ENVIRONMENTAL DRIVERS OF DENGUE, CHIKUNGUNYA AND ZIKA TRANSMISSION AND THEIR MOSQUITO VECTOR, *Aedes aegypti*, IN TWO COASTAL HOTSPOTS IN ECUADOR

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ABV. *Aedes*-borne virus

AIC. Akaike information criterion

BGS. BG-Sentinel trap

C.I. Confidence Intervals

CHIKV. Chikungunya virus

DALYs. Disability-adjusted life years

DENV. Dengue virus

DF. Dengue fever

DHF. Dengue haemorrhagic fever

DNA. Deoxyribonucleic acid

EIR. Entomological inoculation rate

ENSO. El Niño Southern Oscillation

EW. Epidemiological week

GAM. Generalized additive model

GBS. Guillain-Barré syndrome

GLMM. Generalized linear mixed models

HLC. Human landing catch

INAMHI. National Institute of Meteorology and Hydrology of Ecuador

INEC. National Institute of Statistics and Censuses of Ecuador

ITCZ. Intertropical Convergence Zone

LEMMT. Medical entomology and tropical medicine laboratory

LRT. Likelihood ratio test

m.a.s.l. Meters above sea level

MAYV. Mayaro virus

MET. Mosquito electrocuting trap

ML. Maximum likelihood

MoH. Ministry of Health

MRC-CVR. Medical Research Council – Centre for Virus Research

PCR. Polymerase chain reaction

PPK. Prokopack aspiration

RNA. Ribonucleic acid

RVFV. Rift Valley fever virus

SIT. Sterile insect technique

VEEV. Venezuelan equine encephalitis virus

VIF. Variance inflation factor

WNV. West Nile virus

YFV. Yellow fever virus

ZIKV. Zika virus

ABSTRACT

In the Americas, arbovirus transmission is concentrated within urban settings in tropical zones, where high human population densities and environmental conditions enhance the survival and reproduction of *Aedes aegypti*. Since its re-emergence in South America in the 70's, dengue virus has been expanding and increasing in urban settings where it is now endemic. Additionally, the recent arrival of new arboviruses into the region, such as chikungunya (2013) and Zika virus (2015), have triggered major epidemics leading significant public health and economic impacts. These pathogens are linked in sharing a common mosquito vector in *Ae. aegypti*. Given the absence of effective licenced vaccines, vector control is thus the primary strategy for reducing the transmission of all of these pathogens.

Effective vector control and public health preparedness require detailed understanding of vector ecology and human exposure risk within foci of transmission. Both vector populations and viral dynamics are highly dependent on environmental conditions, but the nature of environmental impacts likely depends on local ecological context. Ecuador bears an important burden of arboviral transmission in South America. Most transmission is concentrated in coastal cities where dengue is endemic and rising, and major outbreaks of chikungunya and Zika have recently occurred. However, there has been limited investigation of vector ecology in these rapidly expanding urban settings, and its association with seasonal patterns of arboviral transmission. To address this gap, this study aimed to assess the environmental drivers of *Ae. aegypti* ecology, infection rates and arboviral transmission within two major urban hotspots in Coastal Ecuador. This was accomplished through a series of field studies of vector ecology, laboratory analyses of arboviruses, and modelling investigations designed to identify environmental determinants of human exposure and infection incidence.

The first chapter reviews what is known about the most important *Aedes*-borne viruses and their vectors in South America, with particular focus on Ecuador, vector control, and the global and regional disease burden. The second chapter presents results from a field study carried out in a urban neighbourhood of Quinindé, Ecuador, that evaluated a novel trapping method, the Mosquito

Electrocuting Trap (MET), for direct estimation of human exposure to *Ae. aegypti* bites. The third chapter describes results of a 6-month field study aimed to characterize the environmental determinants of *Ae. aegypti* abundance and distribution, behaviour and arboviral infection rates within two cantons in Coastal Ecuador at the tail end of the 2016-17 Zika outbreak. The fourth chapter presents an analysis of seasonal and annual variation in dengue, chikungunya and Zika virus within these 2 cantons, and associations with climatic and entomological variables. The fifth chapter discusses the key results of each of the chapters and the implications of the findings towards an effective vector control in Ecuador and beyond the country.

It was found that the MET was effective for measuring *Ae. aegypti* host-seeking behaviour, and generated representative estimates of their biting rate and activity time relative to the standard BG-sentinel trap (BGS). Analysis of *Ae. aegypti* ecology indicated that its abundance varied significantly between cantons, neighbourhoods within cantons associated with urbanization gradient, temporal timing of collection and past rainfall. Additionally, there was significant variation in *Ae. aegypti* resting behaviour (resting in or outside houses) between cantons. This demonstrates the existence of heterogeneity in *Ae. aegypti* population dynamics and behaviour between and within the study sites, which highlights the importance of localized surveillance to guide vector control. Likewise, arboviral incidence of dengue and chikungunya (as reported to the health system) also differed between the two study sites, being dengue 1.5 and chikungunya 2.5 times higher in Portoviejo than in Quinindé during the peaking week. The seasonal pattern of disease incidence varied among the three arboviruses, with a difference of 5 weeks between each of their peak of incidence. Intra-annual incidence was also found to be linked with climatic and entomological variables, with dengue and chikungunya incidence being positively associated with temperature and rainfall, while Zika incidence negatively associated with such climatic variables. Outdoor *Aedes* collections with Prokopack aspirators and BGS were positively related to dengue incidence, while indoor Prokopack aspirations were negatively associated with this variable. The interannual incidence of dengue differed between years analysed (2013-2018) with 2015 being the year with highest dengue incidence. Such findings highlight the importance of conducting focalized epidemiological surveillance on each site, but also

differentiating between arboviruses, rather than assuming they all will follow dengue trends. Findings from this work have provided new entomological and epidemiological information to the study sites and despite the short period of study, fine spatial scale heterogeneity was detected in arbovirus transmission dynamics.

CHAPTER 1: INTRODUCTION

1.1. BACKGROUND

Mosquitoes are a group of insects that belong to the family Culicidae (Diptera) and comprise approximately 3,578 species throughout the world [1]. The highest mosquito diversity occurs in the Neotropics, where about one third of all species are present and nine genera are endemic to this region [2]. Females in most mosquito species are hematophagous and require blood meals from vertebrates (reptiles, birds, mammals and amphibians) to obtain nutrients to develop their eggs [3]. This system has allowed pathogenic microorganisms to spread between vertebrate hosts through mosquito bites [4]. Pathogens that are transferred from one organism to another through an intermediary organism, causing a disease to the host from such infection, are defined as "vector-borne diseases" [5]. Vector-borne diseases can be transmitted by a wide variety of arthropods including mosquitoes, biting midges, biting flies, sand flies, ticks, among others; with mosquitoes being of greatest public health importance due to role in causing at least 700 million infections, and more than a million deaths each year [6].

At least half of the world's population lives in areas of vector-borne disease risk [7]. Mosquito-borne diseases affect a wide range of vertebrates including humans [8–10]. Pathogens transmitted by mosquitoes to humans range from parasites (e.g. *Plasmodium falciparum, P. vivax, P. malariae, P. ovale*, and *P. knowlesi*) that cause malaria [11], filarial worms (e.g. *Wuchereria bancrofti, Brugia malayi, B. timori*) that cause lymphatic filariasis [12] and arboviruses (e.g. dengue, chikungunya, yellow fever, Zika, etc.) among others [5]. Among these diseases, malaria affects more people than any other vector borne disease worldwide and caused approximately 229 million cases and 409 thousand deaths in 2019 (~67% were children under 5 years old) [13]. However, mosquito-borne arboviruses also have a huge and expanding impact on public health [14]. Approximately 54% (~287) of all the recognized viruses in the world are transmitted by vectors [15]. Many arboviruses also circulate between human and animal populations, with a few that occur almost exclusively in humans being responsible for serious epidemics worldwide [14]. Arboviruses have been re-emerging in the last few decades in

striking numbers [15]. Some of the most important arboviruses that affect human populations are transmitted by mosquitoes from the genus *Culex* and *Aedes*, with the most important species being *Cx. pipiens*, *Ae. aegypti* and *Ae. albopictus*. *Aedes* mosquitoes are the vectors of dengue (DENV), chikungunya (CHIKV), Zika (ZIKV), and yellow fever (YFV) viruses among others. In combination, these *Aedes*-borne viruses (ABVs) generate the bulk of human arbovirus-related morbidity and mortality [14], and have been rapidly expanding in the last few decades [16–18].

1.2. *AEDES*-BORNE VIRUSES OF GREATEST PUBLIC HEALTH IMPACT, THEIR GLOBAL BURDEN AND MAJOR RISKS

1.2.1. Dengue virus

The arbovirus of greatest public health impact is currently dengue (DENV), a virus that belongs to the family Flaviviridae which presents four different serotypes (1 – 4) [19]. The genome of each DENV serotype is composed by single-stranded RNA of approximately 11,000bp [20], encoding 10 proteins: 3 structural proteins (the membrane, the capsid, and the envelope), and 7 non-structural proteins [21].

DENV is thought to have originated in sylvatic environments, where it still circulates naturally among non-human primate hosts and other *Aedes* vectors [22]. Phylogenetic analyses suggest that a common ancestor of all DENV likely originated in Malaysia, although each DENV serotype evolved independently from their sylvatic counterparts [19] with DENV-2 being the oldest (1000 ± 500 years ago [22]). The first records of a dengue-like illness in human populations come from the 2nd century [23]. Endemic cycles within human populations have increased dramatically in the 1900's [24]. Between 1943 and 1956, the four serotypes of DENV were isolated for the first time, with all subsequent DENV isolations falling within this classification [24]. *Aedes furcifer* has been implicated as the most probable vector linking the sylvatic and urban transmission cycle of DENV [25], while *Ae. albopictus* was probably the main urban vector in Asia before the arrival of *Ae. aegypti* [24]. Now, DENV has been adapted to circulate among human populations, where it is mainly transmitted by *Ae. aegypti* mosquitoes in urban areas, and *Ae. albopictus* in peri-urban and rural areas [26,27]. Dengue fever (DF), the disease caused by DENV, is generally characterized by mild symptoms such as fever, headache, ocular pain and myalgia, which are generally

resolved satisfactorily [28]. However, DF sometimes develops into a severe form known as dengue haemorrhagic fever (DHF), mostly related to hyperendemicity of DENV (i.e., the temporal and spatial co-circulation of two or more DENV strains) [29]. Dengue is currently spreading at the highest speed among the other mosquito borne diseases [30].

DENV is estimated to infect approximately 390 million per year [31], from which 96 million people present symptoms [14], and approximately 9 thousand people are killed [32]. Dengue disease has a huge impact on people's lives as reflected through disability-adjusted life-years (DALYs), estimated as the number of years a person loses due to illness, disability or early death due to this disease [33]. The overall DALYs due to DENV infections was estimated at approximately 1.9 million in 2015, an increase of more than 50% since 2005 [34].

Several vaccine candidates have been under development for DENV, but only one (the chimeric yellow fever 17D virus-tetravalent dengue vaccine [CYD-TDV, Dengvaxia®]) has been licenced in some Asian and Latin American countries [35]. This vaccine however may only be safe for people 9-years or older in endemic settings, and for dengue-seropositive individuals as it may increase the risk of severe dengue in seronegative people [36]. Due to the lack of an effective vaccine that can protect against all four DENV serotypes, vector control remains as the main form of prevention.

1.2.2. Chikungunya virus

CHIKV is a virus from the family Togaviridae that is composed of a single-stranded RNA genome of approximately 11,800bp that encodes for five structural proteins and four non-structural proteins [37]. CHIKV is the causal agent of chikungunya fever; a disease first identified in 1952 during an outbreak in Tanganyika (currently a region of Tanzania) [38]. CHIKV is mainly transmitted to humans by *Ae. aegypti* and *Ae. albopictus* in urban and rural areas, respectively [39]. There is also a sylvatic cycle that has been observed in Africa, involving other *Aedes* mosquito species and other non-human primate reservoirs [40,41]. Furthermore, horizontal and vertical transmission between mosquitoes have been described [42,43] as well as maternal-fetal transmission in human populations [44]. Its main clinical form are fever, arthalgia (i.e., joint pain), back pain, and headache; with a chronic

stage mainly characterized by polyarthalgia (i.e., pain of multiple joints) leading to a limited mobility of patients [45].

Since first discovery in 1952, there have been sporadic CHIKV outbreaks worldwide [18]. Following an outbreak in Kenya in 2004, CHIKV has spread throughout Africa, Asia, Europe and the Americas [18]. In early 2005, an outbreak of chikungunya was reported in the Comoro Islands and Réunion Island in the Indian Ocean [46,47]. Subsequently, the virus spread to Europe in 2007 [48] and the Americas in 2013 [49], causing the initial outbreaks in St. Martin [50], followed by several outbreaks throughout the continent [51]. Since the first detection of CHIKV in the Americas, there were approximately 2.67 million cases until 2017 [52]. One of the most critical consequences of CHIKV is chronic post-infection rheumatism that can severely damage joints, impair daily life and affect mental health [53,54]. Vaccines against CHIKV infection are under development but to date, none have been yet approved [55]. Therefore vector control is currently the only preventive strategy against CHIKV.

1.2.3. Zika virus

ZIKV is a virus from the family Flaviviridae consisting of a positive single-stranded RNA of approximately 10,800bp that encodes for three structural (pre-membrane/membrane, capsid and envelope) and five non-structural proteins [56]. Just like DENV and CHIKV, ZIKV has a sylvatic cycle. It was first identified in non-human primates in 1947, in the Zika Forest in Uganda after took its name [57]. ZIKV has a sylvatic cycle in several African countries where evidence of infection has been found in *Aedes* mosquito species and several non-human primates in remote forested areas [58]. Recently, ZIKV has spilled over into human-to-human transmission in urban areas where it is mainly transmitted by *Ae. aegypti* mosquitoes [59]. *Aedes albopictus* is also a competent vector, but its epidemiological significance is still unknown [60]. *Aedes hensilli* and *Ae. polynesiensis* were implicated as the primary vectors in some of the first human outbreaks in Yap Island and French Polynesia [61,62].

Direct human to human transmission (non-vector mediated) of ZIKV can also occur through maternal-fetal transmission (mainly during pregnancy and breastfeeding) [63,64], sexual transmission [65], and blood transfusion [66]. In humans, the main

clinical manifestations are fever, rash, headache, arthalgia, myalgia (i.e., muscle pain), and periorbital pain [67]. However, microcephaly, a condition of small head size in foetuses that leads to problems with the growth of the brain [68], has been linked to maternal ZIKV infection. ZIKV has also been linked to Guillain - Barré syndrome (GBS), a neurological condition caused by the alteration of the immune system, which leads to motor impairment and sometimes paralysis [69].

The first human cases of Zika infections were discovered from surveys of sera from patients in Uganda and Tanzania, that revealed that the prevalence may be frequent among the population (around a 6.1% seroprevalence of antibodies) [70,71]. Further sera surveys showed that the occurrence of Zika virus in humans was more wide spread in African and Asian countries than previously thought [72]. The first cases of illness caused by Zika virus were reported in 1953 from three people in Nigeria [73]. However, probably due to the mild symptoms exhibited by infected humans and/or due to low rates of transmission, there were very few reported cases in the next fifty years. In 2007, the first outbreak of ZIKV was recorded in Yap Island [74] and then in 2013 in French Polynesia [62,75]. By 2015, ZIKV had spread to Brazil [76,77], and subsequently expanded throughout Central and South America resulting in approximately 867 thousand cases until 2020 [78]. ZIKV caught worldwide attention because of its association with neurological problems and severe congenital malformations [79,80]. Microcephaly cases linked to ZIKV infections were first detected from a temporal and spatial overlap between ZIKV cases and reported microcephaly in Brazil [81]. Then, a retrospective analysis was made using data of microcephaly cases in French Polynesia during their ZIKV outbreak period [82], which finally concluded there was a causal relationship [83]. It is thought that the risk of microcephaly among babies from ZIKV-infected mothers is about 1% [82]. Additionally, the link between ZIKV and GBS, was first described from a case of a woman who developed this syndrome immediately after ZIKV infection during the French Polynesia outbreak [84]. Case studies in Brazil and other countries showing temporal and spatial occurrence of GBS and ZIKV [85–87], led into a retrospective case-study from the French Polynesia ZIKV outbreak, which concluded causality of GBS by ZIKV infection [88].

