 2; p < 0.001) and the highest abundance (χ^2 = 156.5; df = 2; p < 0.001) between the tree study areas. The highest frequency (i.e., 352/393; FP = 89.6%) of *Aedes*-positive breeding sites was observed in the peri-domestic macrohabitats in the urban areas during the rainy season in October 2013.

Table 6.5: Geographical variations in *Aedes* mosquito breeding site positivity across the sites in the rural, suburban and urban areas in south-eastern Côte d'Ivoire from January 2013 to October 2014

Breeding site	**Rural**								**Suburban**								**Urban**							
	Peri-domestic				**Domestic**				**Peri-domestic**				**Domestic**				**Peri-domestic**				**Domestic**			
	N	**n**	**FP**	**PP**	**N**	**n**	**FP**	**PP**	**N**	**n**	**FP**	**PP**	**N**	**n**	**FP**	**PP**	**N**	**n**	**FP**	**PP**	**N**	**n**	**FP**	**PP**
Natural																								
Rock hole	42	6	14.3	0.9	0	0	na	0.0	0	0	na	0.0	0	0	na	0.0	0	0	na	0	0	0	na	0.0
Animal detritus	76	8	10.5	1.3	6	0	0	0.0	6	3	50.0	0.3	0	0	na	0.0	2	0	0	0	0	0	na	0.0
Leaf axil	151	11	7.3	1.7	0	0	na	0.0	19	3	15.8	0.3	0	0	na	0.0	6	0	0	0	0	0	na	0.0
Fruit husk	166	49	29.5	7.7	29	10	34.5	9.8	13	9	69.2	0.8	13	2	15.4	0.8	8	3	37.5	0.2	0	0	na	0.0
Bamboo	45	17	37.8	2.7	0	0	na	0.0	15	4	26.7	0.3	0	0	na	0.0	9	4	44.4	0.2	0	0	na	0.0
Tree hole	56	52	92.9	8.2	13	10	76.9	9.8	11	2	18.2	0.2	0	0	na	0.0	0	0	na	0	0	0	na	0.0
Total	**536**	**143**	**26.7**	**22.5**	**48**	**20**	**41.7**	**19.6**	**64**	**21**	**32.8**	**1.8**	**13**	**2**	**15.4**	**0.8**	**25**	**7**	**28**	**0.4**	**0**	**0**	**na**	**0.0**
Traditional																								
Clay pot	73	36	49.3	5.7	28	8	28.6	7.8	64	34	53.1	2.9	26	9	34.6	3.6	21	14	66.7	0.8	8	4	50.0	1.0
Wood	42	21	50.0	3.3	27	3	11.1	2.9	41	20	48.8	1.7	26	11	42.31	4.4	11	7	63.6	0.4	3	1	33.3	0.3
Metallic pot	32	22	68.8	3.5	12	5	41.7	4.9	72	43	59.7	3.7	33	18	54.5	7.1	36	30	83.3	1.7	12	7	58.3	1.8
Total	**147**	**79**	**53.7**	**12.4**	**67**	**16**	**23.9**	**15.7**	**177**	**97**	**54.8**	**8.2**	**85**	**38**	**44.7**	**15.1**	**68**	**51**	**75**	**2.9**	**23**	**12**	**52.2**	**3.1**
Industrial																								
Tarp	39	27	69.2	4.2	27	14	51.9	13.7	47	38	80.9	3.2	85	15	17.6	6.0	58	32	55.2	1.8	37	14	37.8	3.7
Discarded	213	96	45.1	15.1	41	8	19.5	7.8	578	385	66.6	32.7	167	32	19.2	12.7	691	563	81.5	32.1	76	38	50.0	9.9
Tire	286	172	60.1	27.0	38	11	28.9	10.8	520	462	88.8	39.3	176	63	35.8	25.0	1009	936	92.8	53.4	227	151	66.5	39.4
Vehicle tank	81	40	49.4	6.3	3	1	33.3	1.0	46	34	73.9	2.9	0	0	na	0	91	76	83.5	4.3	3	1	33.3	0.3
Carcasses	167	68	40.7	10.7	4	0	0.0	0.0	224	125	55.8	10.6	13	8	61.5	3.2	124	88	71.0	5.0	7	3	42.9	0.8
Building tool	15	9	60.0	1.4	23	7	30.4	6.9	20	14	70.0	1.2	38	20	52.6	7.9	0	0	na	0.0	39	23	59.0	6.0
Water storage	8	2	25.0	0.3	680	25	3.7	24.5	0	0	na	0.0	816	74	9.1	29.4	0	0	na	0.0	896	141	15.7	36.8
Total	**809**	**414**	**51.2**	**65.1**	**816**	**66**	**8.1**	**64.7**	**1435**	**1058**	**73.7**	**90.0**	**1295**	**212**	**16.4**	**84.1**	**1973**	**1695**	**85.9**	**96.7**	**1285**	**371**	**28.9**	**96.9**
Artificial	**956**	**493**	**51.6**	**77.5**	**883**	**82**	**9.3**	**80.4**	**1612**	**1155**	**71.7**	**98.2**	**1380**	**250**	**18.1**	**99.2**	**2041**	**1746**	**85.5**	**99.6**	**1308**	**383**	**29.3**	**100**
TOTAL	**1492**	**636**	**42.6**	**100**	**931**	**102**	**11.0**	**100**	**1676**	**1176**	**70.2**	**100**	**1393**	**252**	**18.1**	**100**	**2066**	**1753**	**84.8**	**100**	**1308**	**383**	**29.3**	**100**

N: number of wet containers, n: number of *Aedes*-positive breeding sites, FP: frequency of *Aedes*-positive breeding sites among wet containers, PP: proportion of each *Aedes*-positive breeding site type among the all *Aedes*-positive breeding site types. The units of FP and PP are percentage (%).

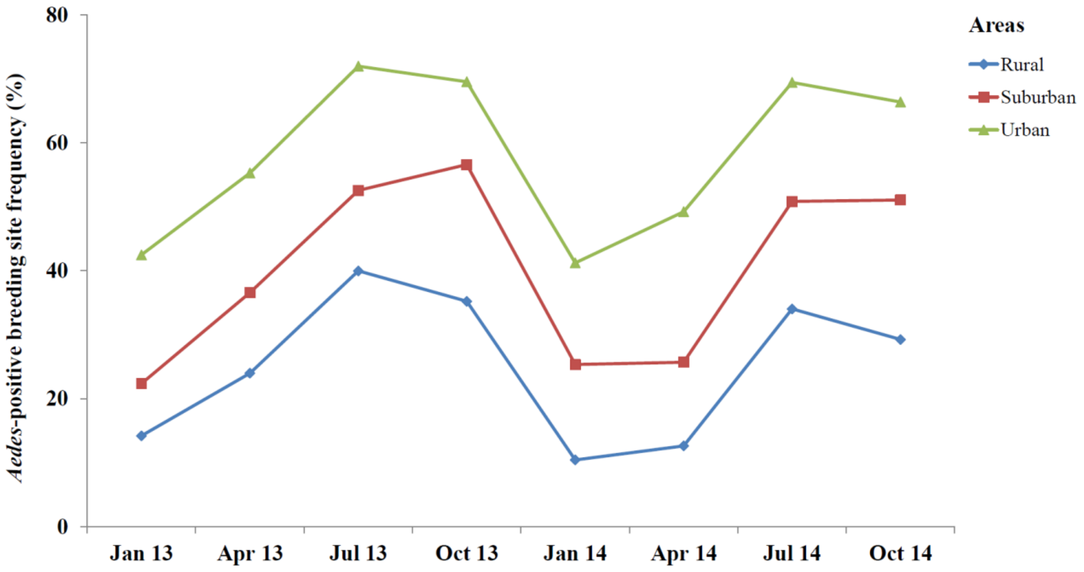


Figure 6.4: Monthly variations in the occurrence of immature stages of *Aedes* mosquitoes in rural, suburban, and urban areas in south-eastern Côte d'Ivoire.

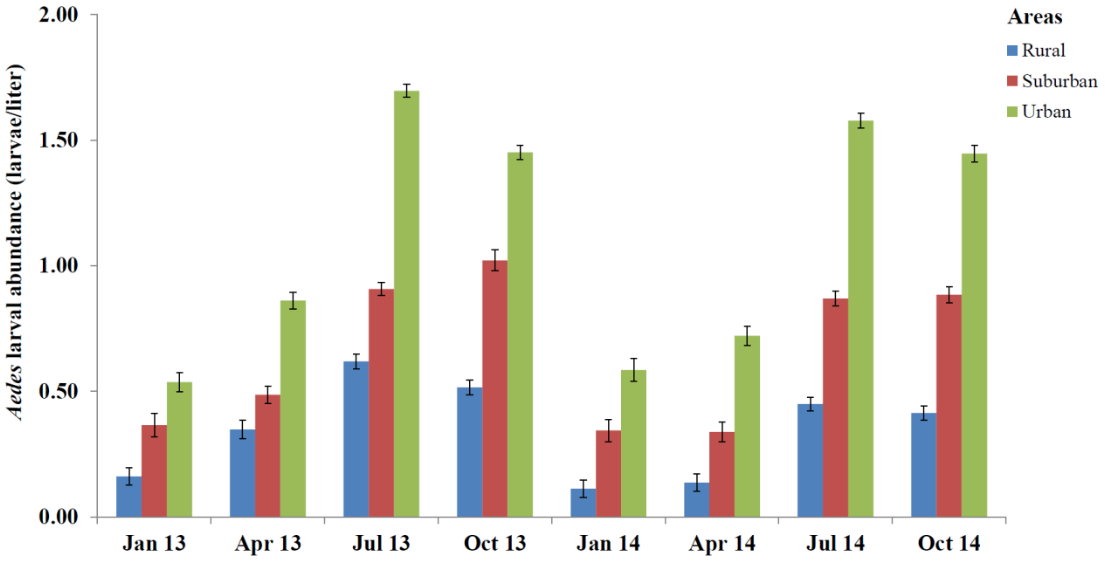


Figure 6.5: Monthly variations in the abundance of immature stages of *Aedes* mosquitoes in rural, suburban, and urban areas in south-eastern Côte d'Ivoire. Error bars show the standard error (SE).

6.5. Discussion

When designing strategies to monitor and control *Aedes* arbovirus vectors in their breeding sites, failure to identify the broad spectrum of potentially available breeding sites will bias the results from field sampling and will thus negatively affect the impact of larval control interventions. Our study pertaining to larval habitats of *Aedes* mosquitoes alongside a rural-to-urban gradient within yellow fever and dengue co-endemic areas in the south-eastern part of Côte d'Ivoire provided strong evidence for influence on species structure, microhabitats, and biological interactions among the immature forms (Figure 6.6).

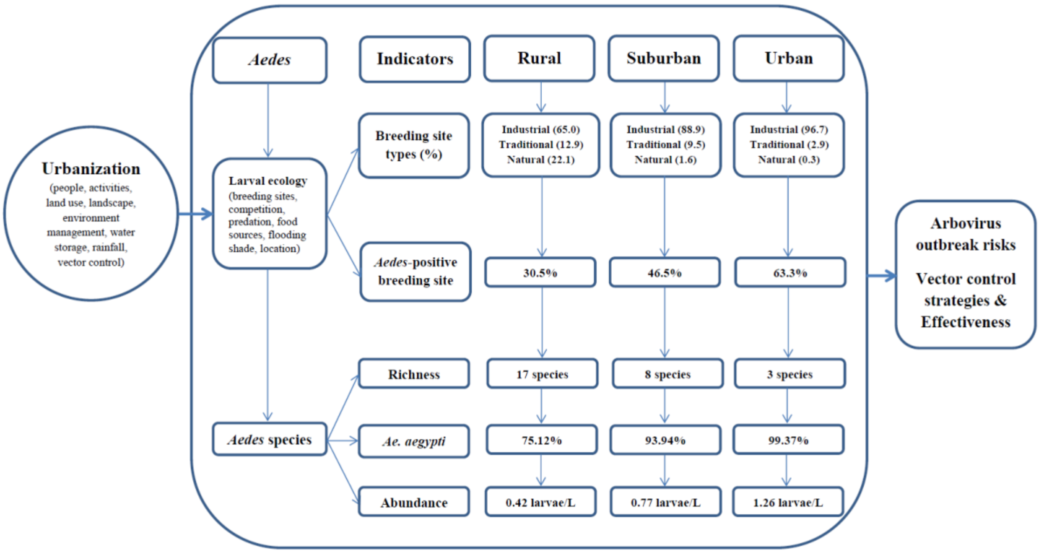


Figure 6.6: Synthesis of how urbanization shapes immature *Aedes* mosquito breeding sites and species in south-eastern Côte d'Ivoire.

Compared to a previous study conducted in the same area of Côte d'Ivoire [16], the current study identified 11 additional *Aedes* species (i.e., *Ae. albopictus*, *Ae. angustus*, *Ae. apicoargentus*, *Ae. argenteopunctatus*, *Ae. haworthi*, *Ae. lilii*, *Ae. longipalpis*, *Ae. opok*, *Ae. palpalis*, *Ae. stokesi*, and *Ae. unilineatus*) and 16 additional non-*Aedes* species that may influence arbovirus transmission patterns. To our knowledge, *Aedes* mosquito species such as *Ae. lilii*, *Ae. stokesi*, and *Ae. unilineatus*, and others such *Cq. fuscopennata* and *Tx. brevipalpis* appear to be reported for the first time in Côte d'Ivoire. *Ae. albopictus* is not native to Côte d'Ivoire, but has previously been reported [22]. Presumably the species has been introduced through the seaport bordering the urban municipality of Treichville. The higher numbers of *Aedes* species is likely due to abundant presence of natural and artificial breeding sites, and their potentials to provide suitable microenvironments. Gravid *Aedes* females select oviposition sites according to their physical, chemical, and biological characteristics [11, 12] and these may change in space and time over the year [16].

The public health relevance of *Aedes* mosquitoes results from their invasiveness and ecologic plasticity, competence for multiple pathogens, potential as bridge vectors due to their opportunistic feeding behavior and adaptation to urban, rural, and forest areas [23]. Almost all of the container-specialist *Aedes* mosquitoes collected as larvae such as *Stegomyia* subgenus, including *Ae. aegypti*, *Ae. africanus*, *Ae. albopictus*, *Ae. angustus*, *Ae. apico-argenteus*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. opok*, *Ae. vittatus*, *Ae. unilineatus*, and *Ae. usambara* species, and *Diceromyia* and *Aedimorphus* subgenera comprising respectively *Ae. furcifer* and *Ae. stokesi* species have been shown to carry and/or to transmit in nature over 24 viruses, including yellow fever, dengue, Zika, chikungunya, and Rift Valley in the tropical regions [5, 6]. In addition, *Ae. (Aedimorphus) argenteopunctatus* in South Africa [24] and *Ae. (Neomelaniconion) palpalis* [25] which show vector competence for Rift Valley fever virus *in vitro* and the other *Aedes* species like *Ae. (Stegomyia) dendrophilus*, *Ae. (Stegomyia) lilii* and *Ae. (Aedimorphus) haworthi* which belong to the same subgenera of species involved in the transmission of the arboviruses thus could be suspected as potential vectors of diseases. Still, *Ae. (Finlaya) longipalpis* belonging to the same *Finlaya* subgenus with *Ae. niveus* which has been the principal vector of dengue virus in Malaysia [26] may potentially transmit arboviruses in Côte d’Ivoire. Among non-*Aedes* mosquitoes, *Er. chrysogaster*, *Er. inornatus* and *Cq. fuscopennata* have been found to have natural infection while *Er. quinquevittatus* has exhibited laboratory competence with yellow fever virus in Africa [6]. Moreover, *An. coustani* has been found to be infested with Zika virus [27], while O’nyong-nyong and chikungunya viruses have been isolated from *An. gambiae* [28]. *Cx. quinquefasciatus* [25] and *Cx. poicilipes* [26] have been shown susceptible to transmit Rift Valley fever virus. In conclusion, as in Senegal [12], the collections of immature stages of non-anthropophagic, unexpected and new potential vectors in rural areas suggest the co-existence of several still unidentified arbovirus cycles in south-eastern Côte d’Ivoire.

Our results also revealed that, urban areas showed higher capacity to support *Aedes* breeding sites and larvae than suburban and rural areas. The higher numbers of positive breeding sites and higher abundance of *Aedes* mosquito larvae may be due to the destruction of natural vegetation coverage for infrastructure buildings in the urbanized areas that may affect biological factors (e.g., fauna and flora), and increase the radiation budget thus modifying the microenvironments within and around the microhabitats [29]. Increased exposure to sunlight probably accelerates *Aedes* mosquito larval development and thus increases the size of adult vectors that possibly find more opportunities of blood feeding sources from larger human populations in urban areas [16, 29]. Still, urban *Aedes* populations are probably less exposed to the pressures from agricultural insecticide and predators (e.g., *Eretmapodites* spp. and *Toxorhynchites* spp.) compared to rural communities. We also found that less than two-thirds of breeding sites were infested with *Aedes* larvae thus suggesting that not all available containers filled with water were occupied by at least one larva or pupa of *Aedes* mosquitoes and the immature *Aedes* mosquitoes were not randomly distributed [12]. The presence of empty containers might imply that the gravid females of *Aedes* mosquitoes select their egg-laying sites carefully according to their physical characteristics (e.g., depth, color, clearance, surface, location, height, shade, sun exposure, and food sources) [12, 29], and biological interactions (e.g., competition and predation) [10, 11, 30] at play within the water-holding container systems.

In our larval surveys, we documented distinct geographic and seasonal variations in terms of the proportions of positive breeding sites and abundance of *Aedes* mosquitoes in all areas. Indeed, the highest proportions and relative abundance of *Aedes* mosquitoes were observed among vegetated peri-domestic breeding sites and during the rainy seasons in all areas. The shade of the vegetation reduces the water temperature [12], thus protecting breeding sites from drying out. Moreover, leaves supply organic detritus and associated microorganisms that may serve as food sources for the mosquito larvae [10]. The geographic and seasonal patterns in *Aedes* breeding sites are important from an epidemiologic perspective and suggest that the rainy season is the best period of time to identify breeding sites, while during the dry season it would be an ideal period of time to control immature *Aedes* mosquitoes, with particular attention for peri-domestic environments.

Our data revealed that the pattern of *Aedes* mosquito breeding sites changes substantially from natural containers to artificial containers along a rural-to-urban gradient. Although artificial breeding sites dominate in all areas, there is a higher proportion of natural containers (e.g., rock holes, animal detritus, leaf axils, fruit husks, bamboo, and tree holes) in rural areas, traditional containers (e.g., clay pots, wood-containers, and metallic pots) in suburban areas. However, in the urban areas, the most productive breeding sites for *Aedes* mosquito were industrial containers (e.g., tarps, discarded tires, vehicle tanks, carcasses, building tools, and water storage containers). The availability of, and the segregation among, *Aedes* breeding sites probably result from the strong impacts of human activities on the environment, while the natural breeding sites are provided by the natural landscape and agriculture [12]. We observed that tree holes, tires, and water storage containers showed higher *Aedes* species richness in rural, higher *Aedes* abundances in all areas, and high *Ae. aegypti* infestation rates in urban areas, respectively. Tree holes, found in the preserved rainforest, seem to provide ideal larval habitats for several species due to their greater stability, various trophic inputs, and retention of rainwater for longer periods of time [12]. Used tires are mostly associated with the palm oil industry in rural areas, production of the local dish "*Attiéké*" in suburban areas, and selling of tires and car repairs in urban areas. Tree holes and tires have bigger volumes and are expected to better protect the immature forms of *Aedes* mosquitoes against flushing during heavy rains [12, 14]. Moreover, tires are black-colored containers that are highly attractive to the gravid *Aedes* females searching for oviposition sites [11, 31]. The high number of water barrels infested with *Aedes* larvae might be due to the water being held for longer periods [32].

Taken together, *Aedes* species diversity, richness, abundance, and dominance signifycantly changed from rural to urban settings. The variations in *Aedes* mosquito species may be explained by the sensitivity of their larvae to environmental changes induced by urbanization [10, 12]. Native species such as *Ae. africanus*, *Ae. argenteopunctatus*, *Ae. longipalpis*, *Ae. stokesi* and *Ae. usambara* were restricted to natural breeding sites in the rural areas. However, other wild species, such as *Ae. furcifer*, *Ae. dendrophilus*, *Ae. palpalis*, *Ae. vittatus*, *Ae. luteocephalus*, and *Ae. metallicus* were also surprisingly frequent in artificial containers. In contrast, our surveillance failed to sample *Ae. fraseri* that have been collected by ovitraps in the rural areas previously [16], probably due to its possible cryptic breeding sites or potential height-dependent oviposition behavior. The existence of multiple types of behavior in the same *Aedes* mosquito species may indicate the existence of generalist species or sibling strains of individuals from various origins [6, 11] that have experienced different selective urbanization pressures.

Lastly, our study showed that *Ae. aegypti*, the primary vector of yellow fever, dengue, chikunguya, and Zika viruses [1-3], was the most prevalent species in all study areas, exhibiting an increasing abundance along rural-to-urban gradient towards an higher abundance in urban areas where larvae mostly inhabit in anthropogenic containers (e.g., tires, discarded containers). However, *Ae. aegypti* displayed behavioral plasticity in that the females lay eggs in a vast array of containers ranging from natural containers such as rock holes, tree holes, and bamboo to a wide range of man-made containers [11], including water storage containers in urban areas [32]. The ecologic variations in oviposition behavior of *Ae. aegypti* and other *Aedes* mosquitoes may be discussed in ecologic, evolutionary, and epidemiologic approaches [11], and suggest possible overlaps of sylvan and urban vector distributions thus linking several potential mixed arbovirus transmission cycles [5, 6, 12, 16]. In addition, if highly infested microhabitats are targeted for removal, *Aedes* mosquito females may possibly adapt to changes in breeding habitats and alternatively oviposit in other containers previously unoccupied [33]. The ability of *Ae. aegypti* to adapt ovipositional behaviors to changing environments possibly enabling to overcome ecological constraints (e.g., instability and disturbance of the breeding sites) imposed by urbanization [10, 11]. *Ae. aegypti*-transmitted yellow fever outbreaks are historically well known in Côte d'Ivoire to have forced the transfer of the capital from Grand-Bassam to Abidjan in 1899 [15]. Since then, several unpredictable resurgences of yellow fever and dengue have been occurring in rural and urban areas causing many suspected, confirmed and fatal cases, and remain presently an unresolved major public health concern [7, 15, 34], with the current outbreak of dengue DENV-3 resulting in one confirmed and 17 suspected cases recorded in Abidjan in May 2017. Our study suggests that the unique removal of artificial containers that is a common practice in arbovirus control programs in Côte d'Ivoire might not effectively control diseases in the south-eastern part of the country. Vector control measures should combine removal of artificial containers [6] and autocidal gravid ovitrap-based on mass trapping [35], and insecticide auto-dissemination approaches [36].