The potentially severe consequences of ZIKV infections on new-borns and adults generated an urgency to develop a vaccine. Several vaccine candidates against

ZIKV infection are under development, but none are currently available [89]. Thus as with DENV and CHIKV vector control is still the main form of ZIKV prevention.

1.2.4. Yellow fever virus

YFV is a virus from the Flaviviridae family and its genome is composed of single-stranded RNA with approximately 11,000bp that encodes 10 proteins including 3 structural (core, pre-membrane/membrane, and envelope) and 7 non-structural proteins [90]. YFV originated in Africa and was introduced in the Americas during the slave trade, causing the first epidemics in Yucatán, now part of Mexico, in 1648 [91]. YFV circulates in sylvatic and urban cycles, involving different vector species and hosts/reservoirs in each cycle [58]. In the sylvatic cycle, the main vector species in Central Africa are *Ae. africanus* and *Ae. opok*, which keep the transmission cycle of YFV among non-human primates and incidentally transmit to humans when present [90]. However, in East and West Africa, other anthropophilic *Aedes* vectors dominate the transmission cycle in the forest-savannah ecotone, a transition ecosystem between the rainforest and the dry savannah, where the urban cycle occurs [90,92]. Here, mosquito species such as *Ae. furcifer, Ae. taylori, Ae. luteocephalus, Ae. metallicus* and *Ae. africanus*, keep the transmission cycle during the wet season, where humans and non-human primates are the main hosts [93]. The highly anthropophilic *Ae. aegypti* mosquito, which also inhabits these areas, has become the main vector during the dry season in West Africa [94], while *Ae. bromeliae* has possibly been responsible for urban outbreaks in East Africa [95]. This ecosystem has become the main source of urban cycle outbreaks due to the presence of competent vectors and movement of infected humans into urbanized areas [90,92]. While in South America, the main sylvatic vector species are mosquitoes from the *Haemagogus* and *Sabethes* genera, which also keep the sylvatic cycle among non-human primate species, but can also transmit to humans if present [96]. In the urban cycle of YFV in Africa and the Americas, *Ae. aegypti* has been described as the main vector species, making the inter-human transmission a real public health problem, which has caused numerous historical outbreaks in both continents [92]. In the Americas, for instance, the last and largest YFV outbreak of the 21st century occurred in Brazil between 2016 and 2018, reaching the metropolitan region of São Paulo, and causing 2,045 cases and 677 deaths [97].

1.2.5. Aedes-borne diseases of minor epidemiological impact

Other ABVs of note are Mayaro virus (MAYV) and Venezuelan Equine Encephalitis virus (VEEV) in the Americas [98,99], and Rift Valley fever virus (RVFV) in Africa [100]. These arboviruses currently have a considerably less burden than DENV, CHIKV, ZIKV and YFV as described above, but could be prone to future expansion in human populations. For instance, MAYV, which belongs to the family Togaviridae, is actively transmitted by *Haemagogus janthinomys* mosquitoes to birds, non-human primates, and other small mammals [101]; however *Ae. aegypti* and *Ae. albopictus* are also competent and thus have potential to initiate a transmission cycle within human populations [102,103]. Small human outbreaks of MAYV have been detected in some countries and territories in the Americas, including Trinidad and Tobago (where it was first isolated), Colombia, Panamá, Brazil, Perú, Bolivia, Ecuador, French Guiana, Venezuela, and Haiti [104]. VEEV is a virus from the family Togaviridae that normally circulates within rodent populations in enzootic cycles, and within horse populations in epizootic cycle [99]. VEEV is mainly transmitted by mosquitoes from the genera *Aedes, Culex (Melanoconion), Psorophora, Mansonia* and *Deinocerites* [99], and may also have potential for emergence in humans as both *Ae. aegypti* and *Ae. albopictus* are competent vectors [105,106]. In addition, RVFV (family Bunyaviridae), could have potential for spread in the Americas following introduction from importation of infected ruminants or immigration of infected people [107]. The potential risk of RVFV establishment in the Americas remains unknown, but should be low if appropriate animal importation measures are taken [108]. Studies on the competence of *Ae. aegypti* populations from the Americas are required to assess outbreak risk as have been conducted elsewhere [109].

As it has been seen, public health is significantly impacted by *Aedes* borne diseases with DENV being a recurrent cause of yearly epidemics and CHIKV, ZIKV and YFV having the potential threat to re-emerge and contribute to the increasing public health impact. Given that most of the *Aedes* borne diseases have no vaccines available, vector control strategies would have multiple benefits as all of them share the same urban vector species. Thus, the ecology and control strategies of the main *Aedes* vector species are reviewed in the next section.

1.3. ECOLOGY AND CONTROL OF *AEDES* VECTORS

As reviewed above, the most common *Aedes* vectors of human arboviruses are *Ae. aegypti* and *Ae. albopictus*. These vectors share some common feature of their ecology and behaviour including the predisposition to bite during the day [110]. However, they differ in other aspects of their ecology, distribution and vectorial competence that impacts their role in arboviral transmission.

Aedes aegypti is the main vector of arboviruses worldwide. Like most mosquito species, adult *Ae. aegypti* relies on standing water to lay eggs. Eggs are particularly adapted to survive to desiccation, thus they can surpass the dry season and hatch when rainy periods start over again. This allows *Ae. aegypti* populations to survive over seasonal variation in rainfall including long periods in the absence of water. This adaptation has also allowed eggs of *Ae. aegypti* to be transported accidentally in dry conditions and colonize new areas where water is present. In the presence of water, eggs can take up to 3 days before hatching [111]. Larval stage of mosquitoes are subdivided into 4 discrete instar sub-stages, within which larvae will feed and develop. The end of each instar is marked by moulting their external cuticle layer. After the 4th instar, larvae metamorphosise into pupae which are still aquatic but do not feed. In the case of *Ae. aegypti*, completion of all 4 instar larval sub-stages and the pupa may take between 7 and 20 days depending on the environmental conditions before emerging as adults [111]. Once emerged, *Ae. aegypti* female mosquitoes may take their first blood meal within 3 days [111], which in this case, is almost exclusively from humans (anthropophily) [112]. This feeding preference is a great advantage for pathogens like DENV that can only replicate in humans and related primates. However, it has been seen that *Ae. aegypti* may also feed on other animals reducing the risk of pathogen transmission among humans [113]. Another aspect of *Ae. aegypti* feeding behaviour that enhances pathogen transmission is their tendency to have more than one blood meal during the same gonotrophic cycle [114,115]; which increases opportunity for viral contact and spread. In field conditions, male adult *Ae. aegypti* live approximately 3 - 6 days [116], and females from 10 – 35 days depending on the parity status [117].

Aedes aegypti is well adapted to urban environments [26,27,118–120], being successful at breeding in artificial containers that are common in and around human dwellings [121,122]. Adult *Ae. aegypti* mosquitoes exhibit a strong preference for resting (endophily) and feeding (endophagy) inside houses [123]. This mosquito species is mainly distributed in tropical and subtropical regions of Africa, Asia and the Americas [124]. Currently, habitat colonization by *Ae. aegypti* is mainly driven by urbanization [118], but its future spread is predicted to be mostly driven by climate change [124] (Figure 1.1).

Aedes albopictus is probably the second most important arbovirus vector in the world, and the primary vector in settings where *Ae. aegypti* is absent. *Aedes albopictus* eggs are more resilient to temperature change (e.g., temperature drop) than *Ae. aegypti* [125], which may have favoured *Ae. albopictus* to colonize more temperate areas [124]. The larval development period of *Ae. albopictus* is between 5 to 10 days, and about 2 days for pupae, but could be longer depending on environmental conditions [126]. Adult mosquitoes of this species may live up to 35 days [127]. Like *Ae. aegypti*, *Ae. albopictus* can take multiple blood meals during the same gonotrophic cycle [115]. However, its host preference is wider than *Ae. aegypti*, including both humans and other animals depending on availability. For instance, in rural areas, *Ae. albopictus* has been seen to feed on other animals more frequently than in urban areas, where humans are more readily available [128,129]. *Aedes albopictus* tends to be more common in rural areas, breeding in natural water basins such as tree holes, bamboo and bromeliads [130,131]. However, it has sometimes been seen to colonize urban areas and breed in artificial containers similar to *Ae. aegypti* [131,132]. Thus, adult *Ae. albopictus* has shown a resting (exophily) and feeding (exophagy) behaviour outside houses [133]. Its future expansion is thought to be linked to climate change which may increasingly facilitate its expansion into temperate areas [134,135] (Figure 1.1).

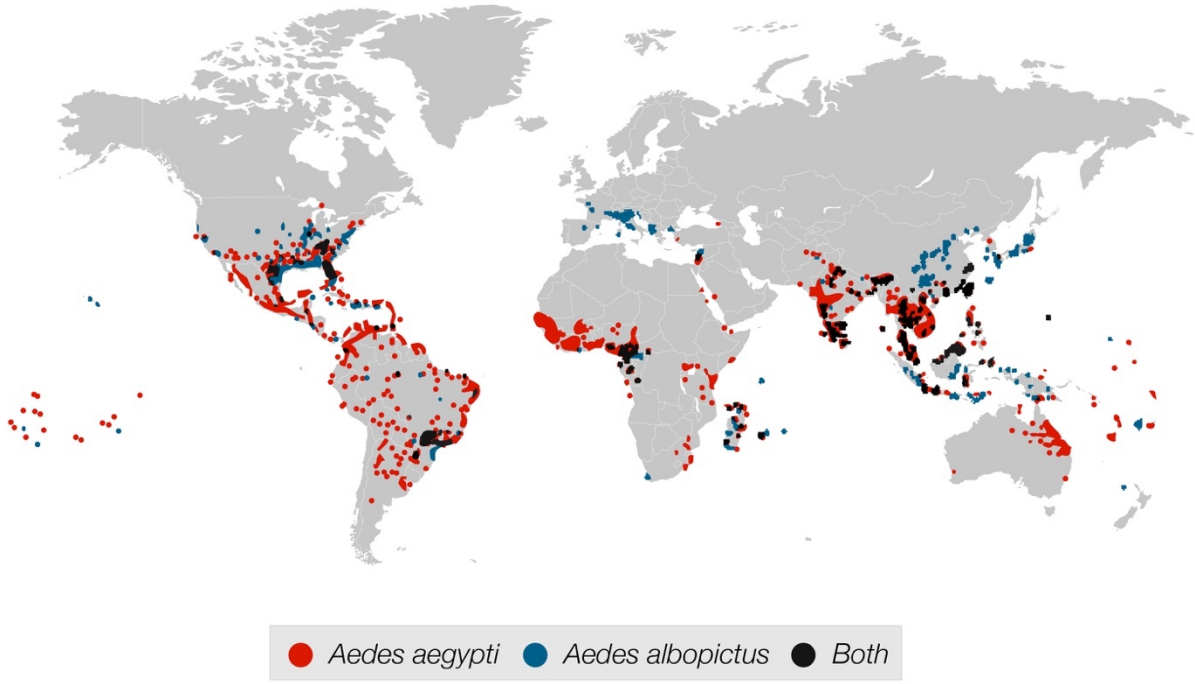

Figure 1. 1. Global current distribution of *Aedes aegypti* and *Aedes albopictus*. Map showing global distribution of *Ae. aegypti* in red, *Ae. albopictus* in blue, and overlapping distribution in black. Map modified from Kamal et *al.* (2018) [124].

Due to the lack of available vaccines for most ABVs, vector control has been widely applied in attempt to control disease transmission [28,51,136,137]. Several different strategies for *Aedes* vector control have been used. The oldest strategy against *Aedes* is larval control and has long been used against this vector species [28,138] which can be achieved either through environmental management to remove aquatic habitats or treating larval habitats with pesticides [139]. The most common larvicides are pyriproxyfen, temephos and *Bacillus thuringiensis israelensis* (Bti) [140–142]. Due to the feasibility of application, such larvicides are mostly targeted to artificial containers in human settlements and thus may be more effective against *Ae. aegypti* than *Ae. albopictus* populations. In addition, several methods have been used to target adult *Aedes*. The oldest and probably most widely used method in current control programmes on space and residual spraying of insecticides in and around houses [143,144]. The effectiveness of these control methods for reducing infection and disease is unclear, generally due to

the lack of conclusive evidence from large scale epidemiological trials [145]. A challenge of these approaches is the emergence of insecticide resistance in *Aedes* populations [146,147]. Resistance of *Aedes* vectors occurs when susceptible populations are able to survive to insecticide doses that have previously been proven to be lethal in susceptible populations [146,147]. This phenomenon has been repeatedly documented in larvae and adult *Aedes* populations, although its impact on the success of vector control is not yet clear. Thus, novel approaches to control *Aedes* populations have been developed such as the development of transgenic mosquitoes to reduce population size, which uses the Sterile Insect Technique (SIT) [148]; and the introduction of *Wolbachia* bacteria into *Aedes* mosquitoes to reduce arbovirus transmission [149].

Currently, the evidence based on vector control for suppression of ABVs is quite patchy and weak [145]. While several studies have evaluated the impact of interventions on *Aedes* population density; relatively few have measured the associated epidemiological impact on infection incidence and disease in humans likely as a consequence of the high cost involved with epidemiological trials. Of the few *Aedes* vector control trials that have measured epidemiological outcomes, few have demonstrated a clear impact [140,150–154]. Thus it remains unclear whether the lack of evidence on effective *Aedes* vector control is because of limited investigation of epidemiological impact, or the limited effectiveness of tools [155].

All existing and newly developing *Aedes* control approaches could benefit from accurate information on vector ecology including the abundance and distribution of potential vector species, their seasonality and temporal dynamics, larval ecology and behaviour. The need for thorough knowledge of vector ecology is even greater in the current context of rapid environmental change and urbanization, which may be driving rapid changes in *Aedes* populations that could impact their ability to spread endemic and newly emerging viruses [156]. Unfortunately, due to resource constraints there is a paucity of up-to-date information on *Aedes* ecology and transmission within many low and middle income countries; including within many urban settings in South America that have been disproportionately affected by recent outbreaks of DENV, CHIKV and ZIKV. Acquisition of contemporary data on vector populations and their transmission potential within

current urban hotspots would be of great value to for selecting and appropriately targeting vector control and other preventive measures.

1.4. BURDEN AND TRANSMISSION OF DENGUE, CHIKUNGUNYA AND ZIKA VIRUS IN ECUADOR

Amongst South American countries, Ecuador has one of the highest incidence of ABVs, which mainly occurs in its Coastal region. The total number of DENV cases in Ecuador has risen sharply in the last 30 years from 63,499 between 1991-2000, 84,259 between 2001-2010 decade, and 145,695 between 2011-2020 decade. These figures support the trend of increasing DENV incidence reported elsewhere [157]. The problem of ABVs in Ecuador was exacerbated by the arrival of CHIKV and ZIKV into the country in the last decade. Following the spread of CHIKV throughout South America starting in 2013, it arrived in Ecuador in December 2014 [158]. Subsequently, a total of 29,007 cases were reported in 2015 [52]. Following on the heels of this CHIKV epidemic, Ecuador reported the first two cases of imported ZIKV in January 2016 [159]. This virus spread in primarily coastal urban settings resulting in two consecutive outbreak years with 3,547 cases in 2016, and 3,183 cases in 2017 [160]. Although these ZIKV case numbers are moderate compared to other high burden countries in South America (e.g Brazil, [160]), they likely underestimate the real burden considerably due to the inability to detect asymptomatic cases, and frequent misdiagnosis due to the similarity of its symptoms with DENV [161,162]. Thus, there is an urgency to increase diagnostic and disease surveillance capacity in this country.

The high burden of arboviruses in Ecuador may be further increased by recent changes in vector ecology. Both *Ae. aegypti* and *Ae. albopictus* are present in South America [163], but *Ae. albopictus* was only confirmed in Ecuador in 2017 (in Guayaquil [164]). Thus, although *Ae. aegypti* was likely the exclusive historical vector of DENV, CHIKV and ZIKV outbreaks in Ecuador, *Ae. albopictus* may play a role in disease transmission in the future. At present *Ae. aegypti* remains the dominant arboviral vector in urban settings in Ecuador. Understanding the local ecology of *A. aegypti* in these hotspots is fundamental for guiding the appropriate selection of appropriate vector control and health system preparedness.