6.6. Conclusions

In south-eastern Côte d'Ivoire, urbanization is associated with larval habitats of *Aedes* species at a finer scale by driving their breeding sites from natural to artificial containers, and at the larger scale by transforming rural to urban areas. *Ae. aegypti* is most prevalent in urban areas, suggesting that urbanization is a driver for producing suitable breeding sites for this mosquito species, and hence related disease outbreaks. However, rural settings still support irremovable containers such as natural breeding sites that host several wild *Aedes* species and *Ae. aegypti*. Therefore, even effective removal of discarded containers in urban areas (a common practice in arbovirus control programs) might not be sufficient to control arboviral diseases. Instead, vector control strategies should embrace a more holistic approach, combining different tools and methods of proven efficacy [6, 35, 36].

6.7. Supporting information

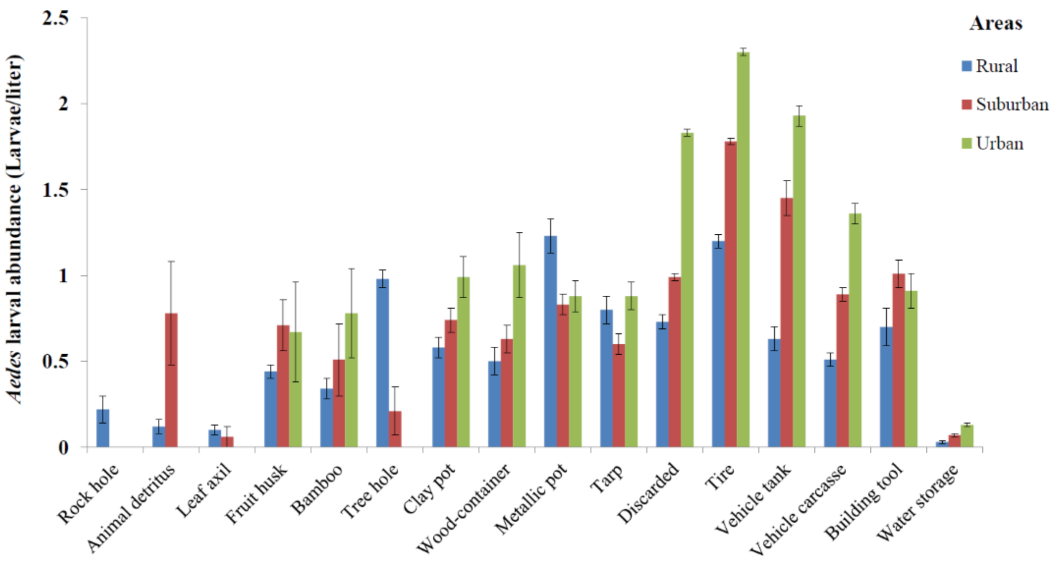
I. Natural conatiners

II.1. Traditional conatiners

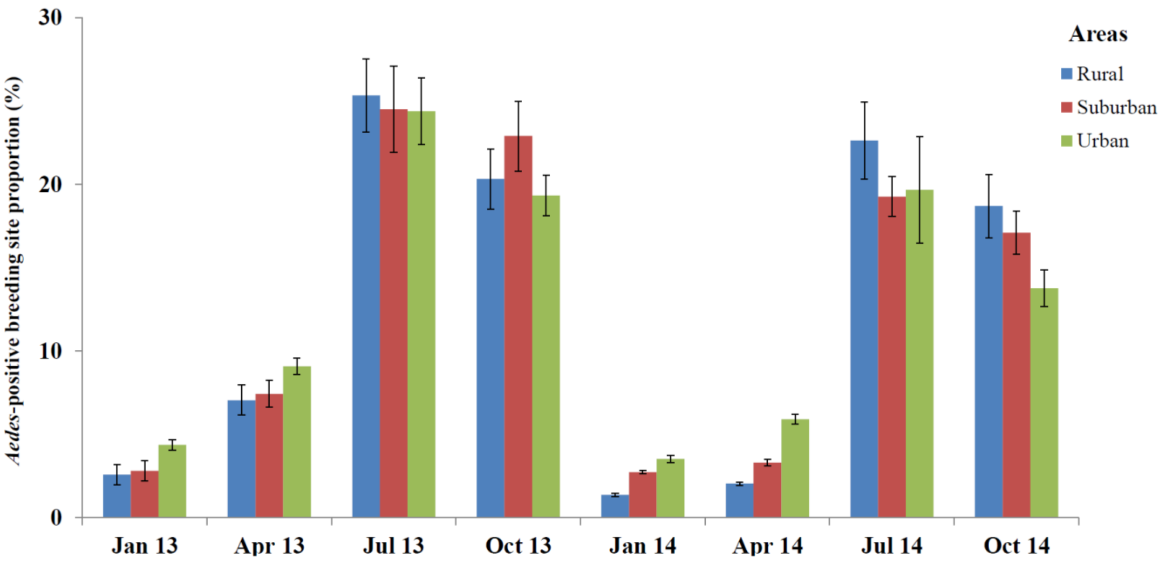
II.2. Industrial conatiners

II. Artificial conatiners

S6.1 Figure: Range of *Aedes* mosquito breeding sites surveyed in rural, suburban, and urban areas in south-eastern Côte d'Ivoire. The container type often reflects the name of the container and the categories include containers that provide comparable larval habitats as follows: I. natural containers: A: rock hole, B: animal detritus, C: leaf, D: fruit husks, E: bamboo, F: tree hole; and II. artificial conatiners composed of: II.1. traditional containers: G: clay pot, H: wood-container, I: metallic pot; and II.2. industrial containers: J: traps, K: discarded container, L: tire, M: vehicle tank, N: vehicle carcasses, O: building tool, P: water storage container.



S6.2 Figure: Variations in abundance of *Aedes* mosquito among breeding sites in rural, suburban, and urban areas in south-eastern Côte d'Ivoire. Error bars show the standard error (SE).



S6.3 Figure: Monthly variations in the proportions of *Aedes*-positive breeding sites in rural, suburban, and urban areas in south-eastern Côte d'Ivoire. Error bars show the standard error (SE).

S6.1 Table: Dynamics of *Aedes* mosquito breeding sites in the rural, suburban and urban areas in south-eastern Côte d'Ivoire from January 2013 to October 2014

Breeding site	**Rural**				**Suburban**				**Urban**			
	N	**n**	**FP (%)**	**PP (%)**	**N**	**n**	**FP (%)**	**PP (%)**	**N**	**n**	**FP (%)**	**PP (%)**
Natural												
Rock hole	42	6	14.3	0.8	0	0	na	0.0	0	0	na	0.0
Animal detritus	82	8	9.8	1.1	6	3	50.0	0.2	2	0	0.0	0.0
Leaf axil	151	11	7.3	1.5	19	3	15.8	0.2	6	0	0.0	0.0
Fruit husk	195	59	30.3	8.0	26	11	42.3	0.8	8	3	37.5	0.1
Bamboo	45	17	37.8	2.3	15	4	26.7	0.3	9	4	44.4	0.2
Tree hole	69	62	89.9	8.4	11	2	18.2	0.1	0	0	na	0.0
Total	**584**	**163**	**27.9**	**22.1**	**77**	**23**	**29.9**	**1.6**	**25**	**7**	**28.0**	**0.3**
Traditional												
Clay pot	101	44	43.6	6.0	90	43	47.8	3.0	29	18	62.1	0.8
Wood	69	24	34.8	3.3	67	31	46.3	2.2	14	8	57.1	0.4
Metallic pot	44	27	61.4	3.7	105	61	58.1	4.3	48	37	77.1	1.7
Total	**214**	**95**	**44.4**	**12.9**	**262**	**135**	**51.5**	**9.5**	**91**	**63**	**69.2**	**2.9**
Industrial												
Tarp	66	41	62.1	5.6	132	53	40.2	3.7	95	46	48.4	2.2
Discarded	254	104	40.9	14.1	745	417	56.0	29.2	767	601	78.4	28.1
Tire	324	183	56.5	24.8	696	525	75.4	36.8	1236	1087	87.9	50.9
Vehicle tank	84	41	48.8	5.6	46	34	73.9	2.4	94	77	81.9	3.6
Carcasses	171	68	39.8	9.2	237	133	56.1	9.3	131	91	69.5	4.3
Building tool	38	16	42.1	2.2	58	34	58.6	2.4	39	23	59.0	1.1
Water storage	688	27	3.9	3.7	816	74	9.1	5.2	896	141	15.7	6.6
Total	**1625**	**480**	**29.5**	**65.0**	**2730**	**1270**	**46.5**	**88.9**	**3258**	**2066**	**63.4**	**96.7**
Artificial	**1839**	**575**	**31.3**	**77.9**	**2992**	**1405**	**47.0**	**98.4**	**3349**	**2129**	**63.6**	**99.7**
TOTAL	**2423**	**738**	**30.5**	**100**	**3069**	**1428**	**46.5**	**100**	**3374**	**2136**	**63.3**	**100**

N: number of wet containers, n: number of *Aedes*-positive breeding sites, FP: frequency of positive breeding sites among wet containers, PP: proportion of each *Aedes* breeding site type among *Aedes*-positive containers.

S6.2 Table: Seasonal variations in *Aedes* mosquito breeding site positivity in the rural, suburban and urban areas in south-eastern Côte d'Ivoire from January 2013 to October 2014

	Rural								**Suburban**								**Urban**							
Breeding site	**Dry season**				**Rainy season**				**Dry season**				**Rainy season**				**Dry season**				**Rainy season**			
	N	**n**	**FP**	**PP**	**N**	**n**	**FP**	**PP**	**N**	**n**	**FP**	**PP**	**N**	**n**	**FP**	**PP**	**N**	**n**	**FP**	**PP**	**N**	**n**	**FP**	**PP**
Natural																								
Rock hole	6	1	16.7	1.0	36	5	13.9	0.8	0	0	na	0.0	0	0	na	0.0	0	0	na	0	0	0	na	0.0
Animal detritus	2	0	0.0	0.0	80	8	10.0	1.2	2	0	0.0	0.0	4	3	75.0	0.3	0	0	na	0	2	0	0.0	0.0
Leaf axil	13	0	0.0	0.0	138	11	8.0	1.7	3	0	0.0	0.0	16	3	18.8	0.3	2	0	0	0	4	0	0.0	0.0
Fruit husk	12	2	16.7	2.1	183	57	31.1	8.9	2	0	0.0	0.0	24	11	45.8	0.9	2	0	0.0	0.0	6	3	50.0	0.2
Bamboo	3	1	33.3	1.0	42	16	38.1	2.5	2	0	0.0	0.0	13	4	30.8	0.3	0	0	na	0.0	9	4	44.4	0.2
Tree hole	11	9	81.8	9.4	58	53	91.4	8.3	2	1	50.0	0.4	9	1	11.1	0.1	0	0	na	0	0	0	na	0.0
Total	**47**	**13**	**27.7**	**13.5**	**537**	**150**	**27.9**	**23.4**	**11**	**1**	**9.1**	**0.4**	**66**	**22**	**33.3**	**1.8**	**4**	**0**	**0.0**	**0.0**	**21**	**7**	**33.3**	**0.4**
Traditional																								
Clay pot	21	11	52.4	11.5	80	33	41.3	5.1	15	3	20.0	1.3	75	40	53.3	3.3	5	2	40.0	0.4	24	16	66.7	1.0
Wood	6	2	33.3	2.1	63	22	34.9	3.4	7	1	14.3	0.4	60	30	50.0	2.5	2	0	0.0	0.0	12	8	66.7	0.5
Metallic pot	3	1	33.3	1.0	41	26	63.4	4.0	17	8	47.1	3.4	88	53	60.2	4.4	9	4	44.4	0.8	39	33	84.6	2.0
Total	**30**	**14**	**46.7**	**14.6**	**184**	**81**	**44.0**	**12.6**	**39**	**12**	**30.8**	**5.2**	**223**	**123**	**55.2**	**10.3**	**16**	**6**	**37.5**	**1.2**	**75**	**57**	**76.0**	**3.5**
Industrial																								
Tarp	12	3	25.0	3.1	54	38	70.4	5.9	5	3	60.0	1.3	127	50	39.4	4.2	23	4	17.4	0.8	72	42	58.3	2.5
Discarded	45	10	22.2	10.4	209	94	45.0	14.6	132	59	44.7	25.4	613	358	58.4	29.9	178	120	67.4	24.7	589	481	81.7	29.2
Tire	66	33	50.0	34.4	258	150	58.1	23.4	163	102	62.6	44.0	533	423	79.4	35.4	299	240	80.3	49.4	937	847	90.4	51.3
Vehicle tank	4	2	50.0	2.1	80	39	48.8	6.1	3	2	66.7	0.9	43	32	74.4	2.7	13	6	46.2	1.2	81	71	87.7	4.3
Carcasses	39	3	7.7	3.1	132	65	49.2	10.1	52	16	30.8	6.9	185	117	63.2	9.8	35	18	51.4	3.7	96	73	76.0	4.4
Building tool	5	2	40.0	2.1	33	14	42.4	2.2	15	6	40.0	2.6	43	28	65.1	2.3	28	19	67.9	3.9	11	4	36.4	0.2
Water storage	318	16	5.0	16.7	370	11	3.0	1.7	386	31	8.0	13.4	430	43	10.0	3.6	409	73	17.8	15.0	487	68	14.0	4.1
Total	**489**	**69**	**14.1**	**71.9**	**1136**	**411**	**36.2**	**64.0**	**756**	**219**	**29.0**	**94.4**	**1974**	**1051**	**53.2**	**87.9**	**985**	**480**	**48.7**	**98.8**	**2273**	**1586**	**69.8**	**96.1**
Artificial	**519**	**83**	**16.0**	**86.5**	**1320**	**492**	**37.3**	**76.6**	**795**	**231**	**29.1**	**99.6**	**2197**	**1174**	**53.4**	**98.2**	**1001**	**486**	**48.6**	**100**	**2348**	**1643**	**70.0**	**99.6**
TOTAL	**566**	**96**	**17.0**	**100**	**1857**	**642**	**34.6**	**100**	**806**	**232**	**28.8**	**100**	**2263**	**1196**	**52.9**	**100**	**1005**	**486**	**48.4**	**100**	**2369**	**1650**	**69.6**	**100**

N: number of wet containers, n: number of *Aedes*-positive breeding sites, FP: frequency of positive breeding sites among wet containers, PP: proportion of each *Aedes* breeding site type among *Aedes*-positive containers. The units of FP and PP are percentage (%).

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7. ARTICLE 3: Effect of land-use changes on the abundance, distribution, and host-seeking behavior of *Aedes* arbovirus vectors in oil palm-dominated landscapes, southeastern Côte d'Ivoire

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Short title: *Aedes* mosquito ecology and land-use changes

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7.1. Abstract

Background

Identifying priority areas for vector control is of considerable public health relevance. Arthropod-borne viruses (arboviruses) spread by *Aedes* mosquitoes are (re)emerging in many parts of the tropics, partially explained by changes in agricultural land-use. We explored the effects of land-use changes on the abundance, distribution, and host-seeking behavior of *Aedes* mosquitoes along a gradient of anthropogenic disturbance in oil palm-dominated landscapes in southeastern Côte d'Ivoire.

Methodology

Between January and December 2014, eggs, larvae, pupae, and adult *Aedes* mosquitoes were sampled in four types of macrohabitats (rainforest, polyculture, oil palm monoculture, and rural housing area), using standard procedures (bamboo-ovitraps, metallic-ovitraps, larval surveys, and human-baited double-net traps). Immature stages were reared and adult mosquitoes were identified at species level.

Principal findings

In total, 28,276 *Aedes* specimens belonging to 11 species were collected. No *Aedes*-positive microhabitat and only four specimens of *Ae. aegypti* were found in oil palm monoculture, The highest abundance of *Aedes* mosquitoes (60.9%) was found in polyculture, while the highest species richness (11 species) was observed in rainforests. *Ae. aegypti* was the predominate *Aedes* species, exhibiting high anthropophilic behavior. Three species (*Ae. aegypti*, *Ae. dendrophilus*, and *Ae. vittatus*) bit humans in polyculture and rural housing areas, with respective biting rates of 21.48 and 4.48 females/person/day. Unexpectedly, all three species were also feeding during darkness. *Aedes* females showed bimodal daily feeding cycles with peaks at around 8:00 a.m. and 5:00 p.m. Host-seeking activities were interrupted between 11:00 a.m. and 2:00 p.m. in rural housing areas, while no such interruption occurred in polyculture. Some rainforest-dwelling *Aedes* species displayed little preference to feed on humans.

Conclusions

In southeastern Côte d'Ivoire, the agricultural land-use/land-cover changes due to the conversion of rainforests into oil palm monocultures influence the abundance, distribution, and host-seeking behaviors of anthropophagic and non-anthropophagic *Aedes* vectors. As a result there is higher risk of humans to arbovirus transmission in polyculture and rural

housing areas. There is a need for integrated vector management, including landscape epidemiology and ecotope-based vector control.

7.2. Introduction

Arthropod-borne viruses (arboviruses) have (re)emerged from their sylvatic reservoirs of Africa and the Americas. Indeed, arboviruses are dispersed globally, and they are responsible for various diseases [1]. Several *Aedes* species act as vectors of arboviral diseases, such as yellow fever, dengue, chikungunya, Rift valley fever, and Zika that are of considerable public health relevance [1]. The resurgence of these mosquito-borne diseases and their geographic expansion has long been associated with human-induced modifications of terrestrial ecosystems [2]. Identifying priority areas for integrated vector management is crucial for public health because the ecology (i.e., abundance, distribution, and behaviors) of *Aedes* mosquito vectors is likely to alter with human-induced land use changes, including deforestation, intensification of agriculture, and urbanization [2- 4].

The expansion of tropical oil palm (*Elaesis guineensis*) plantations is a major driver of deforestation and threatens biodiversity, including arthropods [5, 6]. Wild palm trees have a life-span of up to 200 years, and an economic life-span of 25-30 years, after which trees are cut down and replaced with young palm plants. The planting density ranges from 120 to 160 palms/ha. Changes in land-use can result in the losses of *Aedes* mosquito habitats, hosts, and predators, which, in turn, affect the dynamics, abundance, oviposition, and host-seeking behaviors of vectors searching for alternative habitats and new blood-feeding sources [2]. In contrast, other cultivations such as rubber plantations, and plants with sheathing leaf axils (e.g., banana, bromeliads, and taro), and fruit husks (e.g., coconuts) can be important sources of *Aedes* mosquito breeding as they retain water for larval breeding [7, 8]. Additionally, containers used to supply water to animals and plants support *Aedes* mosquito larval growth [9]. Anthropogenic chemical uses such as pesticides (e.g., insecticides, fungicides, herbicides, and rodenticides) are drivers of changes in mosquito populations [10]. While the transformation of native rainforests into human settlements might destroy natural breeding sites of *Aedes*, it might result in an increase of artificial containers (e.g., tires and discarded water storage containers) that serve as microhabitats for immature *Aedes* [2]. Moreover, open areas directly exposed to sunlight that are created after the removal of natural vegetation accelerate mosquito development and survivorship [4, 8]. Tropical rainforests are rich in biodiversity, including *Aedes* that might breed in tree holes that are protected by foliage and contain microbial food sources for mosquito larvae [2, 7]. Moreover, the diverse fauna in the rainforest [7] serves as blood sources for host-seeking *Aedes* females, thereby maintaining the circulation of arboviruses among non-human primates (sylvatic cycle) [11, 12]. Deforestation, forest-degradation, and forest-fragmentation have been associated with arbovirus emergence or re-emergence [11, 12]. The effects of these multiple anthropogenic changes in land use on mosquito communities and the risk of disease transmission in the tropics may be further amplified by precipitation [2, 13].

In the southeastern part of Côte d'Ivoire, where large parts of rainforests have been converted into oil palm plantations, several outbreaks of yellow fever and dengue have been documented [14]. The outbreaks have been associated with vectors such as *Ae. aegypti*, *Ae. africanus*, *Ae. furcifer*, *Ae. luteocephalus*, *Ae. opok*, and *Ae. vittatus* [15, 16]. At present, Côte d'Ivoire is the third largest African producer of palm oil with an annual production of about 1.8 million tons. Palm oil production generates 3.1% of the national gross domestic product (GDP) [17]. There are plans to further enlarge the national production of palm oil, which might increase human-induced pressures on rainforest [18].

There is a lack of knowledge on how agricultural land-use changes affect the ecology of *Aedes* vectors in oil palm-dominated landscapes of Côte d'Ivoire. It is important to deepen the understanding of this relationship to provide a better land-use strategy for the reduction of arboviral disease risks. We hypothesize that the abundance, distribution, and oviposition and host-seeking behaviors of *Aedes* mosquito species differ depending on the main landscape type.

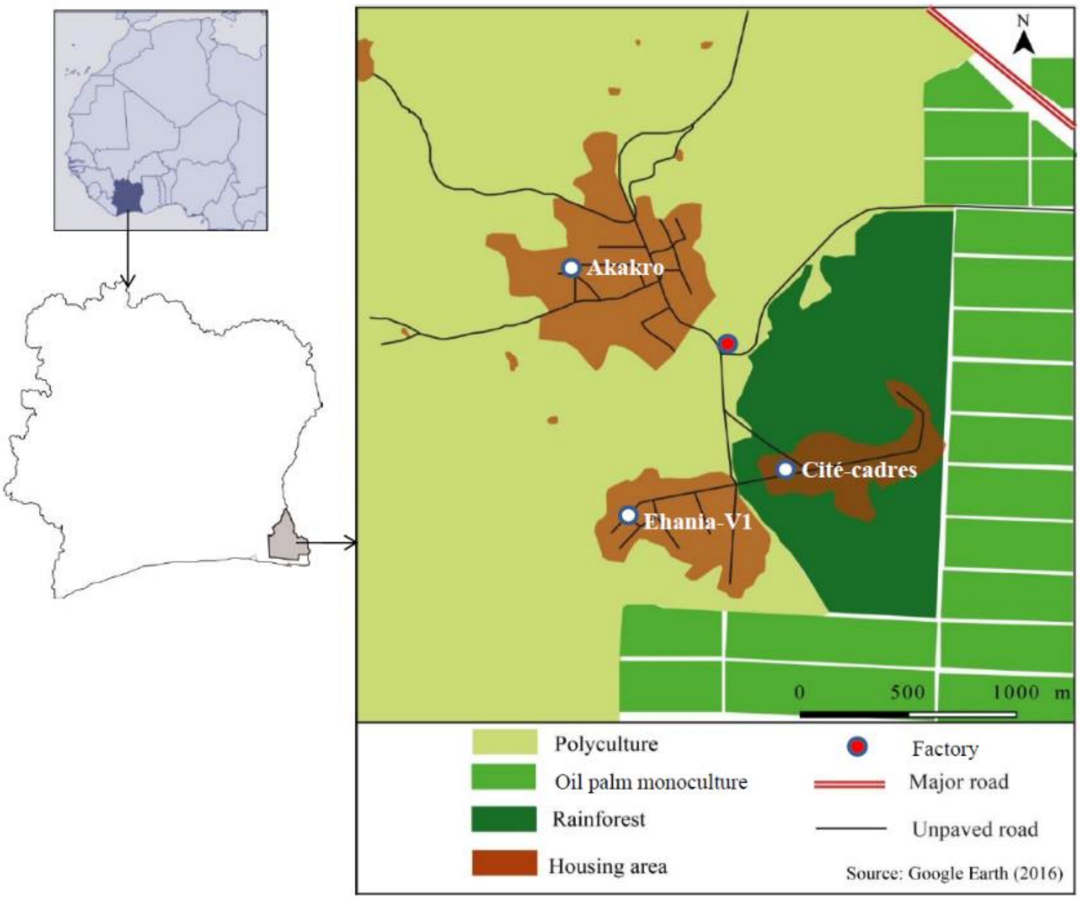
7.3. Methods

7.3.1. Ethics statement

The study protocol was approved by the local health and other administrative authorities. In addition, all entomologic surveys and sample collections carried out on private lands or private residential areas were done with the permission and written informed consent of the residents. Volunteers were vaccinated against yellow fever and protected against malaria with medical prophylaxis. This study did not involve endangered or protected species.

7.3.2. Study area

The study was carried out in the Sud-Comoé region (geographic coordinates 5° 28’ N latitude, 3° 12’ W longitude) located in the south-eastern part of Côte d’Ivoire (Fig 7.1). The estimated human population in the Sud-Comoé region is 642,000 with people mainly living in rural settings. The economic activities are primarily based on subsistence agriculture. Additionally, there is some industrial exploitations of oil palm monocultures (approximately 30,000 ha), managed by the commercial company PALMCI. Chemical products (i.e., insecticides, fungicides, and herbicides) are intensively used for oil palm plantation and crop protection [19].



Source: Google Earth (2016)

Figure 7.1: Location of the study areas in southeastern Côte d’Ivoire. The study was carried out in the villages located in oil palm plantation areas belonging to the Sud-Comoé region. The study area covers the villages of Ehania-V1, Cité-cadre and Akakro situated at the interface between the industrial oil palm plantation and traditional agricultural smallholdings. The industrial exploitations are devoted to the monoculture of oil palm plantations (*Eleasis guineensis*) covering over 30,000 hectares managed by an integrated agro-industrial unit of PALMCI. In the industrial part, a primary rainforest of over 100 ha has been preserved intact and forbidden of any human activities. In the traditional lands, the agricultural exploitation systems are polycultures comprising oil palm trees, rubber trees, banana, taro, bromeliads, and cocoa growing in the same space. Several small villages averaging 20 people are dispersed in these smallholdings.

The natural vegetation mostly constitutes of rainforest. Several small villages are dispersed across the landscape. The rainforest and traditional agriculture host trees, bamboo, and diverse animal species (primates, and birds).

The climate in the study area is characterized by high temperature and precipitation with two rainy seasons. The seasons are distinguished by rainfall rather than temperature. The main rainy season extends from May to July, while the shorter rainy season lasts from October to November, with distinct dry seasons in between. The average annual precipitation ranges from 1,200 to 2,400 mm. The annual average temperature and relative humidity are around 26.5 °C and 80-90%, respectively.

Our study was conducted in the Aboisso department, covering some 625 km^2 and an estimated population of 21,300 people, many of which work for PALMCI. The workers leave the villages in the morning to work in the farms and return back in the afternoon.

7.3.3. Study design

The study area was divided into 10 blocks around the eight villages of Ehania (Ehania-V1-8), Cité-Cadre and Akakro. In each block, four types of macrohabitats of roughly equal size were classified as rainforest, polyculture, oil palm monoculture, and rural housing areas based on the land-cover features (Table 7.1 and S7.1 Figure). The blocks with the villages of Ehania-V1, Cité-Cadre, and Akakro were selected for this study (Figure 7.1). Eggs, larvae, pupae, and adults of *Aedes* mosquitoes were sampled every month during 12 cross-sectional surveys from January to December 2014. There were four defined macrohabitats and we used metallic-ovitraps, bamboo-ovitraps, larvae surveys, and human-baited double-net traps for mosquito collection (S7.2 Figure).

7.3.4. Aedes *mosquito egg collection*

Aedes spp. eggs were collected monthly using 30 bamboo-ovitraps and 30 metallic-ovitraps during the 12 cross-sectional surveys in each macrohabitat. Bamboo-ovitraps were made of cut bamboo, and metallic-ovitraps made of a tin can cut to imitate natural and artificial breeding sites of *Aedes* mosquitoes, respectively. Metallic-ovitraps were painted black, while bamboo-ovitraps were not painted. Both ovitrap types had a volume of 400 cm^3 and were filled to ¾ with water. The water was a mix of distilled water, rainwater, and a 10% hey infusion with *Panicum maximum* to increase the attractiveness of the ovitraps [20]. A 5 cm x 7 cm x 0.3 cm paddle made of hardboard served with its rough surface as an oviposition

Table 7.1: Classification of *Aedes* habitats sampled in oil palm-dominated landscapes in southeastern Côte d'Ivoire from January to December 2014

	Term	Definition
I	**Macrohabitat[1]**	**Landscape covering specific floristic area and presenting ecological or phyto-geographical aspects roughly homogeneous**
A	Rainforest[a]	Area covered with dense forest showing natural ecosystem with strong canopy coverage and comprising big threes, creepers, fixed masses of bamboo (*Bambusae*), and wild vertebrate animals such as primates, birds, and reptiles
B	Polyculture[a]	Area covered with mosaic of oil palm trees (*Eleasis guineensis*) mixed with other multiple crops composed of the plants of several industrial crops such as rubber (*Hevea brasiliensis*), cocoa (*Theobroma cacao*), coffee (*Coffea* spp.), papaya (*Carica papaya*), coconuts (*Cocos* spp.) and avocado (*Persea Americana*), and food-crops such as bananas (*Musa* spp.), taro (*Colocasia* spp.), bromeliads (*Ananas comosus*), yam (*Dioscorea* spp.), maize (*Zea mays*), and cassava (*manihot esculenta*) growing in the same space. Natural trees, fixed masses of bamboo (*Bambusae*), and degraded or secondary forest relics are dispersed in several places in the area
	Oil palm monoculture[a]	Area covered uniquely with the monoculture of oil palm trees (*Eleasis guineensis*)
D	Rural-housing area[a]	Area covered with human-inhabited space comprising buildings such as houses, markets, hospitals, schools, and other social edifices
II	**Microhabitat[1]**	**Containers that have potentials to hold water and serve as breeding sites for *Aedes* mosquito larvae**
II.1	**Naturally-occurring microhabitat[2]**	**Containers created without or by indirect intervention of humans**
E	Natural tree hole[b]	Rot and pan holes of different shapes and volume located from 0 to 2 m above the ground level
F	Bamboo hole[b]	Cut of fixed masses of bamboo (*Bambusae*)
G	Natural plant leaf[b]	Sheathing leaf axils from plants such as *Sanseviera* spp. and *Xanthosoma* spp. and sheets from *Thaumatococcus daniellii* fallen on the floor
H	Other natural microhabitat[b]	Non-ligneous containers such as snail shells and rock holes
II.2	**Agriculturally-occurring microhabitat[2]**	**Containers created by growing crops cultivated by humans**
I	Crop fruit husk[b]	Skins of the coconuts (*Cocos* spp.) and cocoa (*Theobroma cacao*)
J	Crop flower[b]	Flowers of bananas (*Musa* spp.)
K	Crop leaf[b]	Sheathing leaf axils from plants such as bromeliads (*Ananas comosus*), taros (*Colocasia* spp.), and bananas (*Musa* spp.), and fallen sheets on the floor
L	Cultivated plant hole	Growing plant holes of different shapes and volume located from 0 to 2 m above the ground level such as papaya (*Carica papaya*), coffee (*Coffea* spp.), avocado (*Persea Americana*), and cocoa (*Theobroma cacao*)
II.3	**Man-made microhabitat[2]**	**Containers created by direct intervention of humans**
M	Crop collection container[b]	Containers such as ceramic, cemented, glass, plastic and metallic receptacles used to collect crops such as rubber latex collection cups.
N	Husbandry watering container[b]	Containers such as ceramic, cemented, glass, plastic and metallic receptacles used to store water for watering plant or animal husbandry.
O	Discarded container[b]	Worn cans, tires, tarps, broken bottles, buckets, shoes, calabashes, mortars, building tools and debris of abandoned cars and machines
P	Household water container[b]	Containers such as ceramic, cemented, glass, plastic and metallic receptacles used to store potable water or collect rainwater for drinking, cooking or washing

[1]: habitat classe, [a]: macrohabitat type, [2]: microhabitat catergory, [b]:microhabiat sub-category.

substrate and was plunged into each container and left for one week during each of the 12 surveys.

7.3.5. Microhabitat surveys and Aedes *spp. larval sampling*

In a preliminary survey, existing larval breeding sites, such as natural and artificial cavities or containers with a potential to contain water were defined as microhabitats for *Aedes* larvae. Based on this preliminary survey, microhabitats were classified into three categories and 12 sub-categories depending on their occurring process, and the use (Table 1 and S1 Figure). We sampled up to 30 microhabitats of each of the 12 sub-category types among each macrohabitat. Microhabitats were examined monthly for the presence of water and immature stages of mosquitoes during 12 surveys between January and December 2014. If mosquito larvae and/or pupae were present, the content of microhabitat was completely removed using the following equipment: flexible rubber tube connected to a manual suction pump, ladles, and pipettes. Immature forms of *Aedes* and other non-*Aedes* mosquitoes such as *Anopheles* spp., *Culex* spp., *Eretmapodites* spp., and *Toxorhynchites* spp. were sampled and recorded separately. The predacious larvae of mosquitoes, such as *Cx. tigripes*, *Eretmapodites* spp., and *Toxorhynchites* spp. were removed from the samples and preserved separately to avoid predation on the other species. The microhabitats sampled were refilled to their initial volume with the original water, and topped up with distilled water or rainwater according to their flooding mechanism. The presence of shade, predators, and plant leaves in the microhabitats were recorded.

7.3.6. Aedes *adult abundance and host-seeking behavior surveillance*

Adult mosquitoes were sampled using four human-baited double-net traps in each macrohabitat type for three consecutive days from 04:00 a.m. to 08:00 p.m. during 12 monthly cross-sectional surveys in 2014. A double-net trap was a combination of two nets: an inner, smaller net that protected the human bait and an outer, larger net with two openings on each of the four sides which allowed the entry of mosquitoes yet precluded their exit (S7.2 Figure) [21]. For each double-net trap, there was a pair of persons: one person was located inside the small net and served as bait to attract mosquitoes. The other person was located outside the double-net device and collected the mosquitoes trapped within the outer net, once every hour. Each trap was monitored by two teams of two persons each that took turns beginning at 12:00 a.m.

7.3.7. Laboratory treatment procedures

All mosquito samples were stored separately in plastic boxes and transported in a cool-box to a nearby field laboratory. In the laboratory, mosquito larvae were reared until they reached the adult stage. In order to minimize mortality, a maximum of 20 larvae were placed in 200 ml plastic cups, filled with 150 ml distilled water and covered with netting. Larvae of *Aedes* and other mosquitoes were fed each morning between 07:00 a.m. and 08:00 a.m. with Tetramin Baby Fish Food. Predacious larvae (e.g., *Toxorhynchites* spp. and *Cx. Tigripes*) were fed with larvae from additionally sampled mosquitoes from the study areas. Emerging adults and collected adult mosquitoes were identified to species level using a morphological key [20, 22]. As larval mortalities were low, the proportion of mosquito species was estimated on the basis of emerging adults. Adult specimens were stored by species and recorded in an entomology collection database.

7.3.8. Statistical analysis

The proportion of *Aedes* species were calculated as percentage of specimens among *Aedes* fauna. We used Fisher's exact test to determine the relationship between species composition and the macro- and microhabitats. Fisher's exact test was employed because expected numbers of specimens were equal or less than five. *Aedes* species richness was expressed as the number of collected species in each study area and compared using a one-way analysis of variance (ANOVA), followed by Bonferroni's correction. The species diversity, dominance, and community similarity of *Aedes* spp. in the study and among the macrohabitats were estimated by Shannon-Weaver's index (H), Simpson's index (D), and Sorenson's coefficient (CC), and analyzed by Kruskal-Wallis test because the log-transformed data exhibited significant deviations from normality. For the samples collected by bamboo-ovitraps, metallic-ovitrap, and double-net trap, we used repeated measures approaches in generalized linear mixed models (GLMM), in order to take into account possible interactions between the variables "macrohabitats" and "month" [23]. The frequency of *Aedes*-positive microhabitats was calculated as the percentage of water holding containers with at least one larva or pupa and analyzed using a GLM approach. To account for overdispersion due to excessive numbers of zeroes, the data were log-transformed [log (number of specimens + 1)]. A significance level of 5% was set for statistical testing. All statistical analyses were conducted using Stata version 14.0 (Stata Corporation; College Station, Texas, United States of America).

7.4. Results

7.4.1. Mosquito species composition

Table 7.2 shows the species composition of adult mosquitoes collected as eggs, larvae, pupae, and adults using bamboo ovitrap, metallic ovitrap, larval survey, and human-baited double-net trap methods. A total of 30,449 mosquito specimens were collected, comprising different medically important genera, such as *Aedes*, *Anopheles*, *Culex*, *Mansonia*, and predatory larvae of *Eretmapodites* and *Toxorhynchites*. For any sampling method, *Aedes* mosquitoes dominated the fauna, representing 92.9% of the total fauna with 11 species. The proportions, sex, and the numbers of mosquito species varied substantially between sampling methods (Table 7.2). Overall, *Aedes* mosquitoes lacked in the oil palm monoculture, and conversely abounded in the other macrohabitats, with higher abundance in the polyculture (Figure 7.2) and higher species richness in the rainforest (Table 7.2).

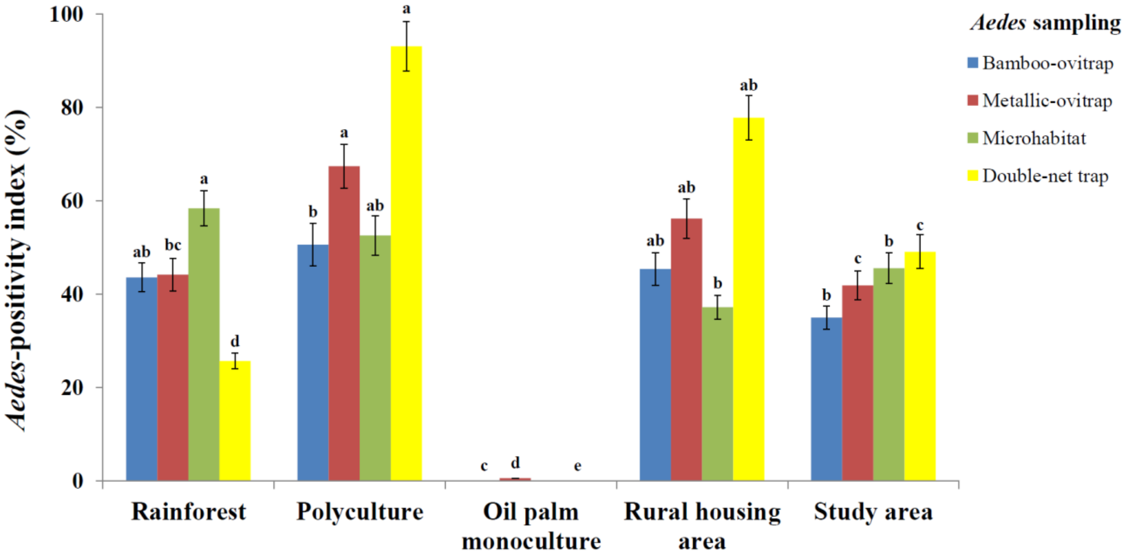


Figure 7.2: *Aedes* mosquito species occurrence among macrohabitats in oil palm-dominated landscapes in southeastern Côte d'Ivoire from January to December 2014. Error bars represent the standard error (SE). Letters indicate the results of the GLMM. Groups that do not share the same letter for the same sampling method are significantly different.

7.4.2. Distribution of Aedes *immature stages across macrohabitats*

Figure 7.2 Table 7.3 immature *Aedes* species occurrence, stratified by macrohabitats. Overall, the study area showed *Aedes*-positivity indices of 35.0% (482/1,378) in the bamboo-ovitraps, 41.9% (577/1,377) in metallic-ovitraps, and 45.6% (801/1,756) in the microhabitats. The highest *Aed*es-positivity indices in the bamboo-ovitraps (177/350; 50.6%) and in the metallic-ovitraps (232/344; 67.4%) were found in the polyculture environment. Conversely, GLMM indicated that *Aedes*-positivity indices were significantly lower in oil palm monoculture compared to the other macrohabitats (p <0.05) (S1 Table).

Microhabitat *Aedes*-positivity indices widely varied from one macrohabitat to another (Table 7.3 and S7.3 Figure). No *Aedes*-positive microhabitats were found in oil palm monoculture. In contrast, the highest *Aedes*-microhabitats positivity index was estimated for the rainforest (94/161; 58.4%), followed by the polyculture (388/737; 52.6%), and the rural housing area (319/858; 37.2%). In the rural housing area, water containers were highly infested with *Aedes* larvae (159/229; 69.4%), and reached 86.4% (19/22) in December 2014 during the long dry season. In the polyculture site, the highest *Aedes*-infectivity index (135/167; 80.8%) was observed among the discarded containers.

Table 7.4 shows the proportions of each type of *Aedes*-positive microhabitats among the whole *Aedes*-positive microhabitats in each macrohabitat. In the rainforest, all the *Aedes*-positive breeding sites (94/94; 100%) were naturally occurring microhabitats, while 95.0% (303/319) of *Aedes*-positive microhabitats were man-made containers in the rural housing area. The polyculture macrohabitat had substantial proportions of all *Aedes*-positive microhabitat types, with 24.2% (94/388) of naturally-occurring, 24.8% (96/388) of agriculturally-occurring and 51.0% (198/388) of man-made microhabitats. Taken together, in the study area, the *Aedes*-positive breeding sites were dominated by man-made microhabitats (501/801; 62.6%), followed by naturally-occurring microhabitats (198/801; 24.7%), and agricultural microhabitats (102/801; 12.7%) (S7.4 Figure). Overall, a part the oil palm monocultures, *Aedes*-microhabitat positivity indices were higher during the dry season (S7.5 Figure), whereas the highest proportions of *Aedes*-positive microhabitats were recorded during the rainy seasons (S7.6 Figure) in the other macrohabitats and the whole study area.

The frequency of microhabitats with shade, plant leaves, and predators varied among the macrohabitats. The highest proportions of shaded microhabitats (n = 607; 96.9%), and microhabitats with plant leaves (92.6%) were found in the rainforest. Wet microhabitats containing at least one of the predatory larvae of *Toxorhynchites* spp., *Eretmapodites* spp.,

Table 7.2: Species composition of mosquitoes sampled in oil palm-dominated landscapes in southeastern Côte d'Ivoire from January to December 2014

Genus	Species	Bamboo-ovitrap				Metallic-ovitrap				Larval survey				Double-net trap				Total			
		F	**M**	**T**	**%**	**F**	**M**	**T**	**%**	**F**	**M**	**T**	**%**	**F**	**M**	**T**	**%**	**F**	**M**	**T**	**%**
Aedes	*Ae. aegypti*	1382	1343	2725	8.9	2052	1952	4004	13.1	3909	3742	7651	25.1	6735	1286	8021	26.3	14078	8323	22401	73.6
	Ae. africanus	163	167	330	1.1	199	193	392	1.3	120	141	261	0.9	59	9	68	0.2	541	510	1051	3.5
	Ae. dendrophilus	410	408	818	2.7	528	481	1009	3.3	405	384	789	2.6	302	58	360	1.2	1645	1331	2976	9.8
	Ae. fraseri	16	11	27	0.1	27	38	65	0.2	16	21	37	0.1	0	0	0	0.0	59	70	129	0.4
	Ae. furcifer	41	35	76	0.2	62	70	132	0.4	145	122	267	0.9	23	3	26	0.1	271	230	501	1.6
	Ae. lilii	26	16	42	0.1	13	15	28	0.1	9	5	14	0.0	0	0	0	0.0	48	36	84	0.3
	Ae. luteocephalus	42	50	92	0.3	67	49	116	0.4	27	27	54	0.2	0	0	0	0.0	136	126	262	0.9
	Ae. metallicus	13	16	29	0.1	44	49	93	0.3	25	23	48	0.2	0	0	0	0.0	82	88	170	0.6
	Ae. opok	13	30	43	0.1	9	1	10	0.0	8	7	15	0.0	0	0	0	0.0	30	38	68	0.2
	Ae. palpalis	6	6	12	0.0	19	13	32	0.1	55	62	117	0.4	3	1	4	0.0	83	82	165	0.5
	Ae. vittatus	29	13	42	0.1	98	80	178	0.6	57	38	95	0.3	119	35	154	0.5	303	166	469	1.5
	Total	**2141**	**2095**	**4236**	**13.9**	**3118**	**2941**	**6059**	**19.9**	**4776**	**4572**	**9348**	**30.7**	**7241**	**1392**	**8633**	**28.4**	**17276**	**11000**	**28276**	**92.9**
Anopheles	*An. pharoensis*	0	0	0	0.0	0	0	0	0.0	8	2	10	0.0	0	0	0	0.0	8	2	10	0.0
	An. gambiae	0	0	0	0.0	0	0	0	0.0	39	48	87	0.3	19	2	21	0.1	58	50	108	0.4
	An. ziemani	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	1	0	1	0.0	1	0	1	0.0
	Total	**0**	**0**	**0**	**0.0**	**0**	**0**	**0**	**0.0**	**47**	**50**	**97**	**0.3**	**20**	**2**	**22**	**0.1**	**67**	**52**	**119**	**0.4**
Culex	*Cx. nebulosus*	19	27	46	0.2	52	43	95	0.3	15	19	34	0.1	6	0	6	0.0	92	89	181	0.6
	Cx. poicilipes	32	36	68	0.2	29	41	70	0.2	73	54	127	0.4	48	5	53	0.2	182	136	318	1.0
	Cx. quinquefasciatus	74	62	136	0.4	89	71	160	0.5	218	176	394	1.3	56	11	67	0.2	437	320	757	2.5
	Cx. tigripes	3	4	7	0.0	13	6	19	0.1	79	95	174	0.6	3	0	3	0.0	98	105	203	0.7
	Total	**128**	**129**	**257**	**0.8**	**183**	**161**	**344**	**1.1**	**385**	**344**	**729**	**2.4**	**113**	**16**	**129**	**0.4**	**809**	**650**	**1459**	**4.8**
Eretmapodites	*Er. chrysogaster*	87	69	156	0.5	76		76	0.2	112	97	209	0.7	48	14	62	0.2	323	180	503	1.7
	Total	**87**	**69**	**156**	**0.5**	**76**	**0**	**76**	**0.2**	**112**	**97**	**209**	**0.7**	**48**	**14**	**62**	**0.2**	**323**	**180**	**503**	**1.7**
Mansonia	*Ma. africana*	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	6	0	6	0.0	6	0	6	0.0
	Ma uniformis	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	2	1	3	0.0	2	1	3	0.0
	Total	**0**	**0**	**0**	**0.0**	**0**	**0**	**0**	**0.0**	**0**	**0**	**0**	**0.0**	**8**	**1**	**9**	**0.0**	**8**	**1**	**9**	**0.0**
Toxorhynchites	*Tx. brevipalpis*	0	0	0	0.0	0	0	0	0.0	47	36	83	0.3	0	0	0	0.0	47	36	83	0.3
	Total	**0**	**0**	**0**	**0.0**	**0**	**0**	**0**	**0.0**	**47**	**36**	**83**	**0.3**	**0**	**0**	**0**	**0.0**	**47**	**36**	**83**	**0.3**
Total	**Abundance**	**2356**	**2293**	**4649**	**15.3**	**3377**	**3102**	**6479**	**21.3**	**5367**	**5099**	**10466**	**34.4**	**7430**	**1425**	**8855**	**29.1**	**18530**	**11919**	**30449**	**100**
	No. of species	**16**				**16**				**19**				**15**				**22**			