In this study, I carried out a detailed investigation of the ecology of *Ae. aegypti* vectors and arboviral transmission within two urban hotspots of transmission in coastal Ecuador. The study took part during the tail end of the 2016-2017 South American Zika epidemic, and had the underpinning goal of understanding the role of spatial and temporal variation on driving human exposure risk and disease dynamics. To achieve this, a series of field, laboratory and modelling investigations were carried out to address the following objectives:

1.5. OBJECTIVES

i. Evaluate the performance of the Mosquito Electrocuting Trap (MET) relative to the BG-sentinel trap (BGS) for estimating human exposure to *Ae. aegypti* and its association with microclimatic conditions (CHAPTER 2).

ii. Assess the effects of environmental determinants on the spatial and temporal variation of *Ae. aegypti* population abundance, behaviour, and arboviral infection rates in two arboviral hotspots in Coastal Ecuador (CHAPTER 3).

iii. Identify climatic and entomological drivers of intra and interannual variation of ABVs in two transmission hotspots in Coastal Ecuador (CHAPTER 4).

iv. Conduct public engagement activities with participants from the study sites and get them involved into participatory activities aimed to improve their understanding on arbovirus transmission and prevention (APPENDIX 2).

With this work, it is aimed to contribute to an improved understanding of environmental and entomological drivers of arboviral transmission in Coastal Ecuadorian settings and generate guidance for vector control and epidemiological surveillance in Ecuador and beyond.

1.6. STUDY SITES

This work was carried out in the cantons of Quinindé (Esmeraldas province), and Portoviejo (Manabí province) (Figs. 1a, 1b). In Ecuador, cantons are generally equivalent to cities and surrounding suburbs, and constitute the second political division of the country after provinces. Ecuador is politically divided into 22 provinces, which are in turn subdivided into cantons, and subsequently into urban and rural parishes. The two cantons of the study are located in the Pacific coastal region of the country which is limited on the West by the Pacific Ocean and on the East by the Andes mountains. Quinindé canton is the second most commercially important canton in Esmeraldas province, the northernmost province in the coastal region, and the canton's territory comprises approximately 3,875 Km^2, with an estimated population density of 36.3 people/Km^2 during 2017 [165,166]. The canton is subdivided into one urban and five rural parishes. All of the work in this canton was conducted in the city of Quinindé (Rosa Zárate), which is the only urban parish within the canton (Fig. 1c). Portoviejo canton harbours the capital city of Manabí province, the city of Portoviejo, making this canton one of the most commercially important of the entire coastal region (Fig. 1d). The size of this canton is approximately 960 Km^2, with an estimated population density of 326.63 people/Km^2 in 2017. The canton contains nine urban and seven rural parishes.

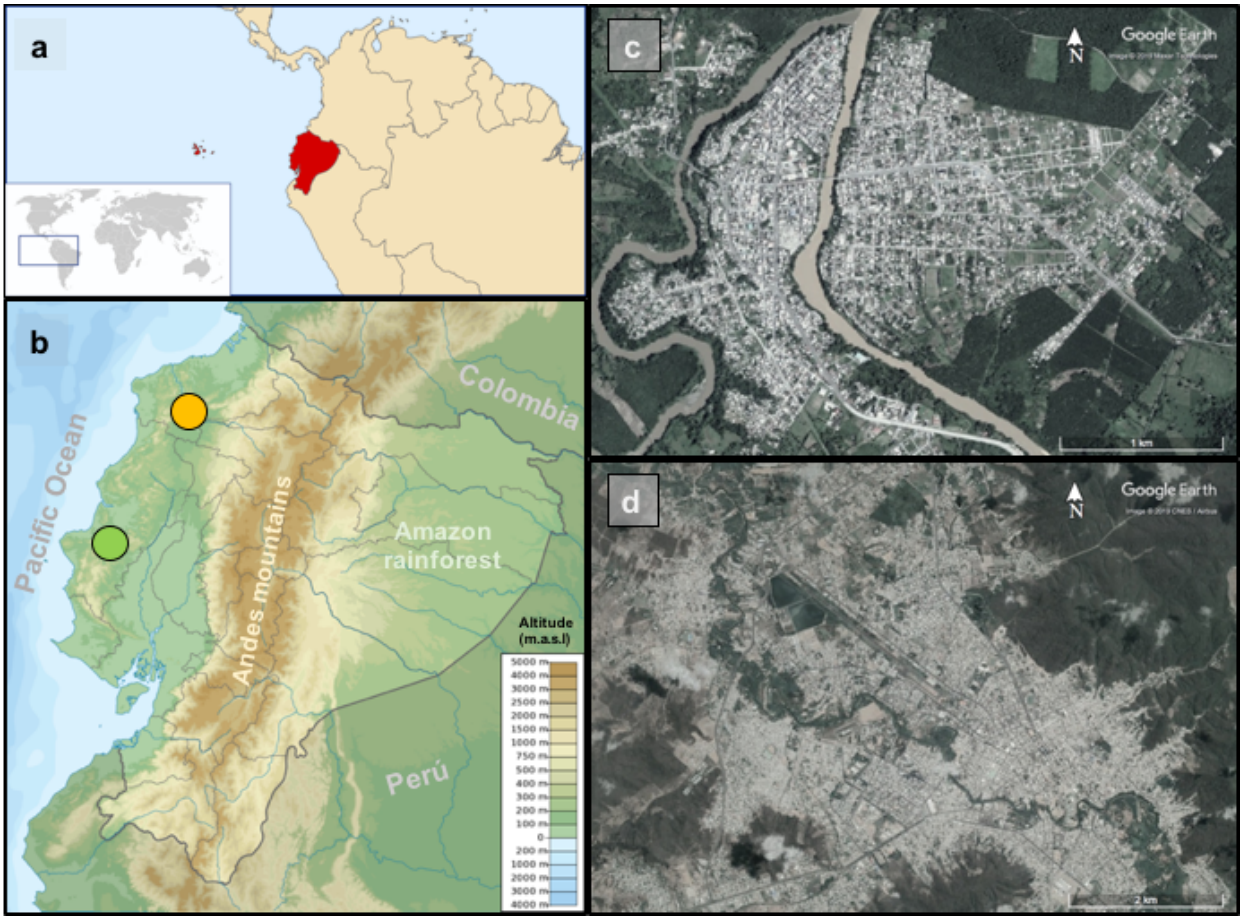

Figure 1. 2. Map study sites. (a) Location of Ecuador in the Americas highlighted in red (taken from [167]); (b) location of the two cantons where the study took place Quinindé (orange circle) and Portoviejo (green circle) situated in the Pacific Coastal region; (c) aerial view of the city of Quinindé, with scale set at 1Km; and (d) aerial view of the city of Portoviejo, with scale set at 2Km.

1.6.1. Climate of the study sites

Portoviejo sits at an altitude ranging between 30 and 150 m.a.s.l., while Quinindé's altitude ranges between 80 and 130 m.a.s.l. The Intertropical Convergence Zone (ITCZ) brings moist air from the Pacific Ocean towards the continent, which cools down as it rises with elevation and hits the western slopes of the Andes cordillera, causing precipitation in the Pacific coastal region [168]. The influence of the ITCZ marks the existence of only two seasons along the year in the coastal region. A wet, warmer season runs approximately from December to May, with an average monthly rainfall of 1600mm and approximately 26°C of mean daily temperature. While from June to November, a dry, cooler season

presents an average monthly rainfall of 450mm and approximately 24°C of mean daily temperature.

1.6.2. Epidemiological impact of arboviruses

Ecuador faces serious challenges with prevention and control of these arboviruses due to constraints found on laboratory capacity for diagnosis, healthcare, and trained personnel for vector surveillance and control [169–171]. In 2019, the Ministry of Health of Ecuador (MoH) developed the "Technical Policy for Vector Surveillance and Control in Ecuador" to serve as official guidelines for vector management in the country [172]. In this document, the MoH acknowledges the need to maintain permanent vector surveillance to identify areas of higher arboviral outbreak risk and direct vector control accordingly. However, this plan has had limited implementation due to human and financial resource limitations. Consequently, effective arboviral surveillance and control is limited by poor understanding and recent data on the ecology and distribution of *Ae. aegypti* within the major urban hotspots of arboviral transmission.

During the study period (2013-2018), the two study sites have experienced high incidence of arboviruses, with DENV occurring every year showing its highest incidence in 2015, and with the arrival of CHIKV in 2015 and ZIKV in 2016. During 2015, the year that DENV and CHIKV co-occurred in the area, Portoviejo and Quinindé reported 1,068.42 and 660.95 cases per 100,000 population of DENV, respectively; while for CHIKV, Portoviejo and Quinindé reported 1,853.88 and 529.49 cases per 100,000 population, respectively. The two cantons ranked within the 15 first places with highest incidence of these two arboviruses among the 52 countries and territories in the Americas [52,157]. For more details about disease burden in the two study sites, see Chapter 4.

As described further in the following chapters, a series of research and public engagement activities were conducted in these cantons throughout this project. This encompassed focalized surveillance of *Aedes* vectors within urban and peri-urban neighbourhoods (Chapter 2 and 3), and analysis of canton-wide disease incidence records spanning the 2013-2018 period. It is hoped that insights gained from these two settings can be applied more widely to other coastal urban settings in Ecuador and beyond.

CHAPTER 2: THE MOSQUITO ELECTROCUTING TRAP AS AN EXPOSURE-FREE METHOD FOR MEASURING HUMAN BITING RATES BY *AEDES* MOSQUITO VECTORS

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Ortega-López, L.D., Pondeville, E., Kohl, A. *et al*. The mosquito electrocuting trap as an exposure-free method for measuring human-biting rates by *Aedes* mosquito vectors. *Parasites Vectors* **13,** 31 (2020). https://doi.org/10.1186/s13071-020-3887-8 (Appendix 3).

2.1. ABSTRACT

Entomological monitoring of *Aedes* vectors has largely relied on surveillance of larvae, pupae and non-host-seeking adults, which have been poorly correlated with human disease incidence. Exposure to mosquito-borne diseases can be more directly estimated using human landing catches (HLC), although this method is not recommended for *Aedes*-borne arboviruses. We evaluated a new method previously tested with malaria vectors, the mosquito electrocuting trap (MET) as an exposure-free alternative for measuring landing rates of *Aedes* mosquitoes on people. Aims were to (i) compare the MET to the BG-sentinel (BGS) trap gold standard approach for sampling host-seeking *Aedes* vectors; and (ii) characterize the diel activity of *Aedes* vectors and their association with microclimatic conditions.

The study was conducted over 12 days in Quinindé (Ecuador) in May 2017. Mosquito sampling stations were set up in the peridomestic area of four houses. On each day of sampling, each house was allocated either a MET or a BGS trap, which were rotated amongst the four houses daily in a Latin square design. Mosquito abundance and microclimatic conditions were recorded hourly at each sampling station between 7:00–19:00 h to assess variation between vector abundance, trapping methods, and environmental conditions. All *Aedes aegypti* females were tested for the presence of Zika (ZIKV), dengue (DENV) and chikungunya (CHIKV) viruses.

A higher number of *Ae. aegypti* females were found in MET than in BGS collections, although no statistically significant differences in mean *Ae. aegypti* abundance between trapping methods were found. Both trapping methods indicated female *Ae. aegypti* had bimodal patterns of host-seeking, being highest during early morning and late afternoon hours. Mean *Ae. aegypti* daily abundance was negatively associated with daily temperature. No infection by ZIKV, DENV or CHIKV was detected in any *Aedes* mosquitoes caught by either trapping method.

We conclude the MET performs at least as well as the BGS standard and offers the additional advantage of direct measurement of *per capita* human-biting rates. If detection of arboviruses can be confirmed in MET-collected *Aedes* in future studies, this surveillance method could provide a valuable tool for surveillance and prediction on human arboviral exposure risk.

2.2. BACKGROUND

Arbovirus transmission to humans depends on multiple factors that involve spatial movement and immunity of human populations [173–175], socio-economic factors and access to basic services (especially water) [176,177], and the ecology and distribution of the mosquito vectors that transmit them [17,124,178]. These factors combine to determine the distribution and intensity of arboviral transmission, and generate often complex and highly heterogeneous patterns of exposure and infection [179,180]. As safe and effective vaccines for DENV, CHIKV and ZIKV viruses are not yet available [36,181,182], control of the *Aedes* mosquito vectors remains a primary strategy for reducing transmission [28,51,137].

Knowledge of where and when humans are at greatest risk of exposure to infected mosquito bites is vital for prediction of transmission intensity and effective deployment of vector control [183–185]. In the case of malaria, this information is used to estimate a time or site-specific “Entomological Inoculation Rate” (EIR); defined as the number of infected mosquito bites a person is expected to receive. This metric is usually derived from conducting Human Landing Catches (HLCs); a method in which a participant collects and counts the number of mosquito vectors landing on them over a given sampling period, then the sample is tested for the presence of a pathogen [186]. By providing a direct estimate of human exposure,

the HLC provides sensitive predictions of malaria transmission [184,187–189]. However, this method raises ethical concerns due to the requirement for human participants to expose themselves to potentially infectious mosquito bites [190]. In the case of malaria, this risk can be minimized by providing participants with prophylaxis [191]. However, such remediation is not possible for arboviruses where often no prophylaxis is available, and therefore HLCs are not recommended for the surveillance *of Aedes*-borne arboviruses (ABVs) [192,193].

Standard entomological monitoring for *Aedes* vectors is usually based on "exposure-free" surveillance of larvae or non-biting adults. This includes surveys of larvae or pupae in water containers [194,195], and collection of adult mosquitoes resting inside and/or around houses to indirectly estimate human-vector contact rates [194,196]. While such surveillance methods are useful for confirming vector abundance and distribution, they are poor predictors of epidemiological outcomes such as disease incidence and outbreak potential [155,197]. Consequently there is a need for vector sampling methods that can provide more reliable entomological indicators of arboviral transmission.

Human exposure to arboviral infection is likely best assessed by surveillance of "host seeking" (human-biting) *Aedes* mosquitoes. Several methods have used to sample host seeking *Aedes* including a variety of fan-operated traps that use visual attraction cues (*e.g.* Fay [198], the Fay-Prince trap [199], the black cylinder suction trap [200], duplex cone trap [201]) and lure-based traps. For the latter, artificial odours and attractants have been developed and tested for use in traps such as kairomone blends [202,203], BG-Lure® cartridges [204,205], and carbon dioxide (CO_2) [206]. Additionally other trapping methods have been developed that use live hosts as lures (*e.g.* animal-baited traps [207] and human-baited traps [208,209]. Only a few studies have directly compared such alternative trapping methods against the HLC with most being outperformed by the latter [208,209]. Out of all these methods, the BG-sentinel (BGS) trap has been demonstrated as one of the most effective and logistically feasible [210,211], and thus often considered a gold standard for *Aedes* surveillance [212,213]. In a range of trap evaluation studies, the BGS outperformed other methods for *Aedes* vectors with the exception of the HLC [214]. Despite these advantages of the BGS, its ability to accurately reflect the biting rates experienced by one person remains unclear.

Consequently, there is still a need for a safe alternative for direct assessment of human biting rates.

Recently, a new Mosquito Electrocuting Trap (MET) was developed as an exposure-free alternative to the HLC for sampling malaria vectors [215–217]. This trap was built on previous work using electrified nets and grids to trap tsetse flies [218,219] and mosquitoes [220,221] attracted to hosts or their odours. Similar to the HLC, this sampling method also uses human participants to lure mosquito vectors and trap them. However, the MET provides participants with full protection from mosquito bites so that no exposure is required. The MET consists of four squared-shaped electrocuting surfaces that are assembled around the legs of a host, with the rest of their body being protected by netting. Host-seeking mosquitoes are attracted towards the host by odour and heat cues as normal, but are intercepted and killed before landing. In previous trials in Tanzania, the MET matched the performance of the HLC for sampling malaria vectors in rural and urban settings [215–217]. This trap has also been used to assess host preference by baiting with human and livestock hosts [217], although it has not yet been evaluated for sampling *Aedes* vectors. If successful in this context, the MET could significantly improve ability to monitor and predict arboviral transmission by facilitating an exposure-free direct estimation of EIR.

This study reports the first evaluation of METs for sampling host-seeking *Aedes* vectors in a hotspot of DENV and ZIKV transmission in coastal region of Ecuador. This region is endemic for such arboviral diseases and has accounted for most of the cases reported in Ecuador. For instance, during the CHIKV outbreak in 2015, a total of 33,625 cases were reported in Ecuador, from which 96.02% was reported in the coastal region [222]. A similar pattern occurred during the ZIKV outbreak in 2016 and 2017, where approximately 98.49% of the cases were reported in this region from a total of 5,303 cases [223,224]. DENV has been reported every year in high numbers and considering 2016 and 2017, 84.78% of cases came from the coastal region from a total of 25,537 cases [224,225].

The objectives of this study were to: (1) evaluate the performance of the MET relative to the BGS trap for sampling host-seeking *Ae. aegypti* and other mosquitoes in the study area; and (2) use the MET to characterize the biting time

of *Ae. aegypti* and other relevant mosquito species and their association with microclimatic conditions. In addition, we took the opportunity to test for the presence of arboviruses in the collected *Aedes* females by both trapping methods to investigate arboviral transmission in the local area.

2.3. METHODS

2.3.1. Location and time of the study

This study was conducted in the neighbourhood of "Los Higuerones" (0°19'34"N, 79°28'02"W, 78 m.a.s.l), located in the city of Quinindé (Rosa Zárate) – Ecuador. This neighbourhood is located in an urban setting dominated by small, closely packed houses (Figure 2.1c), bordering the eastern side with the Blanco river (Figure 2.1d). Quinindé is located in the province of Esmeraldas, the northernmost province in the coastal region of Ecuador. During the 2015 outbreak of CHIKV, this province accounted with the highest disease burden in the country, with a total of 10,477 cases [222]. While for DENV, during 2016, Quinindé alone accounted for 52% of the cases within Esmeraldas province, with a total of 689 cases out of a total of 1,319. In 2017, the number of DENV cases in Quinindé was much lower compared with 2016, where only 87 cases were reported out of 334 in the province of Esmeraldas. Although there is a permanent incidence of arbovirus cases along the year, a higher incidence is usually reported during the first half of the year [177].

The study was carried out across 12 days in May 2017 (4^{th}- 12^{th}, and 16-18^{th}). On each day of the study, mosquito sampling was conducted over 12 hours, from 07:00 – 19:00 hours. Mosquito sampling was conducted within the peri-domestic area (garden/yard) of four households (Figure 2.1d). These houses were selected on the basis of being physically accessible, and having residents present and willing to participate during an initial tour of the area with a local guide. Houses were separated by approximately 90 metres from one another.

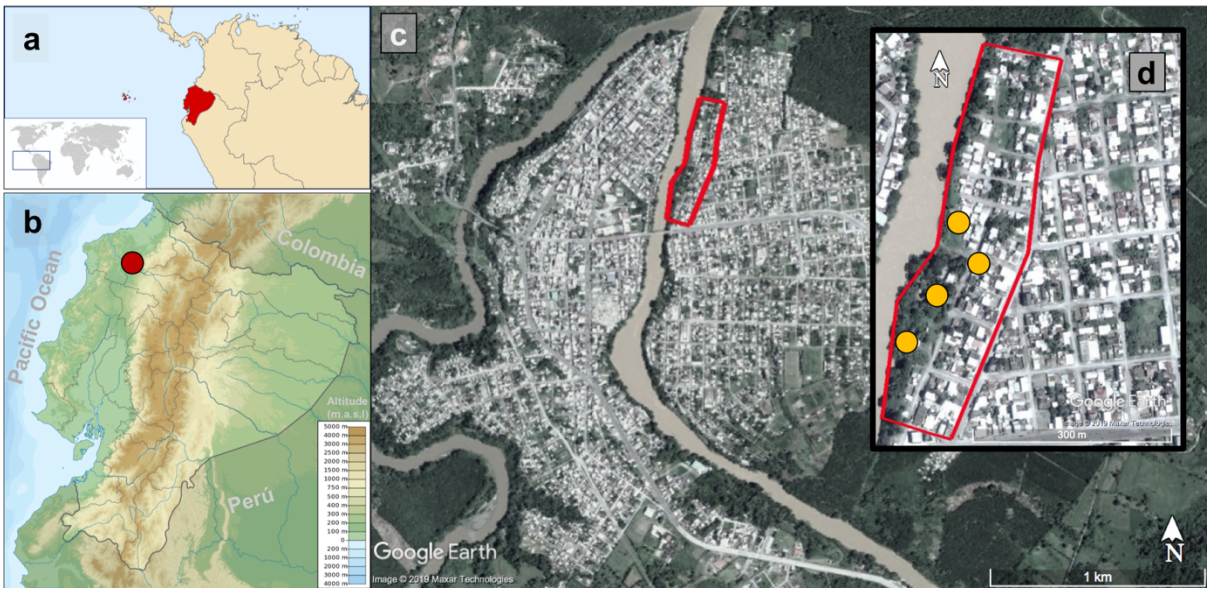

Figure 2. 1. View of the urban area of the city of Quinindé. (a) Location of Ecuador in the Americas highlighted in red (Taken from [167]). **(b)** Location of the city of Quinindé in the Pacific Coastal region, spotted by the red circle. **(c)** City of Quinindé showing Los Higuerones neighbourhood enclosed by the red line. **(d)** Enlarged view of Los Higuerones with the houses sampled spotted by the orange circles.

2.3.2. Trapping Methods

Over the study period, host-seeking mosquitoes were sampled by two different methods as follows:

BG- Sentinel trap (BGS)

The BG-Sentinel® trap (BioGents, Regensburg, Germany) is a white, cylinder shaped trap made of plastic with a gauze cloth covering the top and a hollow black cylinder in the top centre of the trap (Figure 2a). The trap operates with a 12-volt battery that powers an internal fan that produces inwards artificial air currents. In this study, each trap was baited with two BG-Lure® cartridges and a 1.4 litre cooler bottle filled with dry ice in order to maximize the attractiveness of traps to *Aedes*; as it is known that CO_2 increases the catch efficiency of BGS traps [210,211,226]. Mosquitoes are attracted towards the baited traps and then sucked

through the hollow black cylinder into an internal mesh bag that can be easily removed for posterior processing.

Mosquito Electrocuting Trap (MET)

The METs used here consisted of four 30 x 30 cm panels which were assembled into a box around the lower legs of a seated person (Figure 2b). Each panel was made up of stainless steel electrified wires set within a PVC frame. The wires were positioned 5mm apart, which is close enough so that mosquitoes could not pass through without making contact. Wires were vertically arranged in parallel, alternating positive with negative. When mosquitoes try to go through, contact is made and the voltage between wires kills them.

Mosquitoes attracted towards the volunteer were intercepted and killed on contact with these panels. The MET is powered by two 12-volt batteries connected in series to a power source giving a power output of approximately 6 watts (10mA, 600 volts). As an additional safety feature, a protective inner panel made from wide non-conductive plastic grid was fit into each frame preventing accidental contact between users and the electrified wires.

As an additional accessory to the MET, a retractable aluminium frame was built to cover the rest of the volunteer's body with untreated mosquito-proof netting. Thus volunteers were completely protected from mosquito bites during their participation in trapping. A plastic tarpaulin was erected over the MET station at a height of 2m top to protect users from direct rain and sunlight. Each MET was also set up on top of a white plastic sheet to isolate it from the ground and make it easier to see and collect shocked mosquitoes that fell onto the ground after touching the MET.

Figure 2. 2. Trapping methods used in this study. (a) Typical setting up of a BGS trap. **(b)** Technician baiting for the MET.

2.3.3. Experimental Design

Every day of the study, four traps (two METs and two BGS traps) were set up in the peri-domestic area of the four households (one trap per household) at the ground level under shade conditions. Traps were rotated among households each day, so that a different trapping method was used every consecutive day in each house. At the end of the study, this resulted in 6 days of trapping being conducted with each of the 2 methods at all houses.

MET collections were carried out by members of the research team, who were all adult men (30-50 years old). During each hour of the collection period, one member sat within the MET for 45 minutes, with the trap being turned off for the remaining 15 minutes to allow volunteers to take a break. Members of the study team took turns sitting in the trap so that different collectors lured every hour. During the 15-minute period when traps were turned off, mosquitoes were recovered from trap surfaces and the ground below using a pair of forceps, counted and placed in empty 15 ml falcon tubes; which were labelled with a unique code linked to the date, household ID, trap ID, hour period and collector ID. Tubes were stored in a cooler box of 45 L capacity filled with dry ice to kill, preserve and transport the specimens.

Each BGS was baited with two BG-Lure® cartridges on each day of sampling; with lures exchanged between the two BGS traps each day to minimize bias due to differential lure efficiency. BGS traps were further baited with carbon dioxide by adding one 1.2 L Coleman® polyethylene cooler bottle filled with dry ice. Dry ice containers were topped up every day. Like the MET, BGS sampling was conducted for 45 minutes of each sampling hour, with mosquito collection bags being checked and emptied during 15 minute break periods. Mosquitoes from BGS collection bags were emptied into pre-labelled plastic bags and transferred into a cooler box with dry ice to kill and preserve the mosquitoes.

Temperature and relative humidity data were collected every 10 minutes at each mosquito sampling point using TinyTag® Plus 2 TGP-4500 (Gemini Co., UK) data loggers. Data loggers at the BGS sampling stations were tied and hung inside each of the traps, and loggers at MET sampling points were placed on top of the bottom border of the netting frame, next to the MET.

2.3.4. Morphological Analysis

Mosquitoes collected in the field were transported to the Medical Entomology and Tropical Medicine Laboratory of the San Francisco de Quito University (LEMMT-USFQ) in cooler boxes filled with dry ice. At LEMMT-USFQ, mosquitoes were morphologically identified using taxonomical keys [227–229], counted and sorted into different cryo-vials according to date, household, trap type, hour of collection, species, sex and physiological status of females (blood fed/gravid and non-blood fed). All female *Ae. aegypti* specimens were retained for subsequent molecular analysis to test for the presence of ZIKV, DENV and CHIKV. These *Ae. aegypti* samples were grouped into pools of a maximum of 5 individuals.

2.3.5. Molecular Detection of Arboviruses

All pools of female *Ae. aegypti* specimens were screened for the presence of CHIKV, DENV and ZIKV. Details on the RNA extraction, reverse-transcription and PCR procedures are given in the Additional File 1 from Ortega-López et *al.* (2020) [230].

2.3.6. Data Analysis

Statistical analyses were performed in R 3.5.0 and R Studio 1.1.419. Generalized Linear Mixed Models (GLMM) were used to investigate variation in the abundance of host-seeking mosquitoes (per day and per hour) using package lme4 [231]. As mosquito abundance data was overdispersed, all models were fitted with a negative binomial distribution. For all response variables of interest as described below, model selection was carried out through a process of backward stepwise elimination from a maximal model using Likelihood Ratio Tests (LRT) [232].

Statistical analysis was performed for *Ae. aegypti*, and *Culex quinquefasciatus* as the latter was the only other mosquito species found in high abundance in the study area. *Cx. quinquefasciatus* is a nuisance biting mosquito and also a known vector of West Nile Virus (WNV) [233].

The BGS traps functioned continuously across all days and sampling hours. However, the METs stopped running during some sampling hours; generally under conditions of very high humidity due to rainfall which resulted in dampness on the traps and some temporary short circuiting (e.g. observed as plumes of smoke at the bottom junction with the frames). When these malfunctions occurred, the damaged traps were turned off and repaired. This resulted in variation in the total number of hours sampled with each trapping method (MET: 229 hours, BGS: 270 hours). This variation in sampling effort was accounted for in the statistical analysis. Days having less than 9 hours were excluded from the analysis.

Four models were built to assess variation in the abundance of each mosquito species and sex combination respectively. For each of these four response variables, a maximal model was constructed that included the fixed explanatory variables of sampling effort (total number of hours of collection), trap type (MET or BGS), daily mean relative humidity (%RH), and daily mean temperature (°C). In addition, the interaction between daily mean temperature with relative humidity was also included. Sampling day (1 through 12), household ID, trap ID and attractant ID (BG-Lure cartridge ID or MET volunteers ID) were included as random effects.

Mosquito biting activity was assessed through analysis of variation in the mean number of females (*Ae. aegypti* and *Cx. quinquefasciatus*) caught per hour. Here, each mosquito species was analysed separately. Each model included explanatory variables of trap type (MET or BGS), sampling hour, mean temperature (°C) per hour, mean relative humidity (%RH) per hour, and the interaction between hourly temperature and relative humidity. Sampling hour was defined as a continuous variable recoding the first hour of trapping (7-8 am) into 1, and increasing "hour" by one digit for each subsequent hour until 12 (17-18 hrs). Sampling hour was fit both as a linear and quadratic term; with the latter being used to test for peaks in biting time as have been previously reported for these mosquito species [110]. In addition, sampling day, trap ID, cluster ID, household ID (nested within cluster ID) and attractant ID (BG-Lure cartridge ID or MET volunteer ID) were fitted as random effects.

2.4. RESULTS

2.4.1. Mosquito species and abundance

During the 12 day-experiment, a total of five mosquito species were collected by both trapping methods (Table 2.1). *Cx. quinquefasciatus* was the most abundant species (78.6%) followed by *Ae. aegypti* (15.63%), and small number of *Aedes angustivittatus* (2.69%), *Limatus durhami* (2.33%,) and *Psorophora ferox* (0.15%). A small proportion of mosquitoes could not be identified (0.51%, Table 2.1). Overall, more mosquitoes were collected with the BGS trap (60.77%) than with the MET (39.23%), but the numbers of *Ae. aegypti* were relatively similar (Table 2.1).

Table 2. 1. Abundance of mosquito species collected by MET and BGS traps. Mosquito species abundances are split by sex and feeding status of females. The total sampling effort with the two METs was 229 hours, while for BGS traps was 270 hours over the 12 days of sampling.

	Mosquito Electrocuting Trap (MET)				BG-Sentinel (BGS) trap				
Species	♂	♀ unfed	♀ fed	**Total**	♂	♀ unfed	♀ fed	**Total**	**Grand total**
Aedes aegypti	100	99	19	**218**	93	91	27	**211**	**429**
Culex quinquefasciatus	496	238	44	**778**	960	345	77	**1382**	**2160**
Aedes angustivittatus	4	38	6	**48**	0	24	2	**26**	**74**
Limatus durhami	0	22	0	**22**	0	42	0	**42**	**64**
Psorophora ferox	0	1	2	**3**	0	1	0	**1**	**4**
Unknown	0	5	3	**8**	0	5	1	**6**	**14**
		Total MET:		**1077**		**Total BGS trap:**		**1668**	**2745**

In the BGS traps, some non-target insects including house flies, butterflies, crane flies, and many fruit flies were caught. No insect taxa other than mosquitoes shown in Table 2.1 were caught in MET collections.

The mean daily abundance of *Ae. aegypti* was approximately 2 females and 3 males for the BGS trap, and 4 females and 4 males for the MET, but no significant differences between trapping methods were found (Table 2.2, Figure 2.3a,b). The only significant predictor of daily abundance of females *Ae. aegypti* was temperature, which exhibited a negative association (Table 2.2, Figure 2.4a). Similarly, the mean daily abundance of *Cx. quinquefasciatus* females did not significantly differ between trapping methods (Table 2.2, Figure 2.3c,d), however confidence intervals (especially for males) around estimates were very large,

indicating that larger sample sizes may be required to robustly test if there were differences between trap types. The number of female *Cx. quinquefasciatus* per day varied between 16 and 207; with variation being even more pronounced for males where a high of 576 was caught on one day. The daily abundance of female *Cx. quinquefasciatus* was negatively associated with daily temperature (Table 2.2, Figure 2.4b) and positively associated with the number of hours sampled in a day, while no significant differences were found in *Cx. quinquefasciatus* regarding any covariate (Table 2.2).

Table 2. 2 Summary table of statistical significance of terms tested from mosquito daily abundance. Chi-square (X^2), degrees of freedom (df) and p-values (p) are provided for each sex within species.