F: female, M: male, T: total, %: percentage

Table 7.3: ***Aedes*** **mosquito infectivity patterns among the macrohabitats and the study area in southeastern Cote d'Ivoire from January to December 2014**

Term	**Macrohabitat**												**Study area**		
	Rainforest			**Polyculture**			**Oil palm monoculture**			**Rural-housing area**					
	n_1	n_2	**PI**	n_1	n_2	**PI**	n_1	n_2	**PI**	n_1	n_2	**PI**	n_1	n_2	**PI**
Bamboo-ovitrap[1]	346	151	43.6	350	177	50.6	343	0	0.0	339	154	45.4	1378	482	35.0
Metallic-ovitrap[2]	344	152	44.2	344	232	67.4	349	2	0.6	340	191	56.2	1377	577	41.9
Microhabitat[3]	161	94	58.4	737	388	52.6	0	0	NA	858	319	37.2	1756	801	45.6
Naturally-occurring microhabitat[3]	161	94	58.4	148	94	63.5	0	0	NA	47	10	21.3	356	198	55.6
Natural tree hole[3]	54	45	83.3	42	33	78.6	0	0	NA	4	1	25.0	100	79	79.0
Bamboo hole[3]	51	38	74.5	29	21	72.4	0	0	NA	13	4	30.8	93	63	67.7
Natural plant leaf[3]	52	9	17.3	29	7	24.1	0	0	NA	11	0	0.0	92	16	17.4
Other natural microhabitat[3]	4	2	50.0	48	33	68.8	0	0	NA	19	5	26.3	71	40	56.3
Agriculturally-occurring microhabitat[3]	0	0	NA	314	96	30.6	0	0	NA	49	6	12.2	363	102	28.1
Crop fruit husk[3]	0	0	NA	91	47	51.6	0	0	NA	26	6	23.1	117	53	45.3
Crop flower[3]	0	0	NA	68	3	4.4	0	0	NA	16	0	0.0	84	3	3.6
Crop leaf[3]	0	0	NA	96	11	11.5	0	0	NA	0	0	NA	96	11	11.5
Cultivated plant hole[3]	0	0	NA	59	35	59.3	0	0	NA	7	0	0.0	66	35	53.0
Man-made microhabitat[3]	0	0	NA	275	198	72.0	0	0	NA	762	303	39.8	1037	501	48.3
Crop collection container[3]	0	0	NA	57	33	57.9	0	0	NA	6	2	33.3	63	35	55.6
Husbandry watering container[3]	0	0	NA	51	30	58.8	0	0	NA	229	159	69.4	280	189	67.5
Discarded container[3]	0	0	NA	167	135	80.8	0	0	NA	167	105	62.9	334	240	71.9
Household water container[3]	0	0	NA	0	0	NA	0	0	NA	360	37	10.3	360	37	10.3
Double-net trap[4]	144	37	25.7	144	134	93.1	144	0	0.0	144	112	77.8	576	283	49.1

n_1: numbers of ovitraps recovered[1,2], wet microhabitats[3] or double-net traps installed[4], n_2: numbers of *Aedes*-positive ovitraps[1,2], *Aedes*-positive microhabitats[3] or *Aedes*-positive double-net traps[4], PI: *Aedes*-positivity index. PI is expressed as percentage (%).

Table 7.4: Proportions (%) of each *Aedes*-positive microhabitat type among the all *Aedes*-positive microhabitats in macrohabitats and study area in southeastern Cote d'Ivoire in January to December 2014

Term	**Macrohabitat**												**Study area**	
	Rainforest			**Polyculture**			**Oil palm monoculture**			**Rural-housing area**				
	n	**PPM**	**PPSA**	**n**	**PPM**	**PPSA**	**n**	**PPM**	**PPSA**	**n**	**PPM**	**PPSA**	**n**	**PPSA**
Naturally-occurring microhabitat	94	100.0	11.7	94	24.2	11.7	0	NA	0.0	10	3.1	1.2	198	24.7
Natural tree hole	45	47.9	5.6	33	8.5	4.1	0	NA	0.0	1	0.3	0.1	79	9.9
Bamboo hole	38	40.4	4.7	21	5.4	2.6	0	NA	0.0	4	1.3	0.5	63	7.9
Natural plant leaf	9	9.6	1.1	7	1.8	0.9	0	NA	0.0	0	0.0	0.0	16	2.0
Other natural microhabitat	2	2.1	0.2	33	8.5	4.1	0	NA	0.0	5	1.6	0.6	40	5.0
Agriculturally-occurring microhabitat	0	0.0	0.0	96	24.7	12.0	0	NA	0.0	6	1.9	0.7	102	12.7
Crop fruit husk	0	0.0	0.0	47	12.1	5.9	0	NA	0.0	6	1.9	0.7	53	6.6
Crop flower	0	0.0	0.0	3	0.8	0.4	0	NA	0.0	0	0.0	0.0	3	0.4
Crop leaf	0	0.0	0.0	11	2.8	1.4	0	NA	0.0	0	0.0	0.0	11	1.4
Cultivated plant hole	0	0.0	0.0	35	9.0	4.4	0	NA	0.0	0	0.0	0.0	35	4.4
Man-made microhabitat	0	0.0	0.0	198	51.0	24.7	0	NA	0.0	303	95.0	37.8	501	62.6
Crop collection container	0	0.0	0.0	33	8.5	4.1	0	NA	0.0	2	0.6	0.2	35	4.4
Husbandry watering container	0	0.0	0.0	30	7.7	3.7	0	NA	0.0	159	49.8	19.9	189	23.6
Discarded container	0	0.0	0.0	135	34.8	16.9	0	NA	0.0	105	32.9	13.1	240	30.0
Household water container	0	0.0	0.0	0	0.0	0.0	0	NA	0.0	37	11.6	4.6	37	4.6
Total	**94**	**100**	**11.7**	**388**	**100**	**48.5**	**0**	NA	0.0	**319**	**100**	**39.8**	**801**	**100**

n: numbers of *Aedes*-positive microhabitats, PPM: proportions of *Aedes*-positive microhabitat type among the whole *Aedes*-positive microhabitats in each macrohabitat. PPSA: proportions of *Aedes*-positive microhabitat type among the whole *Aedes*-positive microhabitats in the study area. PPM and PPSA are expressed as percentage (%).

and *Cx. tigripes* mosquitoes were also mostly encountered in the rainforest (n = 161; 63.4%). The polyculture area also hosted higher numbers of microhabitats with shade (n = 2,117; 54.5%), plant leaves (n = 2,117; 59.6%), and predators (n = 737; 29.9%) compared with the rural housing areas.

7.4.3. Aedes *species distribution, biodiversity and dynamics*

Table 7.5 presents the geographic distribution and biodiversity of *Aedes* species among the macrohabitats in the study area. *Ae. aegypti* was the dominant species (n = 28,276; 79.2%), and all of the macrohabitats with 49.2% in the polyculture, 25.7% in the rural-housing areas, and 4.3% in the rainforest. Other *Aedes* species such as *Ae. dendrophilus* (10.5%), *Ae. africanus* (3.7%), *Ae. furcifer* (1.8%), and *Ae. vittatus* (1.7%), represented more than 1% of the total *Aedes* fauna in the study area. However, *Ae. africanus* (3.4%) showed its highest abundance in the rainforest, whereas the highest proportions of *Ae. dendrophilus* (7.6%) and *Ae. furcifer* (1.2%) were found in the polyculture area. The proportion of *Ae. dendrophilus* was above 1.0% in the rural housing area.

Aedes species numbers, diversity (F = 17.12; df = 3, p <0.05), and dominance (F = 11.04; df = 3, p <0.05) varied among the study area and the macrohabitats (Table 7.5). The highest *Aedes* species richness (n = 11) and the highest species diversity (Shannon Index H = 1.54) were observed in the rainforest, while oil palm monoculture exhibited the poorest diversity with one species and null Shannon index. The rural housing area displayed significantly higher *Aedes* species dominance (Simpson index D = 0.085) compared with the rainforest (Simpson index D = 0.28), the study area (Simpson index D = 0.64), and the polyculture (Simpson index D = 0.67). The community similarity of *Aedes* species between the macrohabitats also significantly altered (χ^2 = 13.36; df = 3, p <0.05) (Table 7.5). According to Sorenson’s coefficient (CC = 1), *Aedes* mosquito community in the study area were similar to those inhabiting the rainforest. Compared with the rainforest, the polyculture showed the highest community similarity with Sorenson’s coefficient of 0.95, followed by the rural-housing area with a Sorenson’s coefficient of 0.85. In contrast, the *Aedes* communities in the rainforest and oil palm monoculture showed with 0.17 the lowest value for the Sorenson’s coefficient.

Table 7.5: *Aedes* species distribution and biodiversity among macrohabitats in oil palm-dominated landscapes in southeastern Côte d'Ivoire between January and December 2014

Species	**Macrohabitat**								**Study area**	
	Rainforest		**Polyculture**		**Oil palm monoculture**		**Rural-housing area**			
	Number	**%**	**Number**	**%**	**Number**	**%**	**Number**	**%**	**Number**	**%**
Ae. aegypti	1213	4.3	13903	49.2	4	0.01	7281	25.7	22401	79.2
Ae. africanus	948	3.4	61	0.2	0	0.0	42	0.1	1051	3.7
Ae. dendrophilus	544	1.9	2150	7.6	0	0.0	282	1	2976	10.5
Ae. fraseri	129	0.5	0	0.0	0	0.0	0	0.0	129	0.5
Ae. furcifer	24	0.1	352	1.2	0	0.0	125	0.4	501	1.8
Ae. lilii	53	0.2	31	0.1	0	0.0	0	0.0	84	0.3
Ae. luteocephalus	96	0.3	158	0.6	0	0.0	8	0.0	262	0.9
Ae. metallicus	25	0.1	126	0.4	0	0.0	19	0.1	170	0.6
Ae. opok	24	0.1	34	0.1	0	0.0	10	0.0	68	0.2
Ae. palpalis	35	0.1	130	0.5	0	0.0	0	0.0	165	0.6
Ae. vittatus	24	0.1	289	1	0	0.0	156	0.6	469	1.7
Abundance (no. of specimens)	**3115**	**11.0**	**17234**	**60.9**	**4**	**0.01**	**7923**	**28.0**	**28276**	**100**
Species richness (no. of species)	**11**		**10**		**1**		**8**		**11**	
Species diversity (Shannon Index (H))	1.54		0.74		0.00		0.40		0.84	
Species dominance (Simpson Index (D))	0.28		0.67		1.00		0.85		0.64	
Community similarity (Sorenson's coefficient (CC))	1.00		0.95		0.17		0.84		1.00	
	0.95		1.00		0.18		0.89		0.95	
	0.17		0.18		1.00		0.22		0.17	
	0.84		0.89		0.22		1.00		0.84	
	1.00		0.95		0.17		0.84		1.00	

%: proportions of *Aedes* specimens calculated as percentages (%). In each row, a macrohabitat with a Sorenson's coefficient of 1 was used as a reference to calculate the Sorenson's coefficients for the other macrohabitats.

Table 7.6 indicates *Aedes* species abundance among the macrohabitats in the study area. No *Aedes* eggs, larvae, pupae, or adults were collected in the oil palm monoculture using bamboo-ovitrap, larval survey, and double-net trap methods, except the four eggs sampled with the metallic-ovitraps. However, higher mean numbers (mean ± standard error) of *Aedes* specimens with 2.32 ± 0.07 eggs/bamboo-ovitrap/week, 4.18 ± 0.07 eggs/metallic-ovitrap/week, and 26.01 ± 0.12 adults/double-net trap/day were found in the polyculture. The mean numbers in bamboo-ovitrap deployed in oil palm monoculture was significantly lower than the rainforest ($Z = 1.96$, $p < 0.05$) and rural housing area ($Z = 2.06$, $p < 0.05$) (S2 Table). The mean numbers of *Aedes* eggs collected using metallic-ovitrap were significant different between the oil palm monoculture and the rainforest ($Z = -2.04$, $p = 0.041$) (S3 Table), between the polyculture and the rainforest ($Z = -3.45$, $p = 0.001$) (S4 Table). GLMM sowed that the mean numbers of *Aedes* eggs were significantly lower in oil palm monoculture than the other macrohabitats ($p < 0.05$) (S5 Table). The rural-housing area (0.63 ± 0.03 larvae/microhabitat) and the polyculture (0.60 ± 0.02 larvae/microhabitat) showed higher means of *Aedes* larvae compared with the other macrohabitats. In the rainforest, the tree holes were the most *Aedes*-inhabited containers, with 1.87 ± 0.12 larvae/microhabitats. The rainforests were free of any agricultural and man-made microhabitats, while the polyculture macrohabitat hosted all types of microhabitats, except for the household water containers. In the rural housing areas, the water containers were the most important producers of *Aedes* larvae with a mean of 2.47 ± 0.07 larvae/microhabitat. In the discarded containers *Aedes* immatures were also highly abundant with a mean number of 1.46 ± 0.05 larvae/ microhabitats.

Fig 7.3 shows the seasonal dynamics of whole *Aedes* species populations, sampled as eggs, larvae, pupae, and adults, over time among the macrohabitats in the study area. In the study area and macrohabitats, *Aedes* species abundance varied as a function of rainfall over time. *Aedes* abundance reached the first series of peaks in June, during the long rainy season, proportions of 19.1% (n = 28,276) in the study area, 12.4% in the polyculture, 4.6% in the rural-housing area, 2.0% in the rainforest, and 0.01% in oil palm monoculture. The second series of peaks occurred in October, during the short rainy season, with 13.9% in the study area, 9.0% in the polyculture, 3.3% in the rural-housing area, and 1.6% in the rainforest.

Table 7.6: *Aedes* mosquito abundance patterns among the macrohabitats and study area in southeastern Cote d'Ivoire between January and December 2014

Term	**Macrohabitat**												**Study area**		
	Rainforest			**Polyculture**			**Oil palm monoculture**			**Rural-housing area**					
	n_1	**n_2**	**Mean ± SE**	**n_1**	**n_2**	**Mean ± SE**	**n_1**	**n_2**	**Mean ± SE**	**n_1**	**n_2**	**Mean ± SE**	**n_1**	**n_2**	**Mean ± SE**
Bamboo-ovitrap[1]	346	1018	1.28 ± 0.06	350	1899	2.32 ± 0.07	343	0	0	339	1319	1.73 ± 0.06	1378	4236	1.13 ± 0.03
Metallic-ovitrap[2]	344	1198	1.44 ± 0.06	344	2830	4.18 ± 0.07	349	4	0.01 ± 0.004	340	2027	2.72 ± 0.07	1377	6059	1.61 ± 0.03
Microhabitat[3]	607	671	0.36 ± 0.03	2117	5339	0.60 ± 0.02	0	0	NA	1497	3338	0.63 ± 0.03	4221	9348	0.57 ± 0.02
Naturally-occurring microhabitat[3]	607	671	0.36 ± 0.03	435	1537	0.80 ± 0.06	0	0	NA	191	53	0.09 ± 0.03	1233	2261	0.45 ± 0.03
Natural tree hole[3]	92	372	1.87 ± 0.12	82	688	2.40 ± 0.18	0	0	NA	46	8	0.05 ± 0.05	220	1068	1.48 ± 0.09
Bamboo hole[3]	189	257	0.48 ± 0.06	89	377	0.95 ± 0.14	0	0	NA	56	18	0.11 ± 0.06	334	652	0.52 ± 0.05
Natural plant leaf[3]	283	33	0.05 ± 0.02	111	54	0.14 ± 0.05	0	0	NA	28	0	0	422	87	0.07 ± 0.02
Other natural microhabitat[3]	43	9	0.08 ± 0.06	153	418	0.69 ± 0.09	0	0	NA	61	27	0.15 ± 0.07	257	454	0.43 ± 0.06
Agriculturally-occurring microhabitat[3]	0	0	NA	1118	1001	0.22 ± 0.02	0	0	NA	275	51	0.05 ± 0.02	1393	1052	0.19 ± 0.02
Crop fruit husk[3]	0	0	NA	338	556	0.41 ± 0.05	0	0	NA	98	51	0.14 ± 0.06	436	607	0.35 ± 0.04
Crop flower[3]	0	0	NA	266	16	0.02 ± 0.01	0	0	NA	54	0	0	320	16	0.02 ± 0.01
Crop leaf[3]	0	0	NA	360	75	0.06 ± 0.02	0	0	NA	89	0	0	449	75	0.05 ± 0.01
Cultivated plant hole[3]	0	0	NA	154	354	0.69 ± 0.08	0	0	NA	34	0	0	188	354	0.54 ± 0.07
Man-made microhabitat[3]	0	0	NA	564	2801	1.50 ± 0.06	0	0	NA	1031	3234	0.98 ± 0.03	1595	6035	1.15 ± 0.03
Crop collection container[3]	0	0	NA	141	454	0.83 ± 0.10	0	0	NA	39	5	0.07 ± 0.05	180	459	0.63 ± 0.08
Husbandry watering container[3]	0	0	NA	63	303	1.99 ± 0.16	0	0	NA	272	1362	2.47 ± 0.07	335	1665	2.37 ± 0.06
Discarded container[3]	0	0	NA	360	2044	1.74 ± 0.07	0	0	NA	360	1560	1.20 ± 0.07	720	3604	1.46 ± 0.05
Household water container[3]	0	0	NA	0		NA	0	0	NA	360	307	0.24 ± 0.04	360	307	0.24 ± 0.04
Double-net trap[4]	144	228	0.71 ± 0.07	144	7166	26.01 ± 0.12	144	0	0	144	1239	4.89 ± 0.10	576	8633	3.06 ± 0.07

n_1: number of recovered bamboo-ovitraps[1] or number of recovered metallic-ovitraps[2] or microhabiats[3] or double- net trap[4], n_2: number of eggs, larvae or adults of *Aedes* collected, SE: standard error of the mean numbers. Mean is mean numbers of *Aedes* eggs per bamboo-ovitraps[1], mean numbers of *Aedes* eggs per metallic-ovitraps[2], mean numbers of *Aedes* larvae per microhabitats[3] or mean numbers of *Aedes* adults per double-net trap[4], The units are egg/bamboo-ovitrap/week for bamboo-ovitraps[1], egg/metallic-ovitrap/week for metallic-ovitraps[2], larvae/microhabitats for microhabitats[3], and adult/trap/day for double-net traps[4]

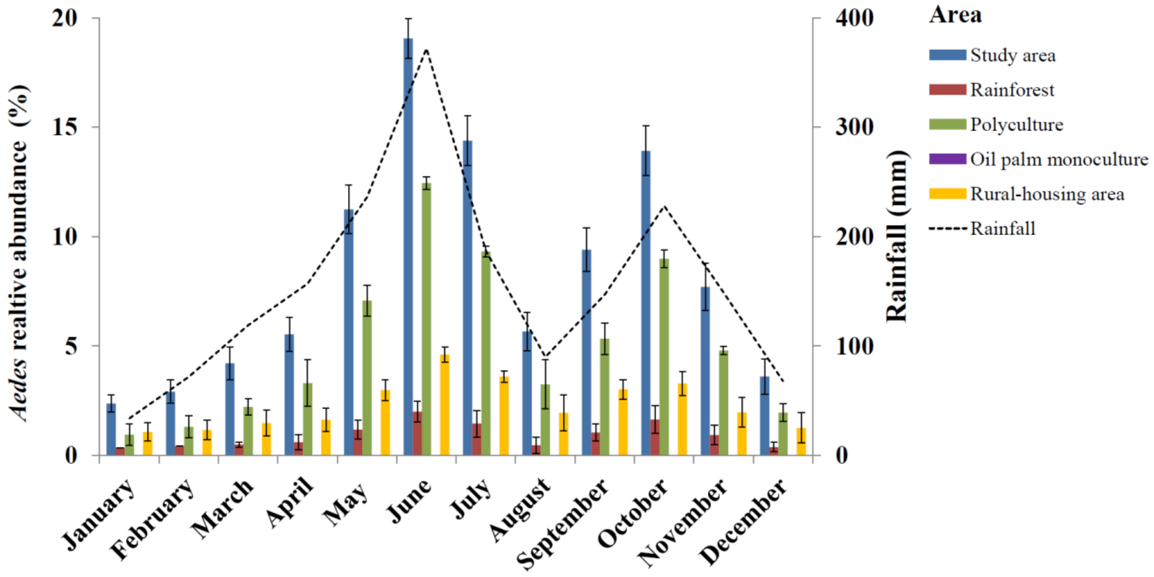


Figure 7.3: Monthly variations in the abundance of *Aedes* mosquitoes in oil palm-dominated landscapes in southeastern Côte d'Ivoire from January to December 2014. Error bars represent the standard error (SE).

7.4.4. Adult Aedes *females' host-seeking behaviors*

The mean biting rates of *Aedes* females were estimated at 2.76 ± 0.07 females/person/day in the study area. Over 93.0% (n = 7,241) of biting was inflicted by *Ae. aegypti*. Conversely, the females of several other species such as *Ae. fraseri*, *Ae. lilii*, *Ae. luteocephalus*, *Ae. metallicus*, and *Ae. opok* were not sampled using the human-baited double-net device (Table 7.2). The highest mean biting rates were found in the polyculture macrohabitat (21.48 ± 0.12 females/person/day), followed by the rural housing areas (4.48 ± 0.10 females/person/day), and the rainforest (0.62 ± 0.6 females/person/day). No *Aedes* females were collected in the oil palm monoculture. GLMM revealed significant differences in the mean biting rates comparing rainforest with polyculture (Z = 2.47, p = 0.014), and rainforest with housing area (Z = 2.37, p = 0.018) (S7.3 Table).

Figure 7.4 presents the seasonal dynamics of *Aedes* host-seeking in the study area and the macrohabitats. GLMM indicated that the biting rates of *Aedes* females significantly varied over the months (p <0.05) (S7.5 Table), and peaked in June during the long rainy season and in October during the short rainy season across all macrohabitats, except for the oil palm monoculture (Figure 7.3). The major biting rate peaks of *Aedes* females averaged 109.54 ± 0.07 females/person/day in the polyculture, 16.14 ± 0.17 females/person/day in the rural housing area, 8.44 ± 0.30 females/person/day in the study area, and 3.18 ± 0.24

females/person/day in the rainforest in June. The secondary most important biting rates occurred in October with 74.5 ± 0.10 females/person/day in the polyculture, 10.7 ± 0.27 females/person/day in the rural-housing areas, 6.33 ± 0.29 females/person/day in the study area, and 2.27 ± 0.32 females/person/day in the rainforest.