Explanatory variables	*Aedes aegypti*						*Culex quinquefasciatus*					
	Males ♂			Females ♀			Males ♂			Females ♀		
	X^2	df	*p*	X^2	df	*p*	X^2	df	*p*	X^2	df	*p*
Sampling effort	3.38	1	0.07	1.95	1	0.16	0.31	1	0.58	15.91	1	<0.001*
Trap type	2.18	1	0.14	0.60	1	0.44	0.95	1	0.33	1.5	1	0.22
Temperature	0.22	1	0.64	4.62	1	0.03*	0.06	1	0.8	6.86	1	<0.01*
Relative Humidity	1.14	1	0.29	2.17	1	0.14	1.23	1	0.27	1.1	1	0.29
Temperature :: Humidity [§]	2.22	1	0.14	1.24	1	0.26	1.07	1	0.3	1.27	1	0.26

* Significant values

[§] Fixed effect indicating interaction term

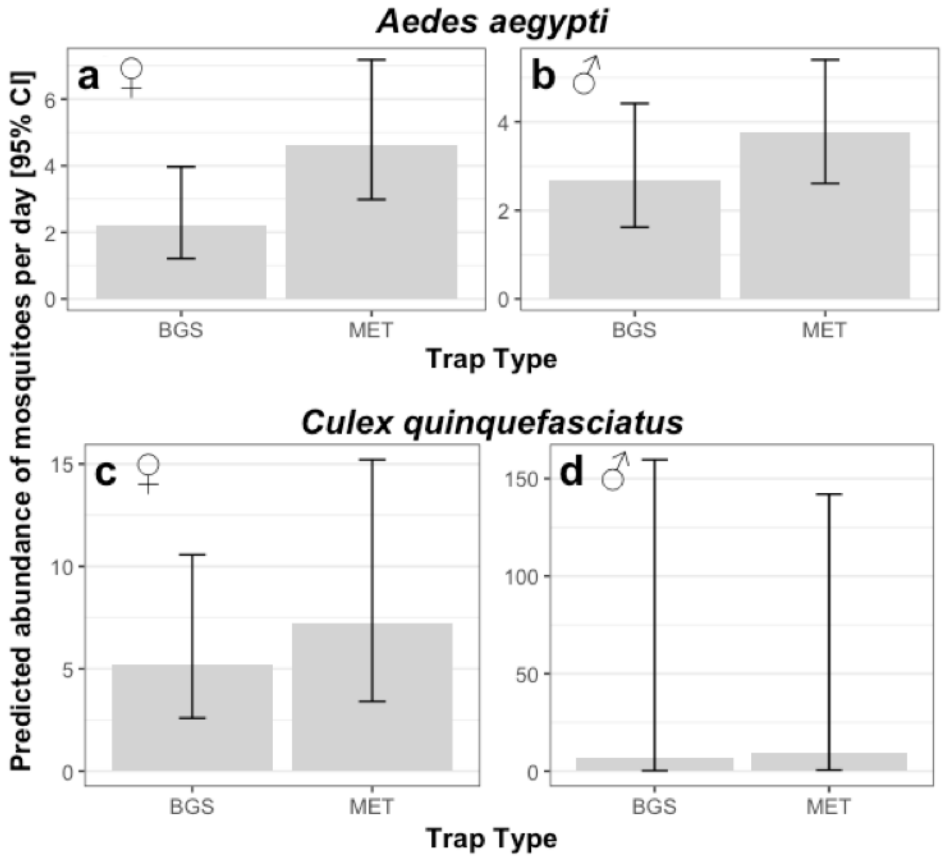

Figure 2. 3. Predicted mean daily abundance of mosquitoes caught with different trapping methods. The upper panels show values for *Ae. aegypti* and the lower panels *Cx. quinquefasciatus*. Panels on the left show data for females (♀) and on the right for males (♂). Error bars indicate the Confidence Intervals (C.I.) at 95%.

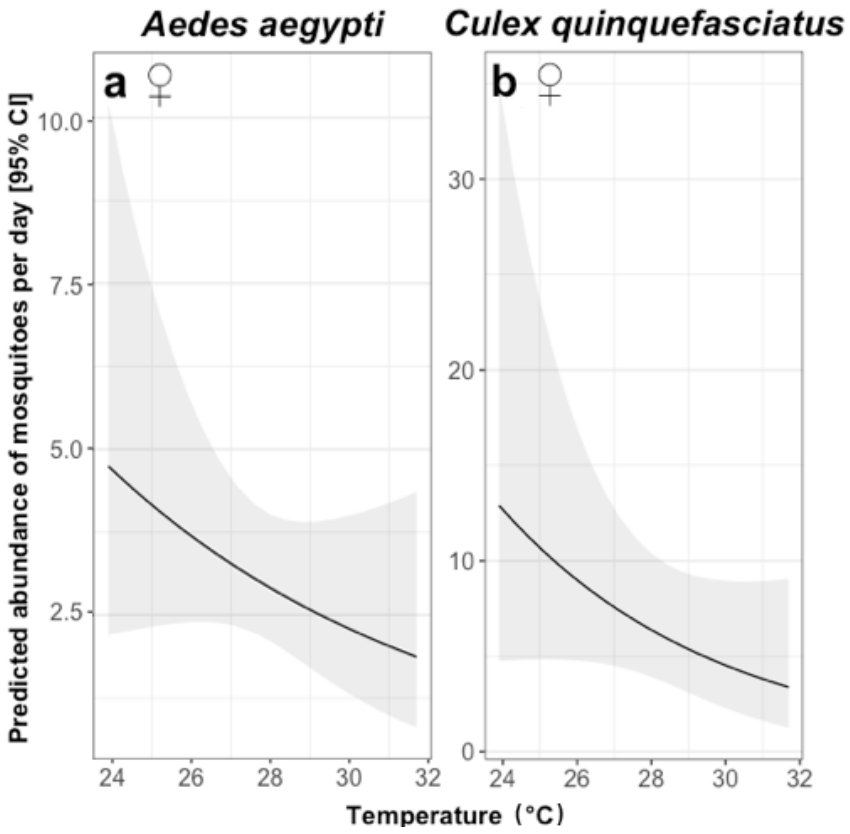

Figure 2. 4. Predicted relationship between mean temperature and number of female mosquitoes collected. Panel **(a)** shows *Ae. aegypti* and **(b)** shows *Cx. quinquefasciatus* females. The black line indicates the mean predicted abundance and the shaded area the Confidence Intervals (C.I.) at 95%.

2.4.2. Mosquito biting activity

Hourly mosquito catches recorded for BGS and METs were used to characterize the biting activity of female *Ae. aegypti* and *Cx. quinquefasciatus*. Variation in the hourly biting activity of female *Ae. aegypti* was best explained by a quadratic association between hourly mosquito abundance and time (Table 2.3), with activity being highest in the early mornings and late afternoon, and little activity during the middle of the day (Figure 2.5a). After taking this hourly variation in biting rates into account, there was no additional impact of trapping method of the number of female *Ae. aegypti* collected per hour (Table 2.3, Figure 2.6). Variation in the hourly biting activity of *Ae. aegypti* was also significantly associated with an interaction between temperature and relative humidity (Table

2.3). This interaction arose because the number of *Ae. aegypti* caught per hour was negatively associated with temperature under conditions of low relative humidity; but the strength of this association was lower as humidity increased (Table 2.3, Figure 2.7), although temperature and humidity were strongly associated (Figure S1, please see additional file from Ortega et *al*. [230]).

The biting activity of female *Cx. quinquefasciatus* also varied significantly across the sampling day. As with *Ae. aegypti*, this pattern was characterized as a quadratic relationship in which mosquito activity peaked during the early morning and late afternoon (Table 2.3, Figure 2.5b). Accounting for this activity pattern, there was no difference in the number of *Cx. quinquefasciatus* caught per hour in different trapping methods (Table 2.3, Figure 2.6b), and no association with temperature or humidity.

Table 2. 3. Summary table of statistical significance of terms tested for association with female mosquito hourly abundance. Chi-square (X^2), degrees of freedom (df) and p-values are provided for females of each species. "N/A" indicates "not applicable" values for which single term significance was not possible because of their involvement in significant higher order terms.

Explanatory variables	*Aedes aegypti* - Females ♀			*Culex quinquefasciatus* - Females ♀		
	X^2	df	*p*	X^2	df	*p*
Trap type	0.60	1	0.44	7e-04	1	0.98
Time (linear)	N/A	N/A	N/A	N/A	N/A	N/A
Time (quadratic)	8.70	1	<0.01*	142.1	1	<0.001*
Temperature	N/A	N/A	N/A	2.07	1	0.15
Relative Humidity	N/A	N/A	N/A	0.09	1	0.77
Temperature :: Humidity §	6.60	1	0.01*	0.09	1	0.76

* Significant values

§ Fixed effect indicating interaction term

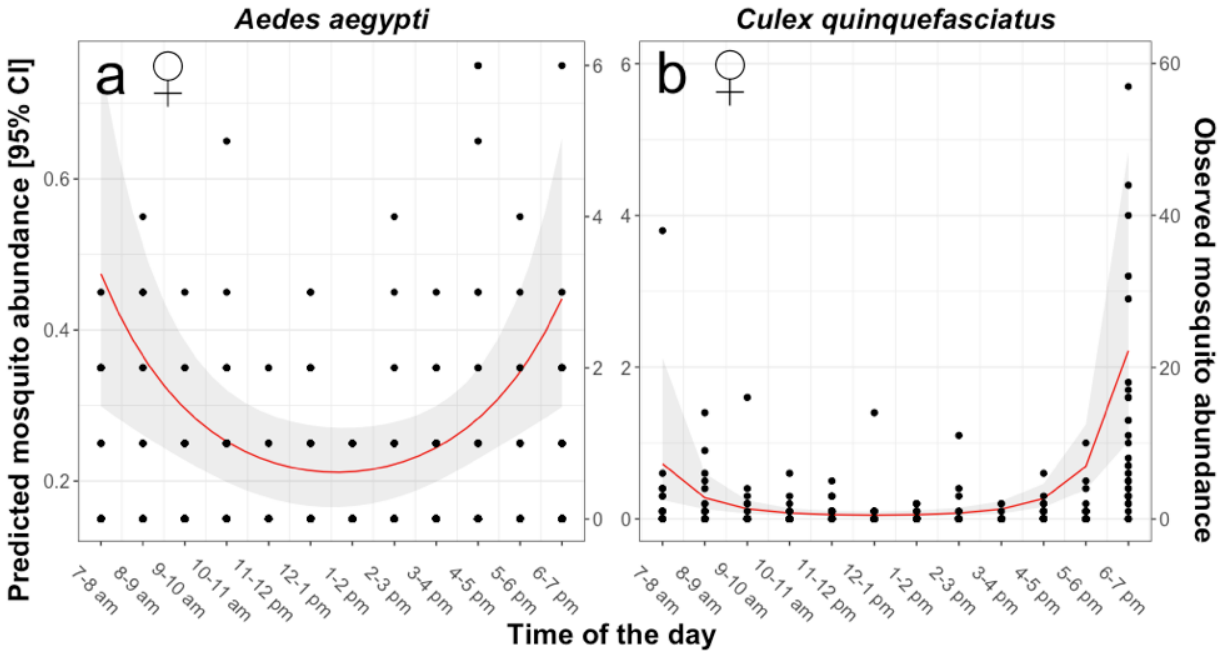

Figure 2. 5. Predicted abundance of biting mosquitoes between 7:00-19:00 hrs. Panel **(a)** indicates *Ae. aegypti* females and **(b)** *Cx. quinquefasciatus* females. Dots represent the observed values which correspond to the right Y axis. The red line corresponds to the predicted mosquito abundance and the shaded area to the Confidence Intervals (C.I.) at 95%; both correspond to the left Y axis.

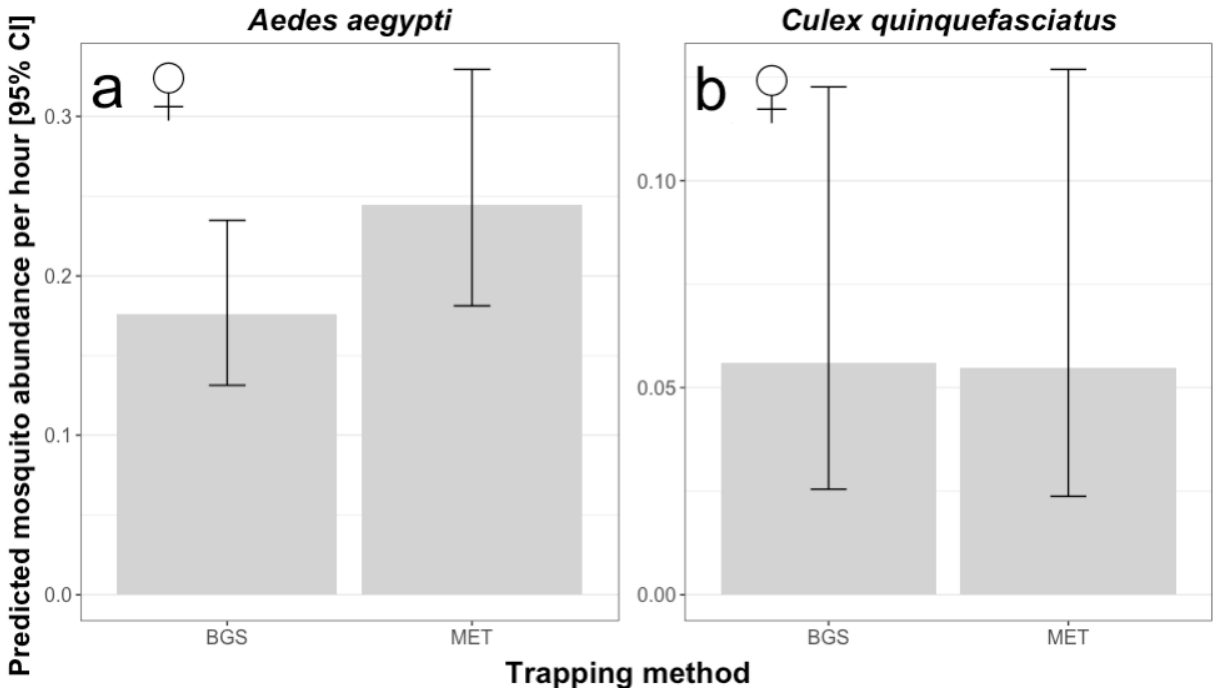

Figure 2. 6. Predicted hourly abundance of mosquitoes using different trapping methods. Panel **(a)** represents *Ae. aegypti* and **(b)** *Cx. quinquefasciatus*. The error bars indicate the Confidence Intervals (C.I.) at 95%.

2.4.3. Molecular screen for ZIKV, DENV and CHIKV

Aedes aegypti females were tested for ZIKV, DENV (1 – 4) and CHIKV and none of the samples were found positive. For a detailed description on the molecular results, please see Additional File 1 from Ortega-López et *al.* (2020) [230].

2.5. DISCUSSION

Identifying an accurate method to predict the exposure of humans to infected mosquito vectors has been an enormous challenge for *Aedes*-borne pathogens [138,234]. Here, we present the MET as a potential alternative for safe measurement of *Aedes* landing rates on humans. When tested in Ecuador, the MET provided similar estimates of *Ae. aegypti* abundance and biting activity as the current gold standard BGS sentinel method. While the BGS uses artificial odour baits and carbon dioxide (CO_2) to lure mosquitoes into a standardized trap; the MET directly estimates the number of *Aedes* host-seeking within the immediate

vicinity of a real host. The MET can also be used to measure biting rates on a range of different host species (e.g. [217]); which currently the BGS and other methods cannot do. The standardization provided by the BGS makes it easy and effective to use in widescale surveillance [212,214], although a limitation is that non-biogenic CO_2 sources are not always available [235]. However, the degree to which BGS collections accurately reflect *per capita* human biting rates is unclear. For example, BGS trapping efficiency may vary with the type and number of lures used, rate of $C0_2$ released (quantity per time), location and colour of the trap (e.g. BGS 1 and BGS 2) [202,210,236], making it difficult to infer how different variants translate into exposure experienced by one person in that environment. An advantage of the MET is that it is more directly analogous to the human landing catch in sampling mosquitoes in the process of host seeking on a person and also estimate variability in attraction between individuals. This could also be seen on the total catches of the other mosquito species when compared to the total numbers trapped by the BGS. The MET could thus provide a useful supplementary surveillance method for estimation and validation of human biting rates and the associated entomological inoculation rate (EIR).

By facilitating a safe and more direct estimation of the EIR for *Aedes*-borne viruses, the MET could provide robust and precise entomological indicators of transmission intensity [215–217]. Such indicators are much needed to understand heterogeneity in transmission [155,237,238], and evaluate the efficiency of vector control interventions. However this relies on the assumption that the MET accurately reflects the true *Aedes* exposure of one person per unit of time. Estimates of human exposure to the malaria vector *An. gambiae s.l.* from the MET were similar to those of the human landing catch in some studies [217,239]; whereas in others mosquito abundance was underestimated by the MET compared to the HLC [216]. Here it was not possible to directly compare the MET to the HLC because of ethical restrictions in using the latter in an area of high arboviral transmission. However we speculate that one factor that could cause the MET to underestimate *Aedes* vectors biting rates is the area of the body protected. Whereas African *Anopheles* vectors generally prefer feeding on the lower legs and feet [240–242]; it is not clear if *Aedes* prefer to bite on specific parts of the body [243,244]. As a next step in validating this approach, we recommend the MET to be directly compared to the HLC under controlled conditions with uninfected

Aedes vectors (*e.g.* semi-field experiments), ideally using a defined *Ae. aegypti* strain and appropriate experimental design to act as a reference standard for future comparison.

Both the MET and BGS trap sampled a similar composition of mosquito species in the study period. However, estimates of the mean daily and hourly abundance of *Ae. aegypti* and *Cx. quinquefasciatus* were slightly but not statistically higher in MET than BGS collections. The relatively short period of this (12 sampling days) may have limited power to detect for minor-moderate differences between trapping methods. We thus conclude the MET is at least as good as the BGS gold standard for sampling host-seeking *Aedes* vectors in this setting, but also recommend further longer-term comparisons over a wider range of seasons, sites and participants to evaluate whether the MET outperforms the BGS. If we assume that MET is equivalent to HLC, these results are also consistent to those shown by Kröckel *et al.*, who also observed that HLC captured more mosquitoes, although not statistically different from the BGS [214].

Mosquito collections conducted here were also used to test for associations between *Aedes* host-seeking activity and microclimatic conditions. The impact of temperature and humidity on the life-history, physiology, behaviour and ecology of *Ae. aegypti* has been extensively investigated under laboratory conditions [125,245–247]. However, relatively little is known about how microclimate impacts the diel host-seeking behaviour of wild *Aedes*. In general, the host-seeking activity *Ae. aegypti* and *Cx. quinquefasciatus* was higher on days when mean temperatures were lower (across range of 25°C to 30°C). Additionally, the hourly biting rates of *Aedes* were negatively associated with temperature but only under conditions of low humidity. As mean hourly temperatures were strongly negatively correlated with relative humidity (Figure S1), these results indicate that *Ae. aegypti* biting activity is highest during relatively cool and humid hours of the day. These microclimatic associations may account for the observed biting activity of *Ae. aegypti* and *Cx. quinquefasciatus*. A comprehensive review [110] of *Ae. aegypti* biting behaviour indicates that bimodal and trimodal activity patterns are often reported, with evidence of specific adaptations to other ecological features (*e.g.* artificial light availability) [110]. Such variability seems to be common and

related to optimal humidity and temperature conditions available during such hours [248,249].

A key feature of any method for estimating EIR is its ability to estimate human biting rates and infection rates in mosquitoes. While the results here presented indicate that the MET could be used to estimate the human biting rates, the infection rates could not be measured as none of the *Aedes* mosquitoes collected with either trapping method were positive for arboviruses. Reported rates of arboviruses in *Aedes* vectors are generally very low (0.1% to 10%) even in high transmission areas (*e.g.* [250–257]). Thus failure to detect arboviruses within the relatively small sample size of vectors tested here (*e.g.* 207 individuals tested in 122 pools) is not unexpected.

Although promising, the MET has a number of limitations relative to the BGS for sampling host seeking *Aedes*. First, although both trapping methods require a power supply, the current version of the MET requires two 12-volt batteries compared to the one required by the BGS), requires human participants and the trap itself is heavier, which is more labour intensive than using BGS. Also, as the METs used here are still research prototypes produced on a bespoke basis without a licenced manufacturer, their production cost is currently more expensive than BGS traps (approximately £650 versus £170 per trap, respectively). In addition, some technical problems were experienced including a tendency to short circuit under conditions of high air humidity. These limitations are expected to be improved if manufactured at scale as manufacturing costs would fall and technical improvements should make the MET suitable for humid environments. The primary advantage of the MET is therefore, its potential ability to directly estimate the EIR for arboviral infections. This advantage could be leveraged to calibrate other existing trapping methods that are less labour intensive and more feasible to be deployed at large scale. Additionally, the MET could be used in combination with other trapping methods to identify hot spots of transmission before large scale deployment with other traps is carried out.

2.6. CONCLUSIONS

Here we evaluated the MET as a tool for estimating human biting rates of the arboviral vector *Ae. aegypti* in a high transmission setting in coastal Ecuador. The MET performed at least as good as the current BG-Sentinel trap gold standard for estimating the mean abundance per hour of host-seeking *Aedes*, and provided a realistic representation of hourly activity patterns. We conclude MET is a promising tool for *Ae. aegypti* and other mosquito species surveillance, which could uniquely enable a relatively direct estimate of the arboviral entomological inoculation rate experienced by communities.

CHAPTER 3: RESTING SITE BEHAVIOUR AND ARBOVIRUS PREVALENCE OF *AEDES AEGYPTI* POPULATIONS ACROSS TIME AND URBANIZATION GRADIENTS IN TWO TRANSMISSION HOTSPOTS IN COASTAL ECUADOR,

3.1. BACKGROUND

As described in Chapter 1, dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) virus have placed a huge public health burden on countries in the Americas. A common feature of DENV, CHIKV and ZIKV in this continent is their reliance on *Aedes aegypti* for transmission. Consequently, *Aedes* vector control combined with entomological and epidemiological surveillance, and raising public awareness have been the main strategy to limit arbovirus transmission [150,258,259]. As vector control practices have been demonstrated to be more effective when their application is sustained over time rather than as an emergency response to an epidemic [150,260], sustained vector surveillance is recommended to detect and respond to potential arbovirus outbreaks [260].

Entomological surveillance can guide vector control programmes by directing efforts to areas and periods of highest vector abundance and/or human exposure risk. Most *Aedes* surveillance systems measure presence and abundance of larvae, pupae and adult mosquitoes as indices of entomological risk [155,194]. However, due to the complexity of arbovirus transmission dynamics, most standard *Aede*s indicators are poorly correlated with epidemiological outcomes such as human infection or disease incidence [155,197]. Consequently, the epidemiological consequences of vector control programmes may be difficult to anticipate [261]. However surveillance of adult female *Aedes*, the only life stage capable of transmission, provides the closest link to arboviral transmission [262]. Detailed monitoring of adult female *Aedes* populations including their seasonal dynamics, geographical distribution, behaviour, and arbovirus infection rates is thus the best currently available indicator of the transmission potential of local mosquito populations and associated human exposure [139,258,259].

Aedes aegypti populations are known to be highly heterogeneous in time and space; with distribution and abundance associated with climate, land cover, human host density, water-breeding sites availability, and socio-economical factors, among others [263–267]. In general, *Ae. aegypti* are highly associated with urban environments in tropical countries, where poor water and waste disposal infrastructure provide ample breeding sites [268]. Although primarily urban, this species can also occur in peri-urban and rural areas in some settings [26,27,118–120,269]. Other mosquito species are more abundant in less populated areas (e.g. peri-urban and rural areas), and may generate interspecific competition that limits *Ae. aegypti* together with a reduced density of human hosts (given the anthropophilic host preference of this species) [27,118–120,269]. Within urban centres, *Ae. aegypti* are also highly heterogeneous [26,119,270]; often resulting in concentrated clusters at the household or neighbourhood level [271,272]. Clusters tend to be up to 30 metres away from their centre [271,272], matching with the known flight range of *Ae. aegypti* [207,273]. Such local heterogeneity is important for implementation of vector control. For example, as large-scale vector control across an entire city is time and resource consuming, focalized targeting at the neighbourhood or household level may be more cost effective [274]. Investigation of fine-scale variation in *Aedes* abundance within urban settings and its association with local environmental factors may thus guide to a more efficient vector control [270,272,275].

In addition to their abundance, arboviral infection rate in adult females *Aedes* can be a useful predictor of arbovirus outbreak potential and human exposure risk [150,276–278]. Molecular techniques such as PCR can provide rapid, sensitive and specific detection of particular arboviruses [279]. These virus-specific approaches can be combined with next generation sequencing for detection and molecular characterization of other untargeted arboviruses that may be co-circulating among vector populations [276,280,281]. Such data could be used to establish phylogenetic relationships between the detected arboviruses and their ancestral isolates, and relate them in terms of timing and routes of introduction [282].

As mentioned in Chapter 1 (Section 1.6.2), a lack of understanding of *Ae. aegypti* behaviour (resting and host seeking) and population dynamics (spatial and temporal distribution), hampers an adequate strategy for an effective vector

control at the national scale. To address this gap, this study investigated the distribution, abundance, behaviour (indoor versus outdoor resting) of adult *Ae. aegypti* population within two coastal cities of Ecuador where arboviral transmission is high. Aims were to (1) measure temporal variation in *Ae. aegypti* populations across six months during the wet season in coastal Ecuador, (2) assess variation in *Ae. aegypti* abundance between urban and peri-urban neighbourhoods (3) investigate resting behaviour preference of *Ae. aegypti* between indoor and outdoor areas, (4) measure arbovirus prevalence in *Ae. aegypti* females and assess their phylogenetic relationship with other South American arbovirus sequences. The study coincided with the tail end of a major ZIKV outbreak that occurred throughout the Americas in 2015-2017; which captured the arrival of ZIKV into Ecuador in 2016 and 2017. In addition to assessing the transmission potential of these Ecuadorian vector populations, viral detection in mosquitoes was conducted with the aim of assessing the origin of newly arrived ZIKV strains.

3.2. METHODS

3.2.1. Study sites and period of study

The study was carried out in two cantons in coastal Ecuador: Portoviejo (1.0°S, 80.4°W), province of Manabí, and Quinindé (Rosa Zárate) (0.3°N, 79.4°W), province of Esmeraldas (Figure 1). Details of these study sites are given in Section 1.6.

The study was designed to sample *Aedes* vectors at households both within urban and peri-urban neighbourhoods in each canton. Neighbourhoods are not officially defined by the canton councils but rather take their definition and delimitations from a cultural perspective and historical belonging from the local communities [283]. Neighbourhoods categorized as “urban” were characterized by having households in a row-housing arrangement, usually organised in blocks surrounded by paved streets. Houses in these neighbourhoods usually lacked open outdoor spaces like internal yards or gardens, and if present, these spaces were surrounded by walls. In contrast, neighbourhoods characterized as “peri-urban” were characterized by having fully detached houses scattered throughout the area, usually accessed by one main earthen road entering the neighbourhood. Wide lawn

lots, crops and gardens surrounded the houses and properties were usually not limited by walls. Therefore, free movement of domestic animals between the properties was possible in these areas unless fences were present.

Mosquitoes were sampled in these cites over four collection periods between November 2016 to April 2017; coinciding with the tail end of the South America-wide ZIKV outbreak which started in 2015 and arrived in Ecuador in early 2016, with a second outbreak in early 2017. Collection periods were approximately 45 days apart from each other, with each consisting of three consecutive days of sampling in each of the two cities. The first collection period in November fell within the dry, cooler season, while the other three periods occurring in the wet, warmer season.

3.2.2. Trapping methods

Mosquito collections were carried out using two methods that target different subgroups of the adult *Aedes* population. First, BG-Sentinel® traps (BGS) were used to target host seeking mosquitoes (BioGents, Regensburg, Germany). Additionally, Prokopack (PPK) aspirators [196] were used to collect mosquitoes resting inside on house walls and ceilings, or in the surrounding outdoor peri-domestic area. The resting mosquito population usually consists of males and recently blood fed females. Both collection methods were used to sample adult mosquitoes during daytime hours (between 9:00 and 18:00 hrs) to coincide with the known pattern of diurnal host seeking in *Ae. aegypti* [111].

The BG-Sentinel® (BGS) trap is a white, cylinder shaped trap that attracts mosquitoes using visual clues and lures (Figure 3.2a). The trap works with a 12-volt battery to power a fan that propels the air inwards and thus pulls approaching mosquitoes inside where they are trapped in an internal collection bag. These traps can be baited with artificial odour lures and carbon dioxide emanators to imitate a vertebrate host, thus attracting adult female mosquitoes who are searching for a blood meal [214]. In this study, each BGS trap was baited with one BG-Lure® cartridge, and a Coleman® polyethylene cooler bottle (1.2 L capacity) placed inside the trap, which contained dry ice that released carbon dioxide as it

evaporated, as this is known to increase the attractiveness to *Aedes* mosquitoes [210,211,226].

Prokopack® aspirators (John W. Hock, Gainesville, USA) are handheld aspirators that can be attached to a 2 metre extendable pole that can be used to reach ceilings and the upper reaches of walls, as well as lower areas (Figure 3.2b). These aspirators are powered by a 12-volt battery that is held in a backpack worn by the user while sampling. The aspirator has an internal fan that pulls air inwards so that insects are sucked into a collection cup on the tip of the nozzle.

Figure 3. 1. Trapping methods used in this study. (a) Typical set up of a BGS trap. (b) Technician aspirating with a Prokopack aspirator.

3.2.3. Experimental design

In each of the two cities, four peri-urban and four urban neighbourhoods were identified for mosquito sampling. Selection of the neighbourhoods was based on informal recommendations from officers from the local Ministry of Health office in each canton, based on areas where cases of DENV had been previously reported, where *Ae. aegypti* was known to be present from previous surveys, and that were considered safe and accessible for the study team to work in.

On each collection trip, mosquitoes were sampled from 24 households per canton, 3 from each of 8 neighbourhoods (Figure 3.2), with different households sampled on different collection trips from the same neighbourhoods. Half of the neighbourhoods were classified as peri-urban, and half as urban; thus 12 households in each category were sampled on each trip. Thus over the course of the study period (4 collection trips per canton) a total of 192 households were sampled. On the first day of each collection period, the study team walked through each neighbourhood with a local guide to identify households for mosquito sampling. These were selected on the basis of having an outdoor area for peri-domestic sampling (garden/yard), residents reporting mosquito nuisance or recent arbovirus infection, and the willingness of residents to participate in the project.