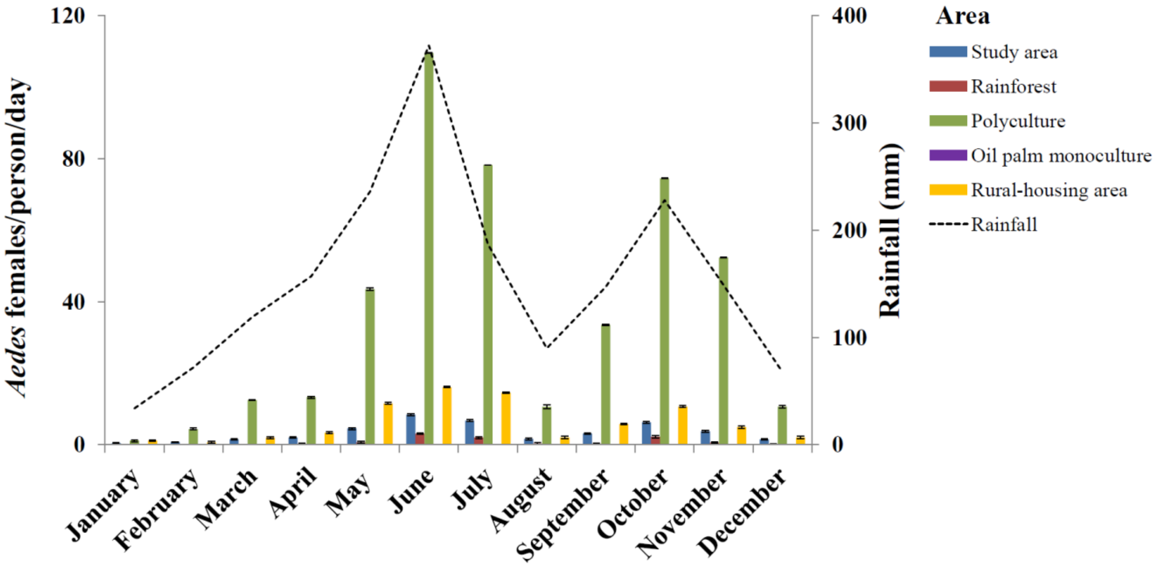


Figure 7.4: Monthly variations in *Aedes* mosquito females' host-seeking activities in oil palm-dominated landscapes in southeastern Côte d'Ivoire from January to December 2014. Error bars represent the standard error (SE).

Figure 7.5 shows the daily host-seeking activity cycles of *Aedes* mosquito females in the study area and across the different macrohabitats. *Aedes* females fed from 04:00 a.m. to 8:00 p.m., covering daytime (06:00 a.m. to 6:00 p.m), and darkness (04:00 a.m. to 06:00 a.m. and 6:00 p.m to 8:00 p.m.) in all macrohabitats, except in the oil palm monoculture (Figure 7.5A). The biting cycles showed two peaks, with the main peak observed between 4:00 p.m. and 5:00 p.m. and a lower peak between 07:00 a.m. and 08:00 a.m. *Ae. aegypti*, *Ae. dendrophilus*, and *Ae. vittatus* followed the same host-seeking patterns (Figure 7.5A) with stronger human biting intensity in *Ae. aegypti* in the study area (Fig 5B), the polyculture (Figure 7.5C), and the rural housing areas (Figure 7.5D). In contrast to these similarities, there was also some dissimilarity in that host-biting activity was interrupted from 11:00 a.m. to 2:00 p.m. in the rural housing area but continued in polyculture macrohabitat (Figure 7.5A).

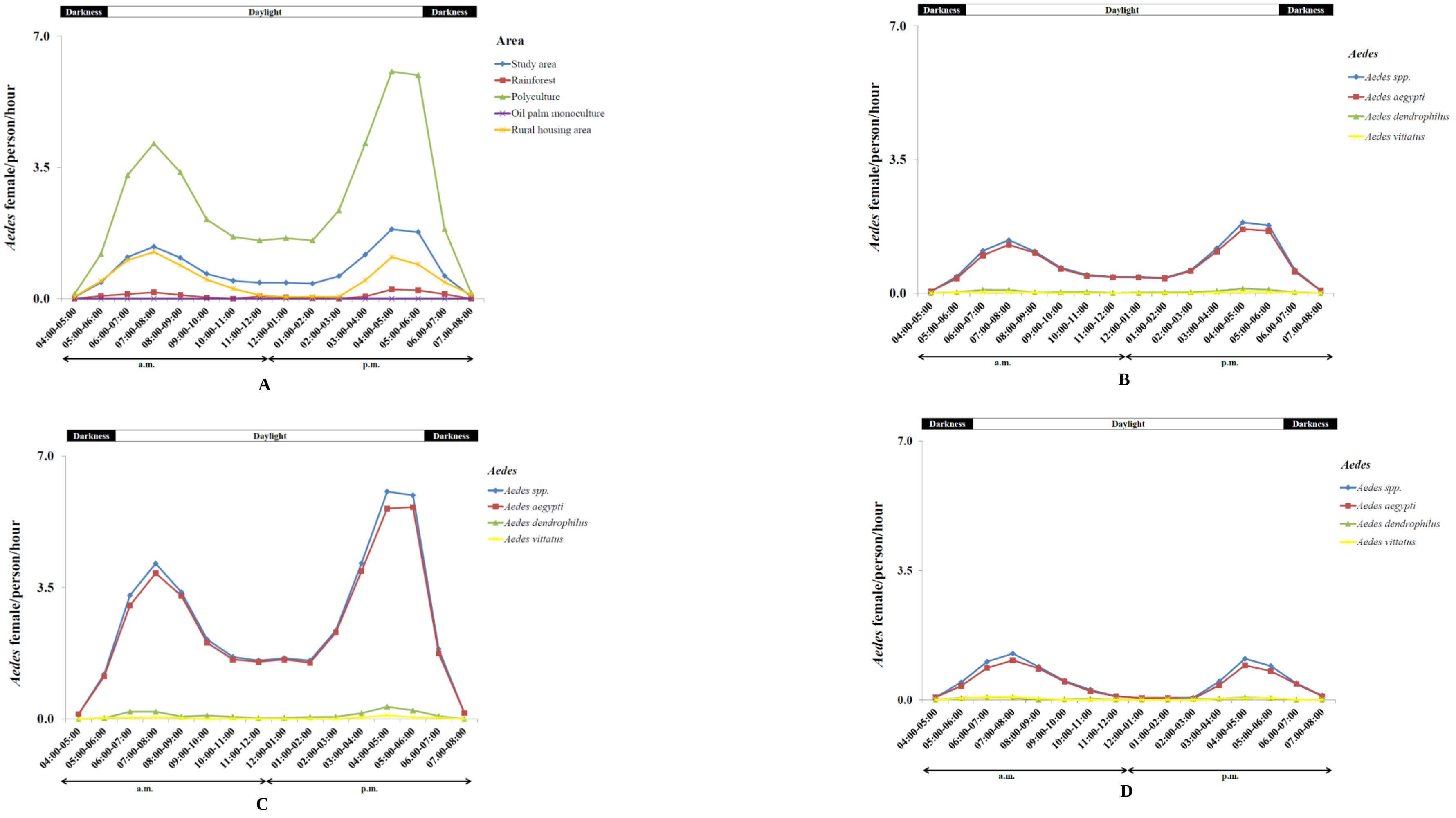


Figure 7.5. Nycthemeral dynamics of *Aedes* mosquito females' host-seeking activities in oil palm-dominated landscapes in southeastern Côte d'Ivoire from January to December 2014. A: All species in all the macrohabitats and the study area, B: Prevalent *Aedes* species (> 1%) in the study area, C: Prevalent *Aedes* species (> 1%) in the polyculture, Prevalent *Aedes* species (> 1%) in the rural-housing areas.

Table 7.7. Synthesis of how land-use changes shape the ecology of *Aedes* mosquitoes in oil palm-dominated areas in southeastern Côte d'Ivoire

	Rainforest	**Polyculture**	**Oil palm monoculture**	**Rural housing area**	**Study area**
Bamboo-ovitrap positivity (%)	43.6	50.6	0.0	45.4	35.0
Metallic-ovitrap positivity (%)	44.2	67.4	0.6	56.2	41.9
Microhabitat positivity (%)	58.4	52.6	0.0	37.2	45.6
Microhabitat type					
Naturally-occurring (%)	100	51.0	0.0	95.0	55.6
Agriculturally-occurring (%)	0	24.8	0.0	1.9	28.1
Made-made (%)	0	24.2	0.0	3.1	48.3
Double net trap positivity (%)	25.7	93.1	0.0	77.8	49.1
Species richness (no of species)	11	10	1	8	11
Abundance (%)	11.0	60.9	0.01	28.0	100
Host-seeking activity (mean ± SE)	0.62 ± 0.06	21.48 ± 0.12	0.00	4.48 ± 0.10	2.76 ± 0.07
Arbovirus-risk	-	++	--	+	+

-- : very low risk, - : low risk, + : high risk, ++ : very high risk; %: percentage; SE: Standard error of the mean. Host-seeking activity is expressed as the mean numbers of *Aedes* females collected per human-baited double-net trap. The unit of host-seeing activity is female/person/day. Overall, there was a lack of *Aedes* microhabitats and species in the oil palm monoculture resulting in very low arbovirus-risk. In contrast, the highest abundance of *Aedes* mosquitoes was found in the polyculture where arbovirus-risk is expected to be very high. The highest species richness was observed in the rainforest where the preference of *Aedes* females to feed on humans was little. The rural housing area and the whole study area hosted substantial numbers of *Aedes* mosquitoes and arbovirus-risk is expected to be high in rural housing area and moderate in the whole study area.

7.5. Discussion

Our study revealed no *Aedes*-positive microhabitats and only four specimens of *Ae. aegypti* in oil palm monocultures, coupled with high *Aedes* species richness in the rainforests, and high biting rates in polyculture and rural housing areas. As identifying priority areas for integrated vector management is of considerable importance for public health [3, 24], this study examined – for the first time – the effects of land-use changes on *Aedes* mosquito abundance, distribution, and human host seeking behavior in oil palm dominated landscapes of yellow fever and dengue foci in the south-eastern part of Côte d'Ivoire. Our data showed that *Aedes* mosquito species displayed several significant differences in community composition, distribution, and host-seeking behavior across different land-covers, with the highest species richness observed in rainforest, highest species numbers in the polyculture macrohabitats, the lowest species richness and numbers in oil palm monoculture, and stronger anthropophagic behaviors in the polyculture and rural housing areas (7.7 Table and S7.6 Figure).

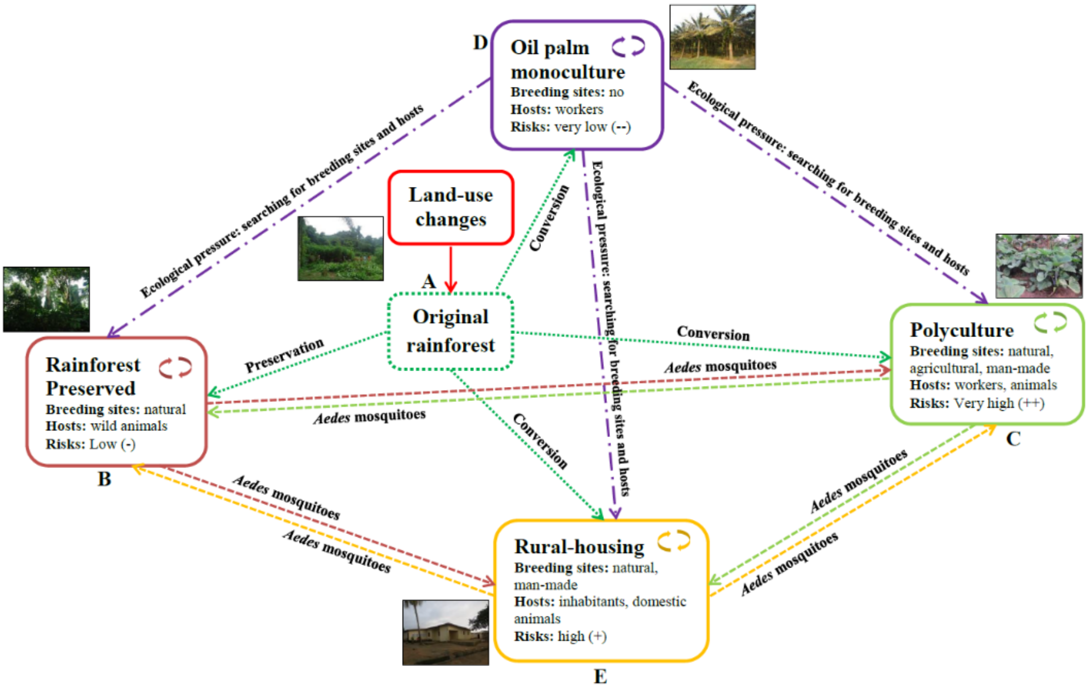


Figure 7.6: Hypothesis on the effects of land-use changes on *Aedes* mosquito ecology and arbovirus-risks in oil palm-dominated landscapes in southeastern Côte d'Ivoire. Human-induced land-use changes into the original tropical rainforests (A) for their conversion into large industrial oil palm plantations have probably resulted in changes in land covers creating four ecologically distinct macrohabitats: rainforest (B), polyculture (C), oil palm monoculture (D) and rural housing area (E). The conversion of the rainforests into large oil palm monoculture has led to the losses of forest-dwelling *Aedes* mosquito breeding sites and hosts thus increasing ecological pressure for searching alternative breeding sites and hosts in the three other macrohabitats. *Aedes* mosquitoes possibly found new breeding sites as anthropogenic containers abundantly encountered in the rural housing area and polyculture where humans (inhabitants and workers) are usually present thus resulting in higher abundance of vectors and high high-risks of arboviruses' transmission in these areas. In contrast, the arboviral transmission risks are low in the oil palm monoculture due to the lack *Aedes* mosquitoes, and in the rainforest due to the low anthropophagy of forest-dwelling *Aedes* species.

Such distributional differences in *Aedes* vectors are likely to shape the distributions of arboviral disease transmission risks between landscapes, with low-risk and high-risk areas (Fig 7.6). The following points are offered for consideration. First, holistically, our study yielded high species richness and high numbers of mosquitoes, with the dominance of medically important *Aedes* species in different anthropogenic landscape-use changes in areas devoted to oil palm plantations. Several *Aedes* species such as *Ae. aegypti, Ae. africanus, Ae. furcifer, Ae. luteocephalus, Ae. opok,* and *Ae. vittatus* have been known to vector at least one of the viruses, including yellow fever, dengue, and chikungunya in Côte d'Ivoire [15, 16] and Senegal [7, 25, 26]. The high *Aedes* species diversity is consistent with previous studies conducted in distinct landscapes in rural areas of Senegal [7, 25, 26]. This could be due to the heterogeneity of landscapes (rainforest, polyculture, oil palm monoculture, and housing areas) that possibly provide a wide range of larval habitats, resting and mating places, and nectar and blood-food sources [7, 25]. Second, we used diverse sampling methods (i.e., bamboo-ovitraps, metallic-ovitraps, larval surveys, and human-baited double-net traps) targeting different development stages (i.e., egg, larvae, pupae, and adults) of *Aedes* mosquitoes during the dry and rainy seasons. Due to logistical limitations, our study only focused on *Aedes* mosquito dwelling up to 2 m above ground, and the anthropophagic populations that are active between 04:00 and 20:00 hours. Some canopy-dweller [26], nighttime-biter [27, 28], and zoophilic [29] *Aedes* species were probably missed by the current sampling techniques. A vertical stratification study, circadian (24-hours period) sampling design, and animal-baited trapping could possibly provide deeper insight into the ecology of *Aedes* mosquitoes living in the canopies, darkness-dependent biting, and zoophagic behaviors, respectively.

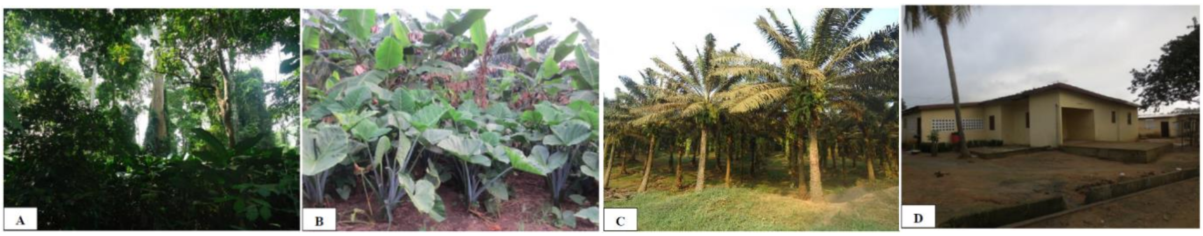
Third, from a reductionist view, we found compositional differences in *Aedes* species among the landscape covers, suggesting ecologically filtering effects of land-use changes on *Aedes* mosquito communities, as observed in arthropods [30]. Bernues-Baneres et al. [31] have observed variations in faunistic diversity of mosquitoes according to the typology of land-covers in Spain. Because of their high sensitivity to environmental changes, mosquitoes have been suggested as bio-indicators of forest degradation level in Brazil [32]. In our study area, *Aedes* species were absent in oil palm monocultures, while they were abundantly present in polyculture environment and rural housing areas. This may suggest the displacement of *Aedes* mosquitoes vectors primarily from the forested areas transformed into oil palm plantations toward preserved rainforest, the polyculture, and rural housing areas for searching alternative breeding sites [33, 34], and blood-food sources [21]. In the first possible scenario, under the increased pressure exerted by *Aedes* mosquito populations, they become highly abundant during the rainy season on the hosts and breeding sites available in the preserved rainforest. The ecologic *Aedes*-rainforest balance is probably interrupted, and hence, leading to the diffusion of forest-dwelling anthropozoophilic *Aedes* species toward the rural human-inhabited areas. Similar findings have been reported in rural areas of Senegal, where *Aedes* vectors have invaded villages from surrounding landscapes and the risk of arboviral infection became highest at the edges of the villages [26]. These wild *Aedes* species that have both horizontal/oral and vertical/transovarial transmission competences for arbovirus probably transmit viruses that they have previously taken from forest-dwelling animals to villagers thus linking the jungle/sylvatic cycles to emergence/rural cycles [12, 20, 21]. Alternatively, the second scenario is that people working in polyculture could be bitten by a virus-infected *Aedes* mosquito, which might carry the virus to rural housing areas that are already colonized by potential competent vectors [20]. These competent vectors may disseminate viruses among the populations. Both scenarios are expected to increase yellow fever and dengue emergence and re-emergence risks, especially since they do not exclude mutually [20], because people live in close proximity to wildlife.

Fourth, *Aedes* mosquitoes still appear to show diverse and atypical breeding patterns across macro- and microhabitats leading to horizontal stratification among species with lack of *Aedes* mosquitoes in the oil palm monocultures and strong colonization of the other macrohabitats (i.e., rainforest, polyculture, and rural housing areas). These findings corroborate previous results showing that land-use changes affect the ecology of immature *Aedes* mosquitoes in the United States of America [2] and in rural areas of Senegal [7]. Ferraguti et al. [3] have reported that mosquito richness is higher in natural areas compared to anthropized areas. Polyculture area has more positive effects on the abundance and species richness of terrestrial arthropod than monoculture in oil palm production landscapes in Peninsular Malaysia [5, 35]. Indeed, oil palm plantations alter ecosystem functioning [36], and reduce species richness and abundance compared with forested areas [37] due to the losses of habitats and hosts [5, 6]. Moreover, the drastic decline in *Aedes* species in oil palm monocultures could probably be exacerbated by multiple and intense uses of chemical products such as insecticides and herbicides for crop protection [19]. *Aedes* species have adapted alternatively their oviposition and blood-feeding behaviors to anthropogenic habitats and hosts that are available in the polyculture and rural housing areas [7]. Polyculture still had naturally-occurring microhabitats (i.e., tree and bamboo holes), developed multiple agriculturally occurring microhabitats (i.e., crop fruit husks, flower, sheathing leaf axils, and cultivated plant holes), and received several man-made containers (i.e., crop collection containers, discarded containers). Indeed, people discarded high numbers of containers such as old tires, parts of vehicles and machines in the maintenance of the agricultural lands, tarps, cans, and other worn items in surrounding polyculture since they directly live in close proximity to their smallholdings. Besides, urbanized housing areas are also incriminated to replace the natural microhabitats (e.g., tree holes, bamboo) by the artificial microhabitats (e.g., tires, discarded containers, water storage containers), increase numbers of microhabitats, expose these breeding sites to higher magnitude of solar radiation and enhance the population size of *Aedes* mosquitoes [38]. In these areas, containers serving as waterers for the poultry husbandry during the dry season were found to be highly infested with *Ae. aegypti* larvae, as observed in bird cages in Malaysia [9]. Anthropogenic environments also act as limiting factors for the *Aedes* mosquito predators (e.g., *Eretmapodites* spp. and *Toxorhynchites* spp.) [4]. Hence, *Aedes* species that uniquely oviposit in natural containers (e.g., tree holes) [7], lay more fragile and desiccation-sensitive eggs, and need rainwater for hatching of the eggs [4], have height-oviposition behaviors [7]. Microbial inputs from predation as food sources for their offspring [2], and wild animal hosts as blood-meals for the adult females [29], were probably restricted to the rainforest [4, 7]. Indeed, the specialists that are strictly ecologic demanding remain confined to particular ecotopes (e.g. rainforest), while the generalists (i.e., *Ae. aegypti*) spread and colonize any environment [4, 7]. All these biotic and abiotic factors interact with the rainfalls that habitually ensure the flooding of the breeding sites to induce significant variations in the abundance and distribution of *Aedes* mosquito species, all which may link the different possible arbovirus transmission cycles and increase exposure of human populations to arbovirus-risks [12].