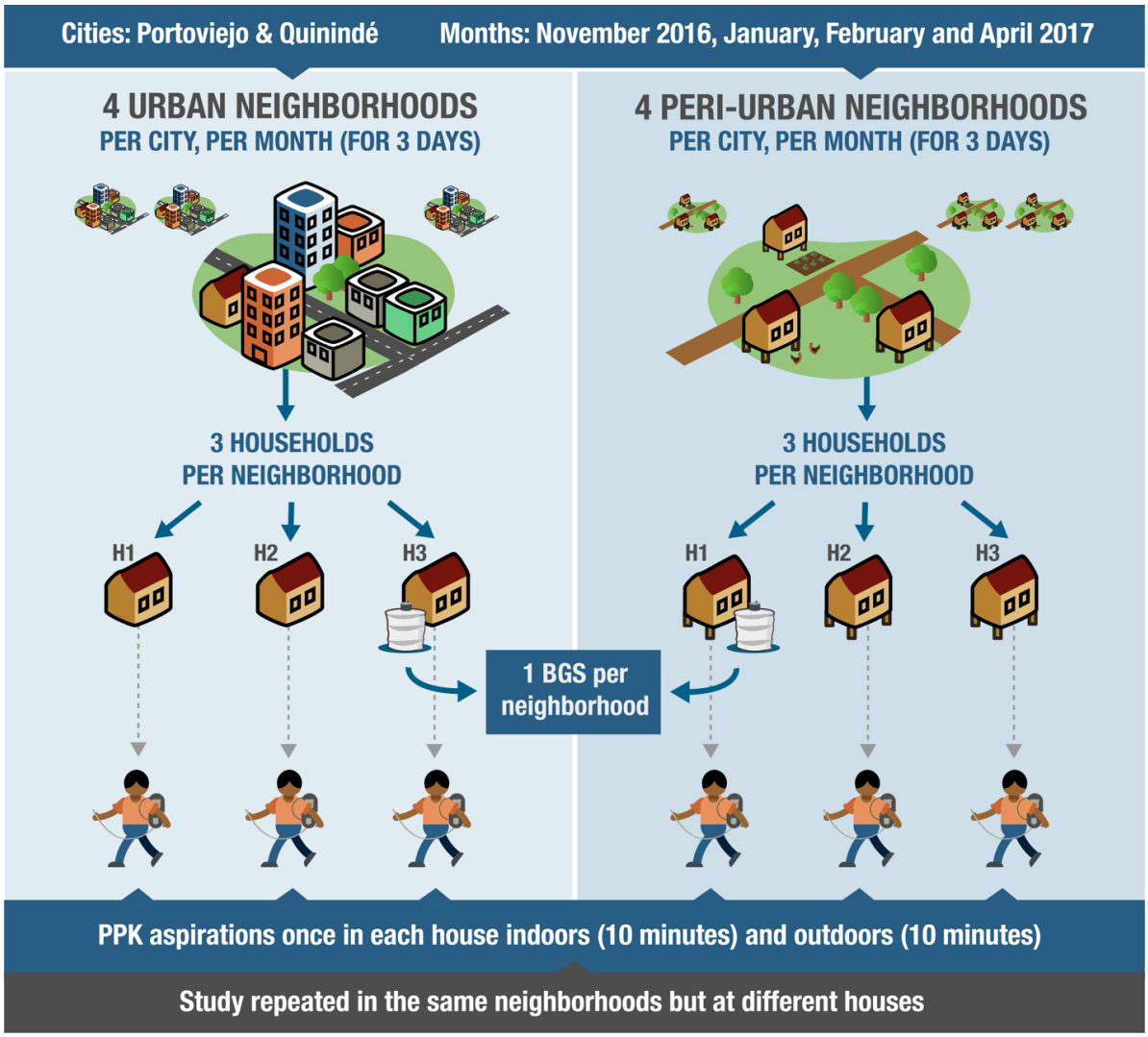

Figure 3. 2. Experimental design. Schematic diagram of the experimental design used to sample mosquitoes from two cantons in Ecuador, Portoviejo and Quinindé, across 4 collection periods: November 2016, January, March and April 2017. The study took place in 4 urban and 4 peri-urban neighbourhoods at each canton. Three households (H1, H2, and H3) were sampled from each neighbourhood with different houses sampled on each of the 4 collection periods. (total of 12 households per neighbourhood over all 4 sampling trips).

Within the three houses selected in each neighbourhood per field trip, one house was allocated for sampling with a BGS trap (daily collection for three consecutive days from the same house, Figure 3.2). This selection was based on finding an appropriate place on the property where the trap would be protected from rain

and theft. BGS traps were set between 8 am and 10 am, with traps being turned off between 5 pm and 7 pm each day. The running time of each trap was recorded, with the mean BGS sampling period being 9 hours per day. The BG-Lure® cartridges used as bait were randomly rotated each day with those that were used in the other BGS traps. Dry ice from the cooler bottles was replenished every morning before the start of sampling. Mosquitoes were recovered from the traps at the end of each day by removing the mesh collection bag and placing it into a plastic zip-lock bag and storing it in a cooler with dry ice to kill mosquitoes. After freezing, collection bags were emptied and mosquitoes transferred into 15 ml falcon tubes, pre-labelled with a unique code related to the sampling unit (i.e. trap type, date, trapping time, canton, neighbourhood type (urban/peri-urban), neighbourhood name, and house ID number) and stored in a cooler with dry ice for laboratory processing.

Prokopack® aspirators were used to sample mosquitoes resting indoors and in peri-domestic areas at all households. Sampling was conducted by two technicians for a standard time of 10 minutes both inside and outside of the house. One technician was randomly assigned to carry out aspiration indoors, and the other outdoors. Indoor aspirations were done by moving the aspirator nozzle along the walls, ceiling and under the furniture until the 10-minute time limit was reached. Outdoor aspiration was carried out by aspirating along the outer sides of the walls, in external facilities like outdoor toilets, storage piles, garages, and laundry washing basins. After each aspiration, mosquitoes were recovered from the Prokopack collection cup with a mouth aspirator and then transferred to a 15 ml falcon tube, which was placed in a cooler filled with dry ice to kill and store mosquitoes for laboratory processing. Falcon tubes were pre-labelled with a unique collection code similar to the format used for the BGS collections (i.e. trap type, collector ID, date, trapping time, canton, neighbourhood type, neighbourhood name, house ID number, and area of the house (indoor/outdoor)).

3.2.4. Environmental data

Microclimate data were recorded using TinyTag® Plus 2 TGP-4500 (Gemini Co., UK) data loggers. Loggers were tied and hung inside each BGS trap during sampling periods. Measurements of air temperature and relative humidity were taken by

loggers every 15 minutes and used to calculate the mean value per day. Macro climate data was obtained from the National Institute of Meteorology and Hydrology of Ecuador (INAMHI), which provided daily temperature and precipitation data from meteorological stations in Quinindé (meteorological station M0156; 0.316°N, 79.469°W, 109 m.a.s.l.) and Portoviejo (meteorological station M1208; 1.164°S, 80.390°W, 60 m.a.s.l.).

3.2.5. Mosquito processing and molecular analyses

All mosquito specimens were stored in coolers with dry ice and transported to the Medical Entomology and Tropical Medicine Laboratory of the San Francisco de Quito University (LEMMT-USFQ) in Quito for further processing. At LEMMT-USFQ, mosquitoes were counted, sexed and morphologically identified to the level of genus or species using taxonomical keys [227–229]. Identification of male specimens of *Cx. quinquefasciatus* was not done through their terminalia features as described by Bram (1967) [284], but rather was assumed from the external features of the thorax, abdomen, head and legs using taxonomic keys [227,228].

Subsequent molecular analyses were performed on specimens identified as *Ae. aegypti* females to test for the presence of arbovirus. Here, pools of up 5 *Ae. aegypti* females were created by grouping on the basis of unique collection code. When more than 5 *Ae. aegypti* females were obtained in a single collection, specimens were split into two or more pools. Pools of *Ae. aegypti* were placed in 1.5 ml cryovials containing TRIzol™ (Invitrogen) labelled with their unique collection code and stored at -80°C at LEMMT-USFQ before being shipped to the MRC - Centre for Virus Research (CVR) at the University of Glasgow for viral screening. At the CVR, samples were transferred to a -80°C freezer. These samples were screened for the presence of Zika (ZIKV), dengue (DENV) and chikungunya (CHIKV) viruses in a three-step process starting with RNA extraction, then reverse-transcription and finally virus-specific PCR as described previously (Chapter 2). The PCR step was modified in this study as samples were not amplified with the DENV1-3 primer. Instead, all samples were tested for DENV presence using individual primers for each serotype (i.e., DENV-1, DENV-2 and DENV-3 primers). The PCR step was done by Sandra Terry, from the Centre for Virus Research (CVR-MRC) from the University of Glasgow.

3.2.6. RNA Sequencing Analyses

Note: These sequencing analyses were done by the Sequencing team, led by Daniel Mair, at the Centre for Virus Research (CVR-MRC) from the University of Glasgow.

In parallel to the PCR step, full genome sequencing was carried out on a subset of pools containing higher numbers of blood fed *Aedes* to maximize the probability of finding an arbovirus. A total of 19 pools of female *Ae. aegypti* were deep sequenced from Portoviejo (9 pools) and Quinindé (10 pools). Most of the selected pools came from indoor Prokopack aspirations obtained during the last collection period (April 2017), which coincided with the timing of rising arbovirus cases in people based on previous years (see Chapter 4). In some cases, where *Ae. aegypti* females from the same collection were split across multiple pools (because >5 mosquitoes per collection were found), individuals were regrouped into one pool for deep sequencing. In addition to the 19 selected pooled samples, one sample containing a lab reared *Ae. aegypti* female mosquito, artificially infected with Semliki Forest virus was used as a negative control, giving a total of 20 samples (Table 3.1).

Table 3. 1. Summary table of samples sent to Next Generation Sequencing (NGS). A total of 20 mosquito pools were sent to NGS to obtain their full genome. All female *Ae. aegypti* sent to NGS were blood fed, with the exception of the negative control that was a lab reared mosquito that had already digested the artificial blood feeding.

Pool ID	Number of females	Month of collection	Location	Habitat	Trap type	Aspiration
1	3	Apr-17	Portoviejo	Urban	Prokopack	indoors
2	3	Apr-17	Portoviejo	Peri-urban	Prokopack	indoors
3	10	Apr-17	Portoviejo	Peri-urban	Prokopack	indoors
4	3	Apr-17	Portoviejo	Urban	Prokopack	indoors
5	7	Apr-17	Portoviejo	Urban	Prokopack	indoors
6	7	Apr-17	Portoviejo	Urban	Prokopack	indoors
7	2	Apr-17	Portoviejo	Peri-urban	Prokopack	indoors
8	2	Apr-17	Portoviejo	Urban	Prokopack	indoors
9	3	Apr-17	Portoviejo	Urban	Prokopack	indoors
10	1	N/A	Lab reared	N/A	N/A	N/A
11	3	Nov-16	Quinindé	Urban	Prokopack	indoors
12	5	Apr-17	Quinindé	Urban	Prokopack	indoors
13	2	Apr-17	Quinindé	Urban	Prokopack	outdoors
14	2	Apr-17	Quinindé	Urban	BGS	N/A

15	6	Apr-17	Quinindé	Urban	BGS	N/A
16	7	Apr-17	Quinindé	Urban	Prokopack	indoors
17	5	Apr-17	Quinindé	Urban	Prokopack	indoors
18	5	Apr-17	Quinindé	Urban	Prokopack	indoors
19	5	Apr-17	Quinindé	Urban	BGS	N/A
20	3	Apr-17	Quinindé	Peri-urban	BGS	N/A

After the RNA extraction step, each pool was aliquoted, described above, pools were sent to the Next Generation Sequencing facility at the CVR (performed by Daniel Mair). RNAs were then processed through Next Generation Sequencing Quality Control (NGS QC). This involved the use of a Qubit® 3.0 fluorometer (Invitrogen, Carlsbad, California, USA) with High Sensitivity reagents to determine RNA and DNA concentration, and a TapeStation 4200 System with High Sensitivity RNA screentape and reagents (Agilent, Santa Clara, California, USA) to obtain a measurement of RNA integrity (RIN). However, as 18s/28s mosquito rRNA are very close in size and so are indistinguishable on the gel, and 18s/28s rRNA is of different size between mosquitoes and humans, the TapeStation traces could not be used to generate RIN quality scores. Instead, the proportion of RNA fragments over 200 nucleotides (also referred to as DV200) were used to give an idea of sample quality for NGS library preparation.

The 20 RNA extracts, were divided into two groups of 10 samples to go into the deep sequencing process separately. The first group of 10 samples (hereafter referred as the “pilot study”) was used to determine the best library preparation method to be suitable for all the samples. Once this method was identified, it was applied to the second group of 10 samples.

To determine the best library preparation method using the first group of 10 samples, each of the samples was DNase treated and then split in two parts, one

of which went through the ribosomal depletion by using the Ribo-Zero® Gold rRNA Removal Kit (Illumina Inc., San Diego, California, USA) and the other did not. This ribosomal depletion aimed to remove the host ribosomal RNA coming from the mosquito genome, as if there was any virus presence, it would had been in too low proportion compared to the mosquito transcript. These paired replicates (with and without ribosomal depletion), formerly derived from each of the 10 original samples, were then processed with the TruSeq Stranded RNA Library Preparation Kit (Illumina Inc., San Diego, California, USA). Reverse transcription (cDNA synthesis) was done with SuperScript™ II Reverse Transcriptase (Thermo Fisher Scientific, Waltham, Massachusetts, USA) followed by double stranded DNA synthesis. Then, A-tailing was carried out to facilitate sequencing adapter binding, which contains flow cell binding regions, sequencing primer binding sites and specific indices for multiplexing, Finally, an amplification of adapter ligated libraries was done to increase concentration for sequencing.

The resulting 20 sequencing libraries were cleaned up using a 0.9V ratio of AMPure XP (Beckman Coulter, California, USA) to remove residual enzymes, buffers and primer or adapter dimers. Sample QC was then performed using Qubit and Tapestation to determine molarity. This was based on the formula: Molarity = concentration in ng/uL, divided by the region average size of the DNA library fragments, divided by the mass of one mole of DNA (660g) multiplied $10^{\wedge 6}$ to convert the value to nanomoles per litre (nM). The samples were then pooled together in equimolar ratios and finally diluted to 4 nM. Subsequently, the pooled sample was denatured with 0.2N NaOH for 5 minutes and then neutralized with 200mM of tris at pH 7. This step was followed by a dilution of the sample to 20 pM with HT1 Hybridization Buffer (Illumina Inc.) and a control of 20 pM denatured PhiX spiked-in at approximately 1%. These samples were further diluted to a final concentration of 1.8 pM.

Sequencing was then performed on the NextSeq in a Mid Output 300 cycle cartridge (150 bp paired-end reads). The flow cell cluster density was approximately 150K/mm2 but only about 60% of the reads achieved a score of Q30 or higher, according to the Phred quality scoring system. A lower cluster density would typically result in higher Q scores due to the better resolution of clusters, which was one of the reasons why it was determined that the run was anomalous.

Therefore, the cartridge was replaced by Illumina due to a possible blockage of fluidics and the sequencing was run again. *Flaviviridae* virus were only found in Ribo-depleted samples screened with the TruSeq method, thus this was selected as the optimal method for use with the subsequent batch of 10 samples. The best method (ribosomal depletion or not) was determined based on bioinformatic analysis related to finding larger contigs and presence of viruses of the *Flaviviridae* and *Togaviridae* families.

Based on this, a further set of 10 samples were prepared using the TruSeq Ribosomal depletion method described above. In this case, the standalone ribosomal depletion kit had been discontinued so instead a TruSeq Stranded Total RNA (Illumina Inc., San Diego, California, USA) containing all of the same components was used. Libraries were sequenced together with the TruSeq ribosomal depleted DNA libraries from the first batch to obtain additional read depth. Analysis of the resulting sequences was performed by Richard Orton (CVR Bioinformatics team).

3.2.7. Statistical analyses

Statistical analyses were carried out in R 3.5.0 and R Studio 1.1.419 using the packages "lme4", "effects" and "multcomp" [231,285,286] with the aim of assessing variation in the abundance of *Ae. aegypti* females between cities, neighbourhoods of different urbanization level, collection months, and trapping methods using Generalized Linear Mixed Models (GLMM) and General Linear Hypotheses tests (GLHT – *Post Hoc* Tukey tests for GLMM). As mosquito abundance data were highly overdispersed, all models were fitted with a negative binomial distribution [287]. All figures were created using the packages "ggplot2" and "ggpubr" [288,289].

GLMMs were constructed to model the mean daily abundance of female *Ae. aegypti* as a function of trap type (BGS, Prokopack-IN, Prokopack-OUT), location (Portoviejo or Quinindé), neighbourhood type (urban or peri-urban), month of collection (November 2016, January 2017, March 2017 and April 2017), and mean daily temperature (taken from INAMHI's weather stations). In addition to these variables, past rainfall obtained from INAMHI's weather stations was also included

to reflect water availability during larval development of *Ae. aegypti* adults caught in traps (Figure 3.3). Based on known *Ae. aegypti* life cycle (Christophers 1960 [111]), adult females caught in a trap would have arisen from eggs laid ~16-39 days previously (Figure 3.3). To coincide with the larval development period, three variables based on cumulative rainfall in the study area 3, 2 weeks and 1 week before collection were calculated. The lagged periods were temporally-discrete estimates of rainfall occurring over a 7-day period: weekly cumulative rain falling 28-22 days, 21-15 days, and 14-8 days before the collection day, respectively. In addition to these main effects as described above, all models tested for interactions between month of collection and location, neighbourhood type and location, trapping method and location, neighbourhood type and trapping method, and mean daily temperature and location. Collection date, neighbourhood, house ID, and trap & collector ID were included as random effects. Two model structures were built which differed in regards to how rainfall was included. The first model structure included all of the described explanatory variables, interactions and random effects, plus rainfall included at three different lags (Table 3.2, Model 1). The second model structure was similar except that rainfall was included as only one covariate, representing the cumulative rainfall over the three-week period before mosquito collection (Table 3.2, Model 2). Maximal models from both model structures were compared using the Akaike Information Criterion (AIC) [290]. The model structure with the lowest AIC was retained for further model selection to assess the significance of covariates through a process of backward stepwise elimination using Likelihood Ratio Tests (LRT) [232].