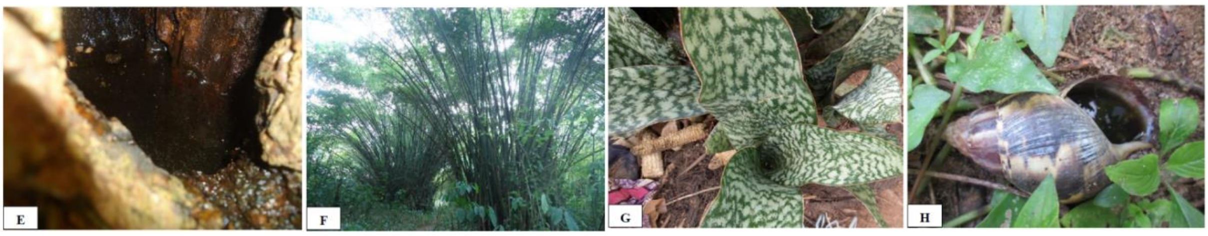
Finally, *Aedes* mosquito females seem to exhibit similarities and dissimilarities in host-seeking behaviors between the types of land cover that acted as a series of ecological filters [30]. *Aedes* mosquitoes were seeking for humans in every land cover type studids here, except for the oil palm monoculture. Moreover, the vectors displayed low preference for feeding on humans in the rainforest. Host-seeking activities were higher in both polyculture and rural housing areas, and biting activity showed one peak in the morning and one peak in the evening. However, biting cycles were interrupted between 10:00 and 14:00 hours in the rural housing areas and maintained in the polyculture. The unexpected ecologic variations in

Aedes biting behavior suggest a complex pattern of arbovirus transmission in the large-scale development of oil palm-planted landscapes. Such outstanding spillovers might be attributable to the adaptation of *Aedes* species to land-use patterns, and human activities and movements. In fact, the absence of aggressive *Aedes* females in oil palm monoculture could be explained by the losses of their habitats and animal hosts [6], while the disinterest of rainforest-dwelling vectors into feeding on humans could be due to their preference to feed on wild animals [29]. When the vector aggressiveness peaked, in the morning and in the evening, humans are generally within housing areas suggesting that high exposures to arboviruses occur in the villages [21, 25]. The interruption of host-seeking activities of *Aedes* females coincided with the migration of workers to the industrial oil palm farming and other people to their own smallholdings. Such an accordance of malaria vector behaviors to human movements has been reported in rubber plantations in Thailand [39]. The gap observed in host-seeking activities also corresponded to the sunlight intensity in the rural-housing areas that are directly exposed to solar radiation due to the lack of natural vegetation coverage. As observed in poikilothermic animals, including insects [40], *Aedes* host-seeking behavior was probably most affected by the sun in the housing area. Conversely, the continuous biting cycles of *Aedes* females in polyculture could be explained by the permanent presence of workers that may habitually serve as blood-food sources [39], and the shade provided by the abundance of vegetation coverage that probably reduces the negative effects of sunlight radiation on host-searching activities. The surprising darkness-biting activities could be interpreted as residual biting activities of *Aedes* mosquitoes that feed nightly on wild animals [21, 26, 29]. The nocturne biting activities of the well-known daytime *Aedes* mosquitoes has been reported on *Ae. aegypti* in Côte d’Ivoire [27] and *Ae. albopictus* in Cameroon [28]. The extent of such atypical host-seeking activity rhythm observed in our study region could have important epidemiologic implications, and needs to be analyzed at greater depth and over larger scales. We conclude that in the southeastern part of Côte d’Ivoire, agricultural land-use is changing as a result of transforming rainforests into oil palm monocultures, which significantly influences the composition, distribution, oviposition patterns, and host-seeking behavior of *Aedes* mosquito species. In turn, there is a lack of *Aedes* mosquitoes in oil palm monocultures and a strong colonization of polyculture and rural housing areas. Hence, humans are primarily exposed to *Aedes* bites and arbovirus risk around their homes and farming plots. In oil palm-planted areas, arboviral disease control strategy should encompass integrated approaches, including landscape epidemiology and ecotope-based vector control.

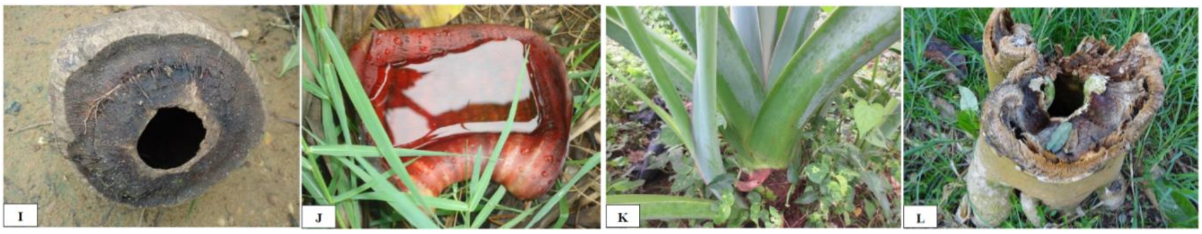
7.6. Supporting information



I: Macrohabitats



II.1: Naturally-occurring microhabitats



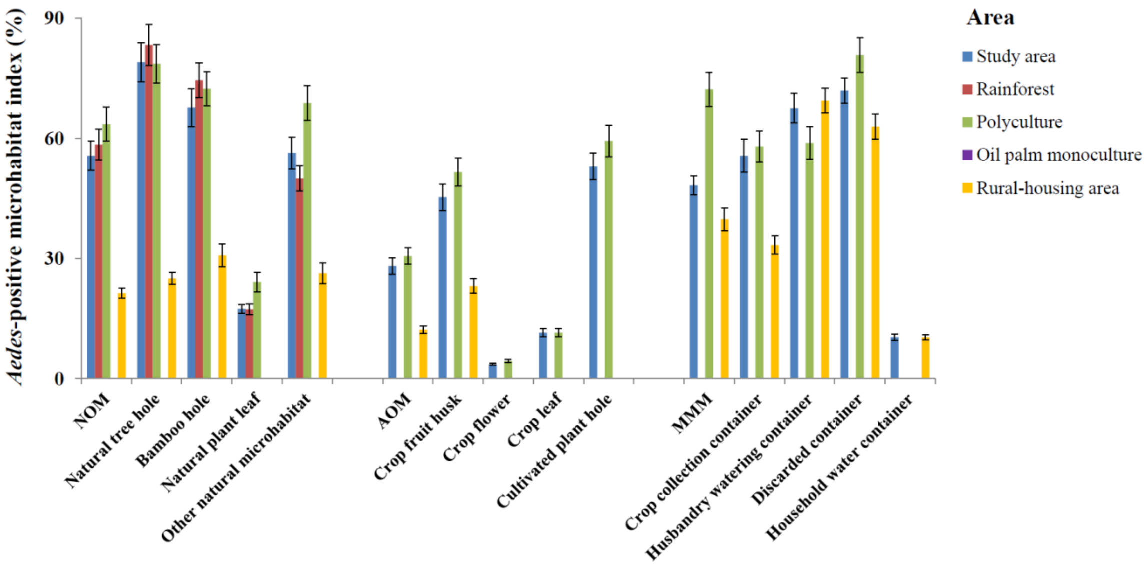
II.1: Agriculturally-occurring microhabitats

II.1: Man-made microhabitats

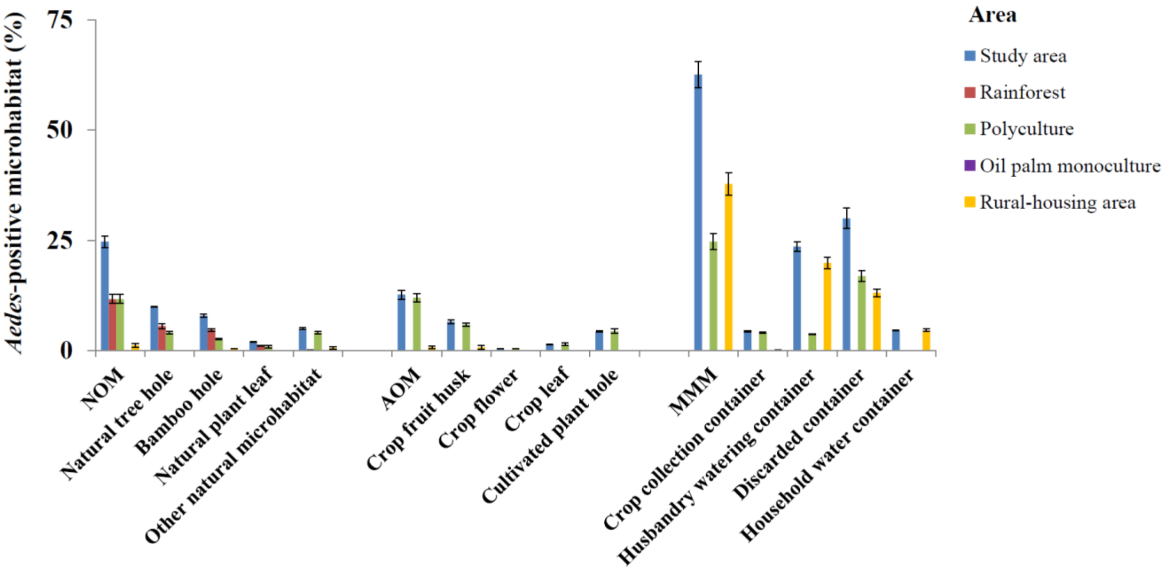
II: Microhabitats

S7.1 Figure: Different macro- and microhabitat types sampled for *Aedes* mosquitoes in oil palm-dominated landscapes in southeastern Côte d'Ivoire. Potential habitats of *Aedes* mosquitoes are stratified into two habitat types: I: macrohabitats, and II: microhabitats. The habitat type often reflects the name of the habitats and the categories include habitats that provide comparable *Aedes* mosquito habitats. The macrohabitats area divided into four ecological blocks: A: Rainforest that was the preserved dense forest hosting several plant species of threes, creepers, and bamboo, and animals; B: Polyculture that covered a mixture of cultivated plants such as oil palm tree, rubber, taro, banana, coconuts, and native threes; C: Oil palm monoculture that was covered uniquely with industrial oil palm trees; and D: rural-housing area that was the human-inhabited space. The microhabitats were summarized into II.1: Naturally-occurring microhabitats that comprised E: Natural tree hole, F Bamboo hole, G: Natural plant leaf, and H: Other natural microhabitats; II.2: Agriculturally-occurring microhabitats that were composed of : I: Crop fruit husk, J: Crop flower, K: Crop leaf, and L: Cultivated plant hole; and II.3: Man-made microhabitats that represented: M: Crop collection container, N: Husbandry watering container, O: Discarded container, and P: Household water container. Containers categorized "other natural microhabitats" were the snail shells and the rock holes.

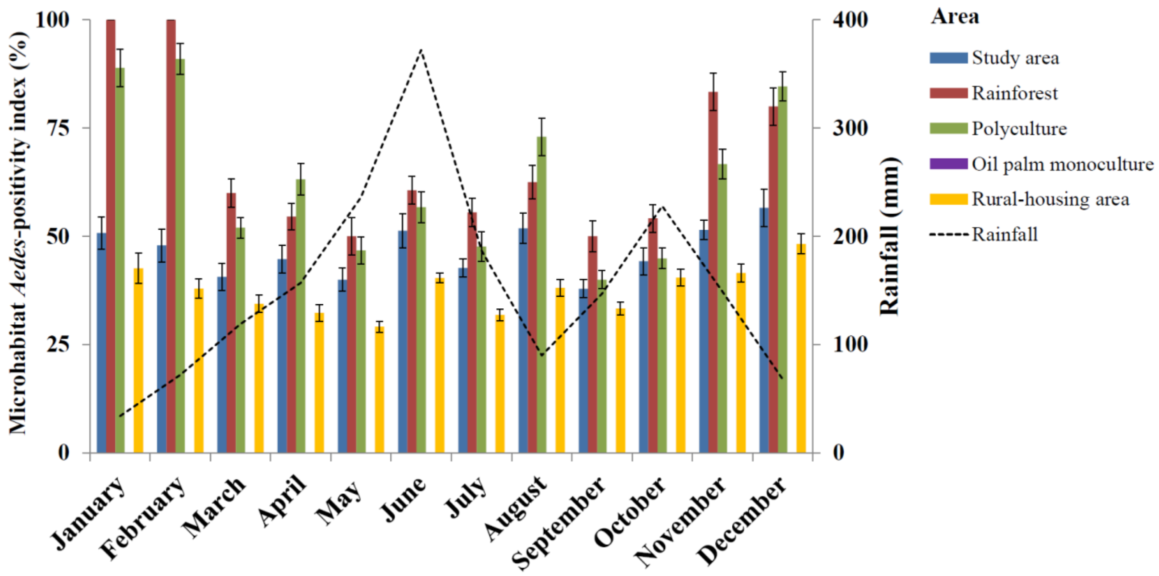
S7.2 Figure: Standardized devices and methods used for sampling different life stages of *Aedes* mosquitoes in the study areas. A: Bamboo-ovitrap, B: Metallic-ovitrap, C: Larval survey, D: Human-baited double net trap.



S7.3 Figure: *Aedes* mosquito species occurrence among the microhabitats in different macrohabitats in southeastern Côte d'Ivoire from January to December 2014. Error bars represent the standard error (SE). NOM: naturally-occurring microhabitat, AOM: agriculturally-occurring microhabitat, MMM: man-made microhabitat.

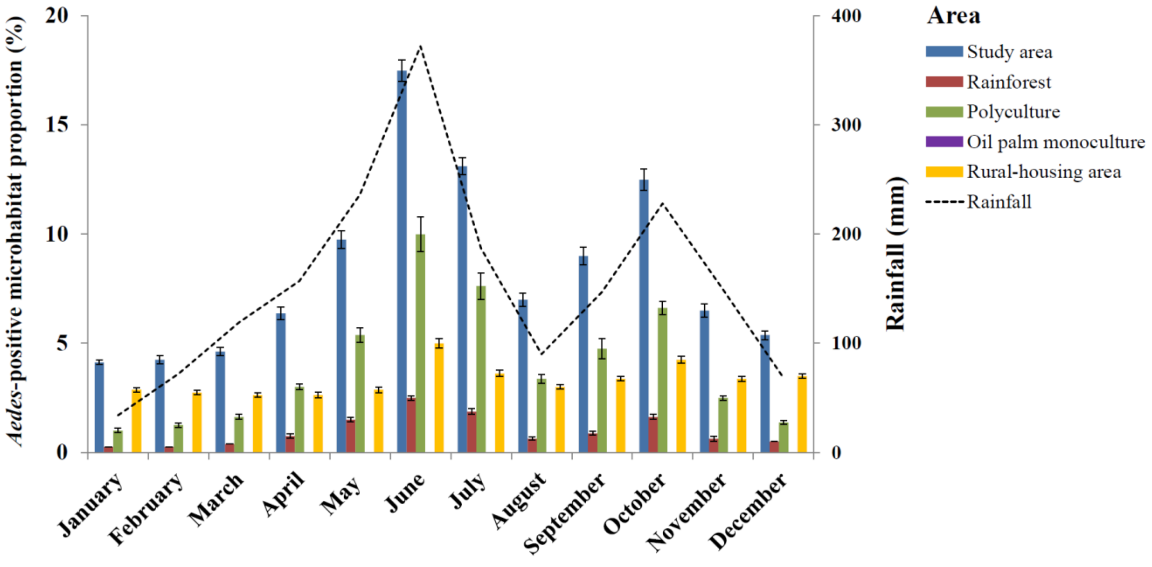


S7.4 Figure: Relative proportions (%) of the different types of microhabitats among *Aedes*-positive microhabitats in the macrohabitats in southeastern Côte d'Ivoire from January to December 2014. Error bars represent the standard error (SE). NOM: naturally-occurring microhabiat, AOM: agriculturally-occurring microhabitat, MMM: man-made microhabitat.



S7.5 Figure: Monthly variations in *Aedes* mosquito species occurrence among the microhabitats in different macrohabitats in southeastern Côte d'Ivoire from January to December 2014.

Error bars represent the standard error (SE).



S7.6 Figure: Monthly variations in different types of microhabitats among *Aedes*-positve microhabitats in the macrohabitats in southeastern Côte d'Ivoire from January to December 2014.

Error bars represent the standard error (SE).

Acknowledgments

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Author contributions

Conceptualization: JBZZ BGK.

Data curation: JBZZ BGK JU

Formal analysis: JBZZ BGK JU

Investigation: JBZZ, YT BGK.

Resources: BGK PM DM YT JU.

Software: JBZZ PM.

Supervision: BGK JU JBZZ.

Validation: BGK JU.

Visualisation: JBZZ.

Writing-review & editing: JBZZ BGK PM DM YT JU.

Writing-original draft: JBZZ.

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8. Discussion

8.1. Overview

Aedes mosquito community dynamics and transmission of arboviral diseases are driven by far-reaching impacts of anthropogenic changes, including human-mediated land-use changes (e.g. urbanization and changing patterns of agriculture) and insecticide uses [1-6]. Land-use changes have been associated with modifications of biodiversity that might be governed by changes in the availability and suitability of mosquito habitats, and thus are a key driver in a range of infectious disease outbreaks, including arboviruses [5, 7, 8]. Urbanization continues at a high pace, particularly in low- and middle-income countries (LMICs) of Africa, and is one of the most ecologically modifying phenomena that influences the larval habitats, the development and survivorship of *Aedes* mosquitoes [9]. Additionally, changing patterns of agriculture, particularly oil palm monocultures, are a threat for tropical rainforests and might enhance the transmission of arboviral diseases [10]. Indeed, tropical rainforests usually host a large diversity of *Aedes* species [11-13], while deforestation and conversion of forested areas into human settlements and agricultural lands can modify *Aedes* species composition and distribution, and has been associated with altered arbovirus transmission [1, 14].

8.2. Key findings and structure of discussion

The aim of this PhD thesis was to generate new evidence on anthropogenic effects (e.g. urbanization and changing patterns of agriculture) on *Aedes* mosquito community dynamics in yellow fever and dengue foci in Côte d'Ivoire. The main focus is on the effects of land-use changes, including urbanization and agricultural practices, on the ecology of *Aedes* mosquitoes. The assessment of *Aedes* mosquito ecology along a rural-to-urban gradient and in an industrial oil palm monoculture in Côte d'Ivoire are showcased by (i) exploring the oviposition ecology of *Aedes* mosquitoes and the dynamics of *Ae. aegypti* in variously urbanized settings (rural, suburban and urban) (Chapter 5); (ii) characterizing the larval ecology of *Aedes* mosquitoes alongside a gradient of increasing urbanization (rural, suburban and urban) (Chapter 6); and (iii) assessing the abundance, distribution and host-seeking behaviours of *Aedes* mosquitoes in oil palm cultivation-dominated landscapes (Chapter 7).

The main contributions of this PhD thesis are discussed, with an emphasis on three goals, as outlined in section 3.2:

- **Goal 1:** To improve our understanding of how urbanization influences the oviposition ecology and species composition of *Aedes* mosquitoes in arbovirus foci;
- **Goal 2:** To assess how urbanization affects the breeding sites, larval ecology and species composition of *Aedes* mosquitoes in arbovirus endemic areas;
- **Goal 3:** To deepen our understanding of the impacts of agricultural land use changes on the abundance, distribution and host-seeking behaviours of *Aedes* mosquitoes in oil palm-dominated landscapes.

In view of the results presented in this thesis, a set of conclusions and recommendations are drawn. Limitations of our work are discussed and research needs highlighted.

8.3. Main contributions of current PhD thesis

Our data on the risk of *Aedes* mosquito-transmitted arboviral disease outbreaks will inform local populations, health authorities, decision makers and scientists on the risk of emergence and re-emergence of yellow fever and dengue. In turn, possible actions to reduce exposure are revealed. With such practical knowledge and new evidence at hand, collaboration between different stakeholders can be fostered and community engagement into *Aedes* vector surveillance and control further enhanced in order to reduce the burden of arboviral diseases.

8.4. Social-ecological characteristics in rural, suburban and urban areas

Our social-ecological investigations revealed that several factors varied along a rural-to-urban gradient. These factors include the number and density of people, availability of containers that may serve as breeding sites for *Aedes* mosquitoes and water storage practices in surveyed households (Chapter 5)

Our findings on social-ecological characteristics along a rural-to-urban gradient suggest important variations among *Aedes* mosquito species numbers and abundance. Indeed, the increasing human population density and number of artificial containers in urban settings is likely to go hand-in-hand with a higher abundance of anthropophagic *Aedes* species, such as *Ae. aegypti* [9]. In contrast, the wide range of natural and artificial containers in rural settings may be favourable to the diversity and the richness of *Aedes* species [14]. Additionally, the high proportion of households storing water for long duration is favourable for the infestation of the water storage containers with the larvae of *Aedes* mosquitoes, mainly *Ae. aegypti*, as reported in urban settings of Equator [15] and Porto Rico [16]. Ultimately, the social-ecological factors, including increased human population size, unmanaged environments and water storage practices, are expected to increase the risks of emergence or re-emergence of yellow fever and dengue in areas undergoing rapid urbanization [8, 13, 17, 18, 19].

8.5. Effects of urbanization on *Aedes* mosquito community dynamics

Taken together, our oviposition and larval ecological studies revealed that *Aedes* mosquito species composition, egg-laying patterns, breeding site characteristics and the biological interactions among species varied considerably along a rural-to-urban gradient (Chapters 5 and 6).

8.5.1. Aedes *species composition*

Urbanization strongly affects *Aedes* mosquito ecology. For example, we found that *Aedes* mosquito species richness was higher in rural compared to suburban and urban areas. Conversely, *Aedes* mosquito abundance was higher in urban compared to suburban and rural areas. *Ae. aegypti* was the predominant species in the three different types of study areas and exhibited particularly high dominance in the urban areas. In rural and suburban areas, several other wild *Aedes* species were recorded, including *Ae. africanus*, *Ae. angustus*, *Ae. Apico-argenteus*, *Ae. argenteopunctatus*, *Ae. dendrophilus*, *Ae. fraseri*, *Ae. furcifer*, *Ae. haworthi*, *Ae. lilii*, *Ae. longipalpis*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. opok*, *Ae. palpalis*, *Ae. stokesi*, *Ae. unilineatus*, *Ae. usambara* and *Ae. vittatus*.

The observed variations in anthropophagic and non-anthropophagic *Aedes* mosquito species diversity and abundance along a rural-to-urban gradient in yellow fever and dengue foci within southeastern Côte d'Ivoire suggest the existence of several arboviral transmission cycles [11, 13, 14]. Indeed, the forest-dwelling *Aedes* species that are still present in the rural area may play a key role as bridge vectors between the sylvatic cycles of dengue, yellow fever and other viruses among non-human primates and humans, while *Ae. aegypti*, which is the main vector and has high anthropophilic behaviour in urban areas, is expected to ensure human-to-human transmission of arboviruses that may cause outbreaks [11, 13]. The species belonging to the *Stegomyia* subgenus, such as *Ae. aegypti*, *Ae. africanus*, *Ae. albopictus*, *Ae. angustus*, *Ae. apicoargenteus*, *Ae. fraseri*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. opok*, *Ae. vittatus*, *Ae. unilineatus* and *Ae. usambara*, and the *Diceromyia* and *Aedimorphus* subgenera comprising *Ae. furcifer* and *Ae. stokesi*, respectively, have been shown to carry and/or transmit in nature over 24 viruses, including yellow fever, dengue, Zika, chikungunya, and Rift Valley in tropical regions [11, 12]. In addition, *Ae. (Aedimorphus) argenteopunctatus* in South Africa [20] and *Ae. (Neo-melaniconion) palpalis* [21], which show vector competence for Rift Valley fever virus *in vitro* and the other *Aedes* species like *Ae. (Stegomyia) dendrophilus*, *Ae. (Stegomyia) lilii* and *Ae. (Aedimorphus) haworthi*, which belong to the same subgenera involved in the transmission of the arboviruses, could be suspected as potential vectors. Of note, *Ae. (Finlaya) longipalpis* belonging to the same *Finlaya* subgenus with *Ae. niveus* that is the principal vector of dengue virus in Malaysia [22], may potentially transmit arboviruses in Côte d'Ivoire. The collection of several non-anthropophagic species – unexpected and new potential vectors in rural areas – suggest the co-existence of several still unidentified arbovirus cycles in southeastern Côte d'Ivoire.

The current PhD thesis suggests that, while vector control should primarily focus on urban settings, rural areas are important as they may serve as transition zones for the introduction or reintroduction of arboviral diseases through sylvatic bridge vectors. Because rural areas hosts various wild vectors, they act as a potential reservoirs and originators of arboviruses from which urban areas might be (re-)infected. Hence, rural settings also need to be considered when elaborating and applying arbovirus vector surveillance and control strategies.

8.5.2. Aedes *oviposition patterns*

Aedes oviposition patterns substantially varied between rural, suburban and urban settings. The mean number of *Aedes* collected as eggs was higher in urban settings compared to suburban and rural settings, while the diversity of *Aedes* species was highest in the rural setting, especially in the sylvatic zone.

Changes observed in *Aedes* species composition and diversity might be governed by specific desiccation-resistance levels of their eggs to environmental changes induced by urbanization [23]. Eggs laid by *Ae. aegypti* mosquitoes – the predominant species in urban settings – are expected to be particularly desiccation-resistant [24, 25]. Hence, this mosquito species might raise the ability to survive in deforested environments that are exposed to direct sunlight. It follows that the capacity of eggs of *Ae. aegypti* increases the species geographical spread, which, in turn, results in higher abundance and enhanced risk of arboviral outbreaks [11-13]. Conversely, wild *Aedes* species, such as *Ae. africanus*, *Ae. dendrophilus*, *Ae. fraseri*, *Ae. furcifer*, *Ae. luteocephalus*, *Ae. metallicus*, *Ae. usambara* and *Ae. vittatus*, which were collected in both rural and suburban settings, probably originate from the surrounding rainforests where they possibly ensure the maintenance of arboviruses among wild animals. Their drastic decline in urban settings might be explained by the destruction of natural environments in the face of house construction and other infrastructures. The removal of vegetation due to house constructions and other infrastructure developments results in direct exposure of *Aedes* breeding sites to solar radiation. The wild *Aedes* species eggs could be protected against solar radiation by rainforest canopy since they are laid in tree holes and bamboo holes filled by rainwater and maintained at relatively low and relatively stable temperatures [14, 26]. It is conceivable that wild *Aedes* species that lay more fragile and desiccation-sensitive eggs remain confined in rural areas, mainly in the rainforest [24, 25].

The research pursued in the current PhD shows that urbanization induces segregation among *Aedes* species by exposing their eggs to different types of environments, leading to specific geographical distributions of vectors, which govern the transmission of arboviruses.

8.5.3. Aedes *breeding sites*

Aedes mosquito breeding sites change profoundly from natural containers to artificial containers and from rural to suburban and urban areas. A common feature is the higher proportions of artificial breeding sites in the three types of typical study areas. However, urban areas show higher capacity to support artificial containers and *Ae. aegypti* larvae, while rural areas show strong ability to host natural containers that harbour several wild *Aedes* species.

Shifts in *Aedes* mosquito breeding sites, their availability and diversity, as well as the number of larvae revealed strong anthropogenic impacts on the environment induced by urbanization. The dominance of artificial breeding sites of *Aedes* mosquitoes is consistent with the findings reported in China [9], and could be explained by the destruction and the conversion of natural environments (e.g. rainforests) into human settlements that are likely to increase from rural to urban. Natural landscape provide abundant *Aedes* breeding sites (e.g. rock holes, animal detritus, leaf axils, fruit husks, bamboo and tree holes), while wild *Aedes* species are mostly found in rural areas. Human activities provide anthropogenic containers, including traditional containers (e.g. clay pots, wood-containers and metallic pots) and industrial containers (e.g. tarps, discarded tires, vehicle tanks, building tools and water storage containers) in urban settings that are suitable breeding sites for *Aedes* mosquitoes, mainly for *Ae. aegypti* [27]. Moreover, *Ae. aegypti* exhibit ecological plasticity breeding in natural containers (i.e. tree holes) in rural areas and artificial containers (e.g. tires and discarded containers) in urban areas and predominated in all areas thus suggesting the existence of the sylvan form, *Ae. aegypti formosus*, and the urban form, *Ae. aegypti aegypti* known in West Africa [28-30]. Tree holes in the rainforests showed the highest *Aedes* species richness, probably because of their ability to provide ideal larval habitats, greater stability, various trophic inputs from leaves of diverse plant species, retention of rainwater under relatively lower temperature for longer periods due to forest canopy coverage, protection against flushing of egg and larvae, and protection against solar radiation and human mediated disturbances [7, 14]. The availability of discarded tires that have been recorded as the most productive *Aedes* breeding sites was attributable to specific devices (e.g. vehicles and machines) used in oil palm industrial plantations in rural areas, the production of the local dish "*Attiéké*" in suburban areas and selling of tires and other car devices in urban areas. Discarded containers (e.g. cans, boxes, coolers and toys) that exhibited high *Aedes*-infestation rates were provided by the byproducts from human activities that have been phrased "human civilization wastes" [24]. In anthropic environments, *Aedes* females can lay their eggs in a large range of ephemeral containers that are susceptible to human-made disturbance [23]. Other important *Aedes* breeding sites were water storage containers in urban areas [15, 16], clay pots in the suburban and rural areas [24], and poultry husbandry watering containers in the rural areas as observed in Malaysia [31].

Taken together, the current PhD highlights that urbanization strongly impacts on *Aedes* mosquito habitats, by shifting the breeding sites from natural to artificial containers, offering suitable conditions for *Ae. aegypti*, the main vector species of yellow fever and dengue. Notwithstanding, rural areas still support substantial proportions of natural breeding sites for several *Aedes* species suggesting that the unique removals of artificial containers, that are the common practices in arboviral control programmes, might not be efficient to control arboviral diseases. Instead, vector control strategies and systems should embrace a more holistic approach, combining removals of artificial containers [12] and autocidal gravid ovitrap-based on mass trapping [32], and insecticide auto-dissemination approaches [33].

8.5.4. Biological associations among Aedes *breeding sites*

We found multiple ecological associations among *Aedes* species co-occurring in the same breeding sites with other *Aedes* species (competition), *An. gambiae* and *Cx. quinquefasciatus* (sympatry), and the predatory larvae of *Cx. tigripes*, *Eretmapodites* and *Toxorhynchites* mosquitoes (predation) with particularly strong intensity observed in rural settings.

Biological interactions among *Aedes* species may possibly explain their ecological segregation along a rural-to-urban gradient, with the restriction of wild species in rural areas and the invasion of *Ae. aegypti* in urban areas. Indeed, inter- and intraspecific competitions for food and habitat resources probably resulted in the decline of wild *Aedes* species considered as inferior competitors (competitive exclusion) and the spread of *Ae. aegypti* that is known as the superior competitor (competitive displacement) [7, 34]. The sympatric co-existence of *Ae. aegypti* with *Cx. quinquefascitaus* and *An. gambiae* in the same containers could be due to the similar feeding patterns of their larvae on the identical microbial inputs and their adults that are highly anthropophagic [18]. The predatory acts of *Cx. tigripes*, *Eretmopodites* and *Toxorhynchites* mosquitoes probably exerted biocontrol on *Aedes* species and structured communities by maintaining species biodiversity in rural areas, as reported in the United States of America [7, 34-36]. Keystone predation allows the persistence of biodiversity in a community as the predators prey disproportionally on competitively dominant prey, thus releasing the poorer competitors from interspecific competition [34]. Moreover, some *Aedes* females prefer to oviposit in breeding sites containing predator-killed conspecifics because of the microbial byproducts provided by the predation [35], while others avoid containers with predators by applying skipping oviposition behaviours [23].

The research presented in the current PhD thesis suggests that the biological interactions reported in our study region may influence the life-history traits and biodiversity of *Aedes* mosquitoes by the biotic facilitation or resistance to invasion, resilience, persistence, co-existence and exclusion of communities, thus altering the transmission of arboviral diseases [7].

8.5.5. Geographical variations among Aedes *species and breeding sites*

We documented substantial variations in the geographical distribution of *Aedes* species and breeding sites along a rural-to-urban gradient. The highest numbers of *Aedes* specimens and positive containers were observed in peri-domestic zones in three study areas, while higher *Aedes* species richness were found in the sylvatic zone in the rural areas.

As described above, the preference of *Aedes* mosquitoes to oviposit in peri-domestic zones, as already observed in Brazil [37, 38], could be due to their ability to provide ideal larval habitats with greater stability, various trophic inputs from foliage for the larvae, retention of rainwater under relatively lower temperature for longer periods due to vegetation coverage, protection of breeding sites against flushing of egg and larvae and lower exposure to human induced disturbances [7, 14]. Moreover, the breeding sites are natural containers (e.g. tree holes, bamboo holes and coconuts) that result from natural vegetation, as well as artificial containers (e.g. cans and tires) discarded in peri-domestic vicinity [26, 39, 40]. Peri-domestic premises are in close proximity to human residencies, and hence the principal blood-meal sources for adult *Aedes* mosquitoes, particularly the highly anthropophagic species of *Ae. aegypti* [41]. The sylvatic zones in rural areas provide similar conditions, but with greater numbers of natural breeding sites and various organic detritus and microorganisms derived from diverse plant species as food sources for *Aedes* larvae, and wild animals as hosts for adult *Aedes* blood-meal sources. Natural ecosystems supply for wide range of opportunities of resting and mating places, shade and nectar sources for *Aedes* mosquitoes [14, 42].

Our findings show that the peri-domestic zones have higher potentials for hosting *Aedes* species, and merit particular consideration for dengue and yellow fever surveillance.

8.5.6. Seasonal variations among Aedes *species and breeding sites*

We found that *Aedes* mosquito diversity, abundance and the availability of their breeding sites were strongly associated with rainfall patterns and other social-ecological features. Overall, the higher numbers of *Aedes* species and specimens, and positive breeding sites were found during the rainy seasons in all study areas. However, we recorded sometimes strong declines in *Aedes* abundance during rainy seasons and increases in water holding container infectivity with *Ae. aegypti* larvae during the dry seasons in urban areas.

The seasonal fluctuations in *Aedes* mosquito abundance could be influenced by seasonal flooding-drying cycles of their breeding sites alternatively flooded by rainfalls and dried by solar power, as reported in Côte d’Ivoire [43] and Brazil [38]. *Aedes* mosquito eggs probably enter into a dormant stage and are maintained as “germ banking” to withstand desiccation periods during the dry seasons until return of rainy seasons, which might flood the breeding sites and increase the abundance of *Aedes* mosquitoes [23, 44]. However, heavy precipitations may result in the flushing of their eggs, larvae and pupae due to the lack of protective vegetation in the built-up environment and the decline of *Aedes* mosquito numbers during the rainy season in urban settings, as observed in October 2013 in urban settings [45, 46]. In contrast, *Aedes* species that breed in tree holes in the rural rainforest are less exposed to such rainfall flushing events [14]. Increased infestation rates of water storage containers with *Ae. aegypti* larvae might be due to household water storing practices by holding of water in uncovered receptacles for long duration [15, 16].

In summary, the current PhD thesis shows the complex effects of rainfall, which is the main climatic factor in the humid tropics differently influencing the seasonal variations in *Aedes* mosquitoes. These effects are expected to alter the temporal and geographical distributions of vectors, and hence, the risks of arbovirus outbreaks [45].

8.6. Impacts of oil palm agricultural land use changes on *Aedes* mosquitoes

The ecological study conducted on *Aedes* mosquito communities in oil palm-dominated landscapes of Côte d'Ivoire showed that the agricultural land-use/land-cover changes, as a result of the conversion of rainforests for the expansion of oil palm monoculture, have significantly influenced the composition, distribution, oviposition patterns, and host-seeking behaviours of *Aedes* mosquito species (Chapter 7). Indeed, no *Aedes*-positive microhabitats, larvae and adults of *Aedes* mosquito were obtained in oil palm monocultures. Conversely, high abundance of *Aedes* mosquitoes was found in polycultures, while the highest *Aedes* species richness was observed in the rainforest. The females of *Aedes* mosquitoes exhibited poor preference in feeding on humans in the rainforest. In contrast, they presented strong anthropophagic behaviours in polyculture and rural-housing areas.

8.6.1. Aedes *mosquito species composition*

We found a high species richness and high abundance of *Aedes* mosquitoes with the dominance of medically important species. Indeed, several *Aedes* species sampled (i.e. *Ae. aegypti*, *Ae. africanus*, *Ae. furcifer*, *Ae. luteocephalus*, *Ae. opok* and *Ae. vittatus*) have been known to host or transmit at least one type of viruses among yellow fever, dengue, chikungunya and Zika pathogens in different rural landscapes in Côte d'Ivoire [43, 47] and Senegal [12, 14, 29, 42, 48]. This high *Aedes* species biodiversity could be explained by the heterogeneity of the landscapes (rainforest, polyculture, oil palm monoculture and rural-housing areas) that provides a wide range of opportunities for larval breeding sites, microbial food sources for immature forms, and resting, refuge and mating places, nectar and blood-food sources for adults [14, 42].

The diversity and abundance of *Aedes* mosquito species revealed by this PhD thesis suggest a high level of exposure of human population living in oil palm-dominated landscapes to arbovirus transmission [8, 11, 13]. This finding is of direct public health relevance.

8.6.2. Landscape-based distribution of Aedes *mosquitoes*

This PhD study documented several important differences in the composition, distribution and behaviours of *Aedes* mosquito species across specific landscapes. For example, several *Aedes* species were absent in oil palm monocultures, while they were present and abundant in surrounding ecosystems, namely, rainforest, polyculture and rural-housing areas. These compositional and distributional differences may be due to the high sensitivity of mosquitoes to changing environments, as reported in Spain [49], and have been suggested as bio-indicators of forest degradation level in Brazil [50]. Indeed, oil palm monocultures alter ecosystem functioning [51], and reduce species richness and abundance, compared with native forested areas [52] due to the losses of habitats and hosts [53, 54]. The destruction of the rainforests for the creation of the large industrial oil palm plantations have probably induced the migration and the invasion of the polyculture and the rural housing compounds by *Aedes* mosquitoes searching for alternative habitats and hosts. The polycultures still have naturally-occurring microhabitats (e.g. tree holes), develop multiple agriculturally-occurring microhabitats (e.g. coconuts, flower and cultivated plant holes) and contain man-made containers discarded by humans (e.g. cans and tires) and provide hosts, such as plantation workers, as blood-food sources. The rural housing areas also provide human blood-feeding sources, and several containers such as discarded containers, tires and discarded vehicle and machine devices used in the maintenance of the oil palm plantations, crop collection containers and husbandry watering containers that were highly infected with the larvae of *Ae. aegypti* and other *Aedes* mosquito species.

The compositional and distributional shifts induced into *Aedes* species by the creation of large industrial oil palm plantations are expected to increase the exposure of inhabitants and workers to high arboviral transmission risks in the polyculture and rural housing areas.

8.6.3. Aede*s females' host-seeking behaviours*

This PhD thesis also revealed that the females of *Aedes* mosquitoes presented poorer preference to feed on humans in the rainforest, and higher anthropophagic behaviours in rural housing areas and polycultures where they adapt their host-seeking behaviours to workers' movements.

The lack of aggressive *Aedes* females in oil palm monocultures could be explained by the losses of their habitats and animal hosts, and the use of chemals for crop protection [54]. The poor anthropophagy exhibited by *Aedes* females in the rainforest could be due to their preference to feed on wild animals [55]. The high anthropophagy of wild *Aedes* mosquito species, such as *Ae. dendrophilus* and *Ae. vittatus*, could be explained by their probable blood-feeding behavioural adaptation to humans. When the vector aggressiveness peaked, in the morning and in the evening, humans are generally within housing areas, suggesting that high exposures to arboviruses occur in the villages [42, 56]. The interruption of host-seeking activities of *Aedes* females coincided with the migration of workers to industrial oil palm farming and other people to their own smallholdings. Such an accordance of malaria vector behaviours to human movements has been reported in rubber plantations in Thailand [57]. The gap observed in host-seeking activities also corresponded to the intensification of sunshine in the rural housing areas that are directly exposed to solar radiation due to the lack of natural vegetation coverage. As observed in poikilothermic animals, including insects [58], *Aedes* females' host-seeking behaviours were probably affected in housing area by direct sunlight. Conversely, the continuous biting cycles of *Aedes* females in polyculture could be explained by the permanent presence of workers that may habitually serve as blood-food sources [57], and the shade provided by the abundance of vegetation coverage that probably reduces the negative effects of sunlight radiation on host-seeking activities.

The observed ecological variations in *Aedes* females' host-seeking behaviours favour and render complex the patterns of arbovirus transmission in large-scale development of oil palm-dominated landscapes.

8.7. Conclusions

Key findings from this PhD thesis suggest that there is a need for new vector control methods and strategies within southeastern Côte d’Ivoire, especially in rural settings. Indeed, the unique removal of discarded containers in urban areas that is commonly applied in arbovirus control programmes might not be sufficient to control arboviral diseases, because several wild vectors breed in irremovable breeding sites, such as tree holes that are widespread in the rainforest nearby. The high numbers of anthropophagic and non-anthropophagic *Aedes* species in rural areas suggest the co-existence of several and still unidentified arbovirus sylvatic cycles that are possibly linked to the rural cycles by bridging vectors and to urban cycles by *Ae. aegypti*, which is expected to be involved in inter-human transmission of yellow fever and dengue in urban areas [11-13]. As a result, while vector control should focus on urban areas, rural areas may serve as transition zones for (re-)introduction of arboviral diseases through sylvatic bridge vectors. Because rural areas host various wild vector species, they act as a potential reservoir and originator of arboviruses from which urban areas might become (re-)infected. Moreover, large industrial oil palm monocultures, by eliminating *Aedes* habitats and hosts, directed the migration of vectors towards the human population living in rural housing areas and working in polycultures, where arbovirus outbreak risks are expected to further gain in intensity. It follows that the control of arboviral diseases should embrace integrated and approaches, including ecotope-based vector surveillance and control combining different tools and methods of proven efficacy (e.g. lethal ovitrap [59] and autocidal gravid ovitrap-based on mass trapping methods [32]), management of discarded containers [12] and water storage practices [15, 16, 60] in urban areas, and setting-specific oil palm cultivation practices in rural areas. Hence, the government, the national programme for public hygiene, the private sectors (e.g. PALMCI) and scientists alike are called to discuss the key findings presented here, and to elaborate and apply arbovirus vector surveillance and control in Côte d’Ivoire.

8.8. Contribution of the PhD thesis to innovation, validation and application

The findings of this PhD thesis advance various issues of validation and application, two of the three key pillars (i.e. innovation, validation and application) of Swiss TPH in the area of research and development activities in public health (Table 8.1). Innovation refers to basic research in the laboratory, development of new tools (e.g. diagnostics, drugs and vaccines) and elaboration of novel concepts and methods for epidemiology and public health. Through validation, newly developed tools, concepts and methods are tested under 'real-life' field conditions. Research findings and gained knowledge from rigorous validation in the field can then be transformed into policies and applied at larger scales [61].

Table 8.1: Contribution of the PhD thesis to the Swiss TPH nexus of "innovation, validation and application"

Chapter	Innovation	Validation	Application
Chapter 5	-	• Comparison of *Aedes* mosquito oviposition patterns along a rural-to-urban gradient • Determination of potential *Aedes* vector species according to urbanization levels	• Adaptations of vector control to the urbanization levels
Chapter 6	-	• Identification of shifts in *Aedes* mosquito breeding sites along a rural-to-urban gradient • Identification of predacious species	• Limitations in the current vector control measures • Suggestions of additional control measures
Chapter 7	-	• Comparison of *Aedes* mosquito distribution between different landscapes with findings from entomological surveys	• Identification of high risk ecosystems of getting yellow fever and dengue in oil palm-dominated landscapes • Ecotope-based vector control

8.9. References

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9. Conclusions and recommendations

The findings of the current PhD thesis make a contribution to further the understanding of the impacts of anthropogenic changes on *Aedes* mosquito community dynamics and have direct public health implications for the prevention and a better understanding of yellow fever and dengue outbreaks in Côte d'Ivoire and elsewhere in Africa. Two important environmental modifying phenomena were studied, namely urbanization and changing patterns of agriculture (large-scale oil palm cultivation). Our study underscores the importance of having detailed context-specific comprehension of how *Aedes* mosquito ecology is governed under different forces of urbanization and oil palm cultivations in order to propose actions to protect public health. Three main conclusions are offered for discussion:

- First, we showed that urbanization shifts the composition of *Aedes* mosquitoes by driving *Aedes* wild species from rural areas towards a higher abundance of *Ae. aegypti* – the major vector species of yellow fever and dengue – in urban areas. Of note, *Aedes* mosquito breeding sites are governed by natural containers (e.g. tree holes) and artificial containers (e.g. tires and discarded containers). Importantly, the rural areas contain several natural habitats in the rainforest that host many different wild *Aedes* species, while water storage containers are highly infested with *Ae. aegypti* larvae in the urban areas. Hence, the removal of discarded containers, which is the main strategy for vector control, might not efficiently control arboviral disease vectors.
- Second, the study revealed that agricultural oil palm land use changes modify the distribution, oviposition patterns and host-seeking behaviours of *Aedes* mosquitoes. Indeed, oil palm plantations have induced major losses of wild *Aedes* mosquito habitats and hosts that alternatively have adapted their oviposition to artificial containers (e.g. tires and discarded containers) in the rural housing areas, and their feeding-behaviours to humans working the polyculture and living in the rural housing areas where yellow fever and dengue outbreaks are expected to increase.
- Third, our research suggests that urbanization and agriculture have induced a significant segregation among *Aedes* mosquito community dynamics that could be incriminated to have epidemiological bearing with direct public health relevance. The invasion of the polyculture and rural housing areas by the *Aedes* vectors expose the workers and the whole populations to arboviruses that wild species have previously taken from wild animals dwelling in the rainforest, thus bridging the sylvatic/jungle cycles to the

rural/emergence cycles in rural areas. The action of connecting the epidemiological cycles of arboviruses is likely to be extended by urbanization by increasing the abundance of *Ae. aegypti*, which is well known to link the rural emergence cycles to urban epidemic cycles of yellow fever and dengue in tropical Africa.

In view of the conclusions offered above, the following recommendations are proposed to mitigate *Aedes* mosquitoes and reduce exposure of the populations to yellow fever and dengue outbreak risks:

- There is a need for an integrated vector management programme, to be implemented by the National Programme for Public Hygiene. Such a programme should combine the removals of discarded containers with other methods of proven efficacy, such as the deployment of lethal ovitraps and autocidal gravid ovitrap-based on mass trapping in the rural areas.
- The local populations should be trained on detecting *Aedes* mosquito larvae, assessing the risks, environmental management through mosquito control campaigns conducted by awareness and surveillance programs and committees. This would build-up the local capacity for conducting and interpreting assessments on *Aedes* mosquito infestation and risks of yellow fever and dengue epidemics, and makes the best use of the data to implement preventive actions. Such a policy may require additional resources to build and strengthen local capacities, and should be organized and implemented by the municipalities. Developing sanitation safety planning approaches to include in-depth health risk prevention could have high potentials for minimizing public health implications and maximize gains for the efforts of the protection of the population.
- Populations living in urban areas should be sensitized on risks of infestation of containers with *Aedes* larvae by holding water for long duration and educated on better water storage practices, such as covering water holding-containers and daily cleaning of the water containers. The tire-dealers should be informed on the risks of contamination of the rainwater-holding tires left outdoors with *Aedes* larvae that is expected to increase the risks of yellow fever and dengue epidemics.
- Rural dwellers should be sensitized on the risks of yellow fever and dengue by creating settlements near the rainforests surrounded by large oil palm plantations and other polycultures because these environments host several wild *Aedes* species that have potentials to transmit arboviruses.

- There is a pressing need for vaccination campaigns against yellow fever, especially to trigger protective actions for workers and the local populations living in rural areas because the efforts of removals of discarded containers may not be sufficient to reduce *Aedes* mosquito populations at acceptable level, and thus the risks of emergence or re-emergence of yellow fever might remain high after the aforementioned interventions.

10. Outlook and research needs

In face of rapid population growth that goes hand-in-hand with urbanization and changing patterns of agriculture in Africa, including Côte d'Ivoire, managing public health concerns arising from anthropogenic land-use changes is a challenging and burning issue. With the practical knowledge and evidence currently at hand, reaching the reduction of risks of emerging and re-emerging diseases, such as yellow fever and dengue, requires joint efforts and integrated management approaches, involving the local population, disease control managers, scientists and other stakeholders. In light of the experience and findings from the present PhD thesis, the following research needs arise:

- Pursue additional case studies on the effects of anthropogenic changes, including urbanization and changing patterns of agriculture with an emphasis on the ecology of *Aedes* mosquitoes and the transmission dynamics of yellow fever and dengue.
- Analyse each *Aedes* mosquito species for the presence of the yellow fever and dengue viruses and their blood-feeding preference on engorged females in order to establish the transmission cycles of viruses and the diseases they cause.
- Assess the predatory effects of *Cx. tigripes*, *Eretmapodites* and *Toxorhynchites* mosquitoes on *Aedes* mosquitoes in order to develop a biological control tool, method and strategy, mainly against vectors dwelling within the rainforests in natural containers (e.g. tree holes).
- Assess the insecticide-susceptibility in *Aedes* adults and larvae in order to implement modified autocidal gravid ovitrap methods and the auto-dissemination technique using the males as vehicles for larval insecticides to target the *Aedes* species that are out of the spectrum of the discarded container removal methods.
- Evaluate the infection rate of dengue viruses among the populations living in rural and urban areas in the study region.
- Assess the risks of yellow fever and dengue outbreaks using standard entomological indicators, such as house index (HI), Breteau index (BI) and container index (CI).
- Assess the egg hatching rates of each *Aedes* species at different levels of urbanization (rural, suburban and urban) to better understand the segregation induced by urbanization into the oviposition and the species composition.

- Assess the oviposition behaviours of *Aedes* species to deepen our understanding of process involve in the colonization of artificial containers in the rural areas as bio-resistance or resilience to anthropogenic changes.
- Identify the insecticides-susceptibility levels and eventual insecticide-resistance genes in *Ae. aegypti*.
- Determine the molecular forms of *Ae. aegypti* (*Ae. aegypti aegypti* (*Aaa*), *Ae. aegypti formosus* (*Aaf*) or another subspecies) that exhibit important ecological variations in geographical distribution (from rainforest-to-urban settings) and breeding sites (from natural containers (e.g. tree holes) to artificial containers (e.g. discarded containers, tires and water storage receptacles).

11. Appendix

Eggs **Larvae** **Pupa**

Figure 11.1: Immature forms of *Aedes* mosquitoes (e.g., *Ae. aegypti*) encountered in southeastern Côte d'Ivoire.

A: white larva of *Ae. aegypti*; B: brown larva of *Ae. aegypti*

Ae. aegypti ***Ae. africanus*** ***Ae. dendrophilus*** ***Ae. furcifer***

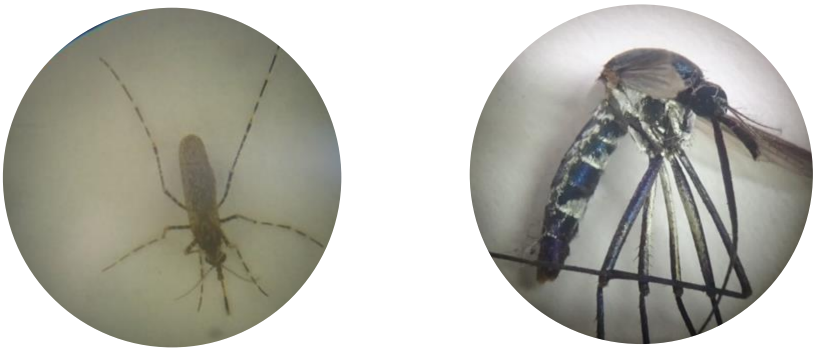
Ae. metallicus ***Ae. opok*** ***Ae. palpalis*** ***Ae. vittatus***

Figure 11.2: Adult specimens of *Aedes* mosquito species commonly found in southeastern Côte d'Ivoire

Toxorhynchites* sp.** ***Eretmapodites* sp.** ***Culex tigripes

Figure 11.3: Predatory mosquito larvae sampled in southeastern Côte d’Ivoire

***Anopheles* sp.** ***Culex* sp.** ***Eretmapodites* sp.**



***Mansonia* sp.** ***Toxorhynchites* sp.**

Figure 11.4: Adult specimens of non-*Aedes* mosquito genera commonly found in southeastern Côte d’Ivoire

12. Curriculum vitae

PERSONAL INFORMATION

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Place of birth	Goulikao / Oumé (Côte d'Ivoire)
Gender	Male
Marital status	Single, no children
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Present employer	Centre Suisse de Recherches Scientifiques in Côte d'Ivoire (CSRS), Abidjan, Côte d'Ivoire: Study Director for GLP (Good Laborratory practices) study

RESEARCH AND COMPETENCE AREAS

- **Epidemiology of tropical mosquito-borne diseases:** arboviruses: yellow fever, dengue, chikungunya, Zika etc.; malaria; and lymphatic filariasis

- **Medical Entomology: Transmission of tropical mosquito-borne diseases, vector ecology** and **testing vector control tools** (Bioassays) in **phase 1** (laboratory trials), **phase 2** (experimental hut trials) and **phase 3** (field works / community-based trials), and community intervention

- **Study Director for the GLP** (Good Laboratory Practices) accreditation and GLP studies at Centre Suisse de Recherches Scientifiques in Côte d'Ivoire (CSRS), Abidjan, Côte d'Ivoire

- **Developping the SOPs** (Standard Operating procedures) in the GLP study implemtation for insecticide testing.

EDUCATION

09/2015 – 06/2017	PhD in Epidemiology; Department of Epidemiology and Public Health (EPH), Ecosystem Health Science Unit, Swiss Tropical and Public Health Institute (Swiss TPH), University of Basel, Basel, Switzerland Thesis title: “*Anthropogenic impacts on* Aedes *mosquito community dynamics in Côte d’Ivoire”*
09/2014 – 09/2015	Fellow of Swiss Government Excellence Scholarships for Foreign Students at Swiss Higher Education Institutions (ESKAS) Epidemiology; Department of Epidemiology and Public Health (EPH), Ecosystem Health Science Unit, Swiss Tropical and Public Health Institute (Swiss TPH), University of Basel, Basel, Switzerland
2008 – 2010	Master in General Entomology: Medical option. Felix Houphouët Boigny University, Abidjan, Côte d’Ivoire Thesis title: “*Evaluation de la transmission du* Plasmodium *par* Anopheles gambiae *s.l. Giles, 1902 en zones de résistance variable des vecteurs aux pyréthrinoïdes et de rizicultures irriguées dans la Région de la Vallée du Bandama (Bouaké, centre de Côte d’Ivoire) : Cas des villages d’Abokro et de Yaokoffikro”*
2007 – 2008	Master in Zoology and Biology of Animals, Felix Houphouët Boigny University, Abidjan, Côte d’Ivoire
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2003 – 2004	DEUG of Chemistry-Biology-Geology (DEUG-CBG), Felix Houphouët Boigny University, Abidjan, Côte d’Ivoire
1999 – 2000	Baccalaureate in Scientific series (D), Modern high school of Oumé, Oumé, Côte d’Ivoire

PROFESSIONAL EXPERIENCE AND POSITIONS

08/2017 – Present	Co-investigator and supervisor of Master students for AnoPest One-Health project in Agboville and Tiassalé, CSRS, Abidjan, Côte d’Ivoire
01/2017 – Present	Study Director in GLP study for insecticide testing in laboratory, experimental huts and field/community level studies with IVCC (Innovative Vector Control Consortium), CSRS, Abidjan, Côte d’Ivoire

09/2014 – 06/2017	PhD candidate in Epidemiology; Swiss TPH, University of Basel, Basel, Switzerland, supervised by Prof. Dr. Jürg Utzinger
09/2014 – 09/2015	Fellow of Swiss Government Excellence Scholarships for Foreign Students at Swiss Higher Education Institutions (ESKAS) Epidemiology; Swiss Tropical and Public Health Institute (Swiss TPH), University of Basel, Basel, Switzerland, supervised by Prof. Dr. Jürg Utzinger

PROJECT EXPERIENCE

08/2017 – Present	CSRS, Abidjan, Côte d’Ivoire; AnoPest One-Health project: vector control strategy based on farmers working in irrigated rice fields in Agboville and Tiassalé, Côte d’Ivoire
01/2017- Present	CSRS, Abidjan, Côte d’Ivoire; GLP Accrediation Project for the accreditation of the laboratory, insectary, experimental huts of CSRS in Abidjan and Tiassalé, Côte d’Ivoire
05/2016 – 04/2017	CSRS, Abidjan, Côte d’Ivoire; RCT (Randomized Control Trial) on indoor insecticide spraying efficacy in Agboville, Côte d’Ivoire
08/2016 – 09/2016	Swiss Centre for International Health, Swiss TPH, Basel, Switzerland; Proof-reading and checking of French-translated version of World Health Organization (WHO) toolkit (consultance)
06/2016 – 10/2016	PNLSGF (Programme National de Lutte contre la Schistosomiase, les geohelminthiases et la Filariose Lymphatique / National program for the control of Schistosomiasis, Geohelminthiasis and Lymphatic Filariasis), Abidjan, Côte d’Ivoire; 2EEFT Project for assessing the transmission of lymphatic filariasis in the boundary areas of Côte d’Ivoire
01/2013 – 12/2016	CSRS, Abidjan, Côte d’Ivoire; arbovirus Project, assessing *Aedes* mosquito dynamics in arbovirus foci within souteastern Côte d’Ivoire.
04/2014 – 12/2014	PNLSGF (Programme National de Lutte contre la Schistosomiase, les geohelminthiases et la Filariose Lymphatique / National program for the control of Schistosomiasis, Geohelminthiasis and Lymphatic Filariasis), Abidjan, Côte d’Ivoire; Mano River Project for assessing the transmission of lymphatic filariasis in the western boundary areas of Côte d’Ivoire
06/2014	CSRS, Abidjan, Côte d’Ivoire; Projet UNICEF : «enquête sur les recours aux soins lors d’épisodes de maladies chez les enfants de moins de 5 ans et les femmes enceintes avec analyse des capacités contributives et de la volonté à payer des ménages dans les districts sanitaires d’Abobo Ouest et Yopougon Ouest, Abidjan, Côte d’Ivoire.

02/2011 – 09/2013	CSRS, Abidjan, Côte d’Ivoire; AvecNet Project, Work Package 2, task 1: “Identification and validation of genes responsible for insecticide resistance” in Tiassalé, Agboville, Taabo, Sikensi, and Aboisso, Côte d’Ivoire
05/2012- 08/2012	CSRS, Abidjan, Côte d’Ivoire; AvecNet Project, Work Package 5, task 4: “Understanding householder practices to prevent mosquito biting in the home”. Tiassalékro and Komenakpé (Tiassalé), and Adiopodoumé and Angré (Abidjan), Côte d’Ivoire
2009 - 2010	CSRS, Abidjan, Côte d’Ivoire, IVCC Project: Community trial project on insecticide-treated nets (ITNs) (Permanet 2.0, Permanet 2.0extra and Permanet 3.0), Bouaké and Tiasalé, Côte d’Ivoire
10/2009 -11/2009	CSRS, Abidjan, Côte d’Ivoire; IVCC project, insecticide efficacy testing in experimental hut trials in Bouaké, Côte d’Ivoire
09/2008 – 09/2009	CSRS, Abidjan, Côte d’Ivoire; Syngenta crop protection project, insecticide efficacy testing in experimental hut trials in Bouaké, Côte d’Ivoire.

STUDENT SUPERVISIONS

07/2017 - Present	**Assamoa Kacou Fidèle & Ekra Kouassi Armand (Master 2, UNA, CEMV (Centre d’Entomologie Médicale et Vétérinaire)):** Assessing vector control strategy based on farmers working in irrigated rice fields in Agboville and Tiassalé, Côte d’Ivoire (co-supervisor; principal supervisor Prof. Dr. Koudou G. Benjamin, University Nangui Abrogoua (UNA), CSRS, Dr. Mouhamadou S. Chouaibou, CSRS)
12/2016 - Present	**Adjobi N’tayé Claver & Alla Koffi Honoré (Master 2, UNA):** Insecticide-susceptibility in the larvae of *Aedes aegypti* from urban and rural settings in rubber and oil palm cultivation areas in southeastern Côte d’Ivoire (co-supervisor; principal supervisor Prof. Dr. Koudou G. Benjamin, Dr. Ouattara F. Allassane, UNA, CSRS)
06/2016 – 10/2016	**Yokoly N’drin Firmin (Thesis, UNA) & Soro Dramane (Master, UNA):** Transmission of *Wuchereria bancrofti* in low lymphatic filariasis endemicity settings in boundary areas of Côte d’Ivoire (co-supervisor; principal supervisor Prof. Dr. Koudou G. Benjamin, UNA, CSRS)
07/2015 – 11/2015	**Dibo Kacou Jean Denis & Tia Bleu Jean Philippe (Master, UNA):** Insecticide-susceptibility in the adult populations of *Aedes aegypti* from urban and rural settings in oil palm cultivation areas in southeastern Côte d’Ivoire (co-supervisor; principal supervisor Prof. Dr. Koudou G. Benjamin, UNA, CSRS)

06/2014 – 12/2014	**Dibo Kacou Jean Denis & Kouamé Jackson (Master, UNA):** Transmission of *Wuchereria bancrofti* in lymphatic filariasis endemic settings in western boundary areas of Côte d'Ivoire (co-supervisor; principal supervisor Prof. Dr. Koudou G. Benjamin, UNA, CSRS)
02/2014 – 07/2014	**Yoboué Aya Charlène (Master 2, UNA).** Deltamethrin-susceptibility in *Culex* sp. and *Anopheles* sp. from human habitations and irrigated rice fields in Tiassalé, south Côte d'Ivoire (co-supervisor; principal supervisor Prof. Dr. Koudou G. Benjamin, University Nangui Abrogoua (UNA), CSRS, Dr. Mouhamadou S. Chouaibou, CSRS).

GRANTS AND AWARDS

04/2017 – 06/ 2017	Support from Swiss TPH, Basel, Siwtzerland (CHF 5,760)
09/2015 – 12/2015	Support from Swiss TPH, Basel, Siwtzerland (CHF 5,760)
09/2014 – 09/2015	Fellow of Swiss Government Excellence Scholarships for Foreign Students at Swiss Higher Education Institutions (ESKAS) (CHF 23,040). Epidemiology; Swiss Tropical and Public Health Institute (Swiss TPH), University of Basel, Basel, Switzerland, supervised by Prof. Dr. Jürg Utzinger.

CONGRESS PARTICIPATIONS

30/08-01/09 /2017	Joint annual meeting 2017 of Swiss Societies for Microbiology (SSM), Infectious Diseases (SSI), Hospital Hygiene (SSHH), Tropical Medicine and Parasitology (SSTMP) and the Swiss Society of Tropical and Travel Medicine (SSTM). Impacts of land use changes on the ecology of arbovirus-*Aedes* mosquitoes in large industrial oil palm plantation areas in southeastern Côte d'Ivoire. **Oral presentation.**
22-23/04 /2017	4th colloquy on malaria organized by National Progamme for Malaria Control, Abidjan, Côte d'Ivoire. Atypical breeding sites of *Anopheles* and transmision of *Wucherreria bancrofti* by *Anopheles gambiae* in Côte d'Ivoire. **Oral presentation.**
09-10/03/2016	4th congress of African Society of Parasitology (Société Africaine de Parasitologie, SOAP) and Ivorian Society of Parasitology and Mycolology (Société Ivoirienne de Parasilogy et de Mycologie, SIPAM) Abidjan, Côte d'Ivoire. Impacts of urbanization on the dynamics of the populations of *Aedes*, vectors of yellow fever and dengue, in southeastern Côte d'Ivoire. **Oral presentation.** Transmision of *Wucherreria bancrofti* by *Anopheles gambiae* in

	the context of mass drug administartion: implications for the elimantion of lymphatique filatiasis in Côte d’Ivoire. **Oral presentation.**
08-10/09/2015	9th European Congress on Tropical Medicine and International Health (ECTMIH), Basel, Switzerland. Urbanisation in Côte d’Ivoire increases *Aedes* mosquito hatching rate and contributes to dengue and yellow fever outbreaks by selecting *Aedes aegypti.* **Oral presentation.**
22-23/04 /2015	3rd colloquy on malaria organized by National Progamme for Malaria Control, Abidjan, Côte d’Ivoire. Behavior of *Anopheles gambiae* in post-conflict areas and risks of outbreaks of malaria in the western boundary areas of Côte d’Ivoire. **Poster presentation.**
03-05/12/2014	2nd congress of African Society of Parasitology (Société Africaine de Parasitologie, SOAP) and Ivorian Society of Parasitology and Mycolology (Société Ivoirienne de Parasilogy et de Mycologie, SIPAM), Abidjan, Côte d’Ivoire. Entomological determinism of emergnece risks yellow fever and dengue in urbanand rural settings in southeastern Côte d’Ivoire. **Oral presentation.** Implications of *Anopheles gambiae* and *Culex quinquefasciatus* in the transmission of *Wuchereria bancrofti* in western bounadry areas of Côte d’Ivoire. **Oral presentation.**
05-07/12/2013	First Scientific Days of African and Malagasy Council for Higher Education (AMCHE) (Conseil Africain et Malgache pour l’Enseignement Supérieur, CAMES), Abidjan, Côte d’Ivoire. Trophic behaviors of *Anopheles gambiae s.s.*, main vector of malaria, in rual and suburban settings in irrigated rice fields in central Côte d’Ivoire. **Oral presentation.**
04-05/12/2013	1st congress of African Society of Parasitology (Société Africaine de Parasitologie, SOAP) and Ivorian Society of Parasitology and Mycolology (Société Ivoirienne de Parasilogy et de Mycologie, SIPAM), Abidjan, Côte d’Ivoire. Behavioral resistance of genetically resistant in *Anopheles gambaie s.s.*, main vector of malaria, in central Côte d’Ivoire. **Oral presentation.**
13-14/11/2013	First Scientific Days of National Institute of Public Heath (Institut National d’Hygiène Publique, INSP), Abidjan, Côte d’Ivoire. Resting behaviors of *Anopheles gambaie s.s.,* in traditonal huts and modern houses in two villages in central Côte d’Ivoire. **Oral presentation.**
22-23/04/2013	2nd colloquy on malaria organized by National Progamme for Malaria Control, Abidjan, Côte d’Ivoire. Simultaneous impacts of habitats, control measures and resistance of *Anopheles gambaie s.s.* on its trophic behaviors in central Côte d’Ivoire. **Oral presentation.**

25/04/2012	1st colloquy on malaria organized by National Progamme for Malaria Control, Abidjan, Côte d'Ivoire. Irrigated rice fields and behavioral polymorphism of malaria vectors: limitations for vector control. **Oral presentation.**
20-24/03/2012	Week for the Promotion of Ivorian Research (Semaine de la Promotion de la Recherche Ivoirienne, SEPRI), Yamoussoukro, Côte d'Ivoire. Irrigated rice field: source of life, but also source of mosquitoes and malaria. **Poster presentation.**

OTHER ACTIVITIES, COLLABORATION AND SKILLS

Memberships	Memer of PhD Programm health Sciences (PPHS) at University of Basel, Basel, Switzerland (10/2016 - Present) Member of Sociéte Ivoirienne de Parasitologie et Mycologie (SIPAM) and Société Africaine de Parasitologie (SOAP), Abidjan, Côte d'Ivoire (12/2014 - Present) Member of Société d'Entomologie de Côte d'Ivoire (SECI) and Société Africaine de Parasitologie (SOAP), Abidjan, Côte d'Ivoire (06/2014 - Present)
Collaboartion	Researcher associated to Centre Suisse de Recherches Scientifiques in Côte d'Ivoire (CSRS), (10/2008 - Present) Collaboration with Swiss Tropical and Public Health Institute (SwissTPH), Basel, Switzerland (10/2016 - Present) Collaboration with the National Programme for the Control of Shistosomiasis, Geohelminthiasis and Lymphatic Filariasis, Abidjan, Côte d'Ivoire (10/2014 - Present).
Computer skills	Microsoft Office (Word, Excel, PowerPoint, etc.), Statistical software (Stata, R, RStudio), ArcGIS, Epi-info, EndNote
Language skills	**French:** reading, writing, understanding, speaking: **excellent** (native speaker) **English:** reading, writing, understanding, speaking: **good** (improved skills by attending 6 certificated lectures at Language Centre in Basel, Switzerland, from September 2014 to December 2015)

PUBLICATIONS

Zahouli JBZ, 2017. Anthropogenic impacts on *Aedes* mosquito community dynamics in Côte d'Ivoire. PhD thesis, University of Basel, Basel, Switzerland.

Zahouli JBZ, Koudou BG, Müller P, Malone D, Tano Y, Utzinger J. Insecticide-susceptibility in urban and rural strains of *Aedes aegypti* from large industrial oil palm plantation areas in Côte d'Ivoire (in preparation; to be submitted to Sci Rep, November 2017).

Zahouli JBZ, Koudou BG, Müller P, Malone D, Tano Y, Utzinger J, 2017. Effects of land-use change on the abundance, distribution and host-seeking behaviors of *Aedes* arbovirus vectors in oil palm-dominated landscapes in southeastern Côte d'Ivoire. Submitted to PLoS One.

Zahouli JBZ, Koudou BG, Müller P, Malone D, Tano Y, Utzinger J, 2017. Urbanization is a main driver for the larval ecology of *Aedes* mosquitoes in arbovirus-endemic settings in south-eastern Côte d'Ivoire. PLoS Negl Trop Dis; 11 (7): e0005751.

Zahouli JBZ, Utzinger J, Adja MA, Müller P, Malone D, Tano Y, Koudou BG, 2016. Oviposition ecology and species composition of *Aedes* spp. and *Aedes aegypti* dynamics in variously urbanized settings in arbovirus foci in southeastern Côte d'Ivoire. Parasit Vectors; 9: 523.

Zahouli JBZ, Tchicaya ES, Nsanzaban C, Donzé J, Utzinger J, N'Goran EK, Koudou BG, 2011. Bio-ecological characteristics of *Anopheles gambiae s.s.* in irrigated rice fields of central Côte d'Ivoire. Med Trop; 71 (6): 575-81.