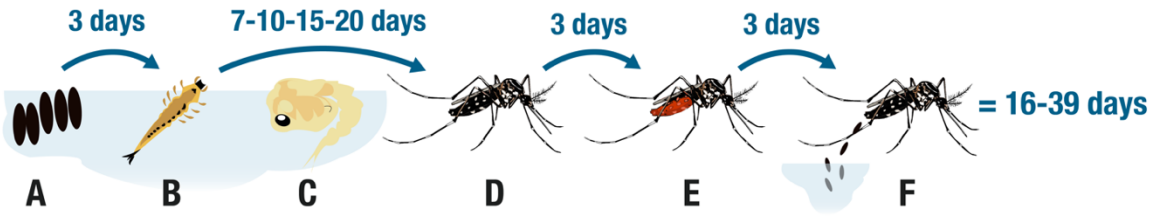

Figure 3. 3. Time of development of *Ae. aegypti* females along their life stages. Development time of *Ae. aegypti* females according to Christophers 1960 [111]. The duration time from when eggs have been oviposited (A) to the first larval instar (B), pupal stage (C), a newly emerged adult female (D), a female that has taken her first blood meal (E), and when that female will oviposit eggs produced from that first blood meal (F).

In order to test the impact of microclimate (as measured at the trapping station) on the host seeking behaviour of *Ae. aegypti*, further analysis was conducted just on the subset of data from BGS traps (used in 3 out of 4 collection trips, in January – April 2017). Here the response variable was the daily abundance of female *Ae. aegypti*, with the explanatory variables being all of the fixed and random variables used with the full dataset except for mean daily temperature as obtained from the INAMHI's weather station, which was instead replaced by mean daily temperature and mean daily relative humidity at the specific trapping locations as measured by the dataloggers at each BGS station. Additionally, interactions between (trap-specific) temperature and humidity, temperature and location, and humidity and location were also included as fixed effects (Table 3.2, Model 3).

Table 3. 2. Full model structures. Three model structures for statistical analyses were tested in this study and full models are shown.

Model structure	**Response variable**	**Fixed effects**	**Random effects**
1	AeAb	Mo + Loc + Nt + Tr + Tmp + Ra_1 + Ra_2 + Ra_3 + Loc*Nt + Loc*Tr + Loc*Mo + Nt*Tr + Tmp*Loc	Dt + Nh + Hs + Col
2	AeAb	Mo + Loc + Nt + Tr + Tmp + $Ra_{1,2,3}$ + Loc*Nt + Loc*Tr + Loc*Mo + Nt*Tr + Tmp*Loc	Dt + Nh + Hs + Col
3	$AeAb_1$	Mo + Loc + Nt + Tmp_1 + Hum + Ra_1 + Ra_2 + Ra_3 + Loc*Mo + Tmp_1*Hum + Tmp_1*Loc + Hum*Loc	Dt + Nh + Hs + Col
Abbreviation		**Description**	
AeAb		Abundance of female *Ae. aegypti* mosquitoes	
$AeAb_1$		Subsample of abundance of female *Ae. aegypti* mosquitoes	
Mo		Month of collection	
Loc		Location (study site)	
Nt		Neighbourhood type (urban or peri-urban)	
Tr		Trapping method (BG sentinel trap (BGS), Prokopack indoor, and Prokopack outdoor)	
Tmp		Temperature as measured from weather stations	
Tmp_1		Temperature as measured from data loggers	
Ra_1		Total rainfall from 14-8 days before sampling	
Ra_2		Total rainfall from 21-15 days before sampling	
Ra_3		Total rainfall from 22-28 days before sampling	
$Ra_{1,2,3}$		Total rainfall from 8-28 days before sampling	
Hum		Relative humidity as measured from data loggers	
Dt		Date of sampling	
Nh		Neighbourhood ID	
Hs		House ID	
Col		Collector ID (for Prokopack aspirations) or Trap ID (for BGS)	

Finally, to determine the pathogen prevalence in *Ae. aegypti* female mosquitoes, the Epitools calculator was used [291]. As pools of unique collections had ≤ 5 mosquitoes per pool, prevalence calculations were done by using a variable pool size assuming a test sensitivity and specificity of 100%.

3.2.8. Phylogenetic Analysis of DENV in Aedes aegypti

As will be described in the results, the only arbovirus detected in *Ae. aegypti* collections was dengue serotype 1 (DENV-1). The full genome sequence of this DENV-1 isolate was compared with other sequences of the same virus collected in South America, Asia and Africa, with and one sequence of DENV-3 from Ecuador used as an outgroup for phylogenetic tree building (Table 3.3). All sequences used in the comparison were downloaded from GenBank and phylogenetic analyses were carried out in MEGA 7 (Molecular Evolutionary Genetics Analysis for macOS [292]). Sequences were aligned by muscle, and DNA model selection was carried out by using the Maximum Likelihood (ML) method with neighbour-joining tree and nucleotide substitution. Gaps and missing data from the sequences were treated by complete deletion from the analyses. A total of 350 bootstrap replicates of phylogenetic reconstruction were carried out by using the ML method based on the General Time Reversible model. To model the evolutionary rate differences among sites, Gamma distribution was applied with 5 categories allowing for some sites to be evolutionarily invariable. Tree inference was obtained by applying the ML heuristic method of Nearest-Neighbourhood-Interchange (NNI) and the initial tree was calculated automatically by using the Neighbour-Join and the BioNJ algorithms.

Table 3. 3 DENV sequences used for the phylogenetic reconstruction analysis. A total of 18 dengue full genome sequences were used for the phylogenetic reconstruction analysis. The sequence marked with (*) corresponds to the DENV-1 sequence obtained in this study, and the sequence marked with (**) corresponds to the DENV-3 sequence used as outgroup of the tree.

Host	Strain / Isolate	Accession #	Year of collection	Country of Origin	Virus
Homo sapiens	HNRG13154	KC692499.1	1999	Argentina	DENV – 1
Homo sapiens	297arg00	AF514889.3	2000	Argentina	DENV – 1
Homo sapiens	12898/BR-PE/10	JX669462.1	2010	Brazil	DENV – 1
Homo sapiens	DENV1_BR/SJRP/484/2012	KP188543.1	2012	Brazil	DENV – 1
Homo sapiens	DENV-1/CO/BID-V3376/1998	GQ868559.1	1998	Colombia	DENV – 1
Homo sapiens	DENV-1/CO/BID-V3382/2006	GQ868564.1	2006	Colombia	DENV – 1
Homo sapiens	D1/H/IMTSSA-ABID/99/1056	AF298807.1	1998	Côte d'Ivoire	DENV – 1
Homo sapiens	CHI3336-02	EU863650.1	2002	Easter Island (Chile)	DENV – 1
Homo sapiens	DENV-3/EC/BID-V2975/2000	FJ898457.1	2000	Ecuador	DENV – 3 **
Homo sapiens	TD-00044-S	KY474303.1	2014	Ecuador	DENV – 1
Homo sapiens	DENV1/EC/Esmeraldas/210/2014	MF797878.1	2014	Ecuador	DENV – 1
Aedes aegypti	EC0426-1/seq/01	MN556095	2017	Ecuador	DENV – 1 *
Homo sapiens	FP0203	DQ672556.1	2010	French Polynesia	DENV – 1
Homo sapiens	DENV-1/8/Thailand/01/2013	KF887994.1	2014	Thailand	DENV – 1
Homo sapiens	DENV-1/VE/BID-V2168/1998	FJ639740.1	1998	Venezuela	DENV – 1
Homo sapiens	VE-61059-2006	HQ332177.1	2006	Venezuela	DENV – 1
Homo sapiens	DENV-1/VE/BID-V1134/2007	EU482609.1	2007	Venezuela	DENV – 1
Homo sapiens	DENV1-VE-IDAMS-910132-2015-10-19	MH450312.1	2015	Venezuela	DENV – 1

3.3. RESULTS

3.3.1. Mosquito species and abundance

During the 6-month period of study, a total of 3987 mosquitoes from 7 different genera were collected. The two most abundant species were *Ae. aegypti* (24.73%) and *Culex quinquefasciatus* (68.15%) (Table 3.4). Other mosquitoes collected included *Ae. angustivittatus* (0.2%), *Anopheles pseudopunctipennis* (0.08), *Limatus durhami* (1.83%), *Psorophora ferox* (0.75%), *Aedes spp.* (0.68%), *Anopheles spp.* (0.6%), *Sabethes spp.* (0.08%), and *Wyeomyia spp.* (0.25%) (Table 3.4). A small proportion of mosquitoes (2.66%) could not be identified due to damage or loss of diagnostic features (Table 3.4). Due to the morphological similarities such as the golden-brownish colour of the scales, some of the *Ae. angustivittatus* may have been misidentified as *Cx. quinquefasciatus*.

Most mosquitoes were collected by Prokopack aspiration inside houses (47.6%), followed by aspirations outdoors (34.49%), and then BGS traps (17.91%). Focusing on *Ae. aegypti,* most individuals were collected by Prokopack indoor aspirations (49.79%) followed by BGS collections (36.6%) and Prokopack outdoor aspirations (13.59%) (Table 3.4). Most *Cx. quinquefasciatus* were collected by Prokopack aspirations inside (49.72%) and in the outdoor area around houses (41.52%), followed by BGS (8.76%).

Table 3. 4. Abundance of mosquitoes collected with BG-Sentinel (BGS) traps and Prokopack (PPK) aspirations in Portoviejo and Quinindé between November 2016 and April 2017. Mosquitoes are broken down by sex (♂ = males, ♀= females), with females further split by blood feeding status. Prokopack aspirations were carried out inside houses and in the outdoor area for 10 minutes at each house area, while BGS collections were carried out outdoors for approximately 9 hours during the day.

	Trapping methods									Total counts			
	BGS traps			PPK aspirators									
				Indoors			Outdoors						
Species	♀ fed	♀ unfed	♂	♀ fed	♀ unfed	♂	♀ fed	♀ unfed	♂	Total ♀ fed	Total ♀ unfed	Total ♂	**Grand Total**
Aedes aegypti	68	197	96	242	82	167	31	33	70	341	312	333	**986**
Aedes angustivittatus	0	0	0	2	1	0	3	2	0	5	3	0	**8**
Aedes spp.	0	11	0	2	2	0	1	10	1	3	23	1	**27**
Anopheles pseudopunctipennis	0	0	0	2	1	0	0	0	0	2	1	0	**3**
Anopheles spp.	3	0	0	6	0	6	4	1	4	13	1	10	**24**
Culex quinquefasciatus	42	64	132	342	274	735	312	207	609	696	545	1476	**2717**
Limatus durhami	0	25	0	1	3	0	6	38	0	7	66	0	**73**
Psorophora ferox	2	20	0	1	2	0	0	4	1	3	26	1	**30**
Sabethes spp.	0	2	0	0	0	0	0	1	0	0	3	0	**3**
Wyeomyia spp.	0	5	0	0	2	0	0	3	0	0	10	0	**10**
Unidentified	10	9	28	0	13	12	19	0	15	29	22	55	**106**
									Total	**1099**	**1012**	**1876**	**3987**

3.3.2. Population dynamics and behaviour of Ae. aegypti

The two alternative model structures for analysing variation in *Ae. aegypti* abundance were compared *via* AIC to assess which one had greater explanatory power. The difference in AIC between these two competing was models was smaller than 2 units (Δ=0.096), which can be interpreted as evidence that they were not significantly different from each other [290]. However, the model with slightly lower AIC was retained for further analysis and evaluation of covariates. This was the model that incorporated rainfall as three separate time-lagged covariates (Table 3.2, Model 1).

Using this model, *Ae. aegypti* abundance was significantly associated with the month of collection, cumulative rainfall 28 to 22 days before the collection day, neighbourhood type, and an interaction between location and trap type (Table 3.5). Pairwise *post hoc* analysis of the final model indicated that *Ae. aegypti* female abundance was significantly higher in March 2017 than in November 2016 (Figure 3.4, Table 3.6, GLHT Tukey: Z= 2.56, *p*= 0.04) and January 2017 (Figure 3.4, Table 3.6, GLHT Tukey: Z= 2.88, *p*=0.02). There was no difference in mean abundance between months of collections in the rest of the pairwise combinations.

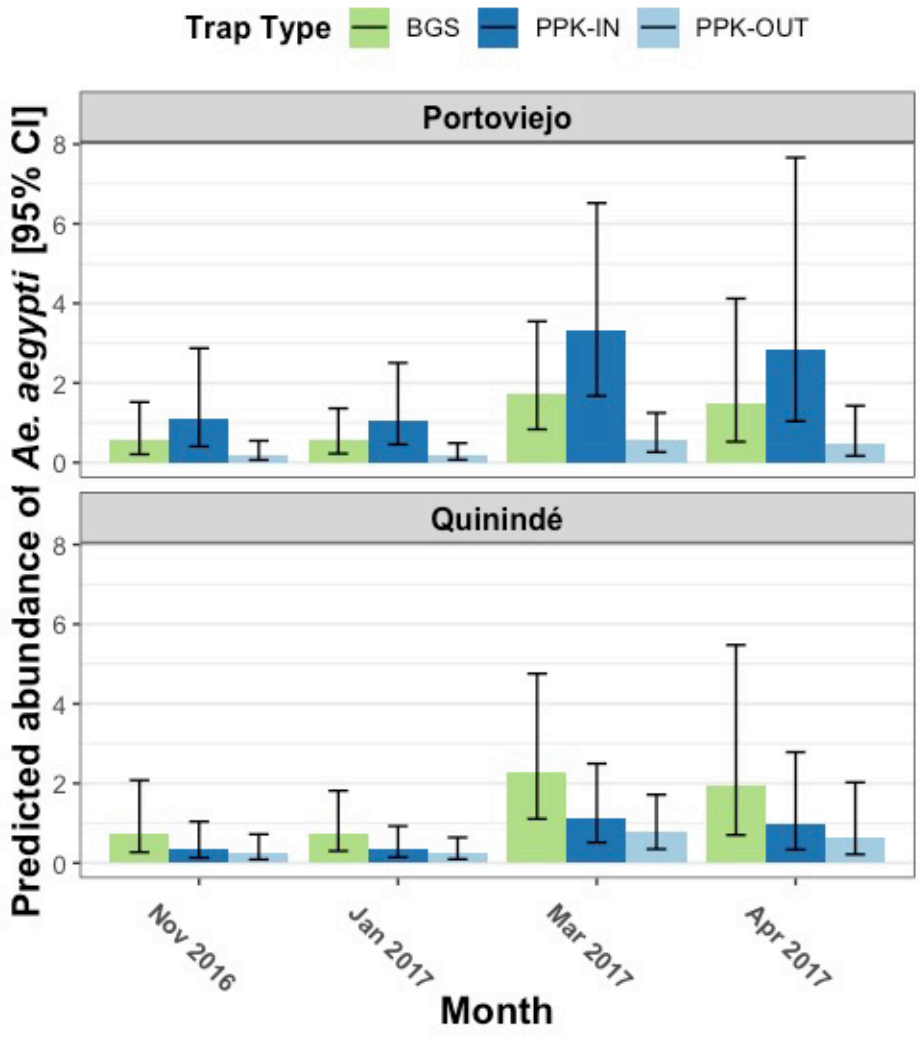

Figure 3. 4. Predicted *Ae. aegypti* female abundance according to month of collection per canton. Height of columns indicate the estimated mean of *Ae. aegypti* females, while error bars indicate the 95% CI. Different colours of bar represent different trapping methods, being BG-Sentinel trap (BGS), Prokopack aspirations made inside (PPK-IN) or outside of houses (PPK-OUT).

The abundance of female *Ae. aegypti* was approximately two times higher at households in urban than peri-urban neighbourhoods (Figure 3.5, Table 3.5 and 3.6).

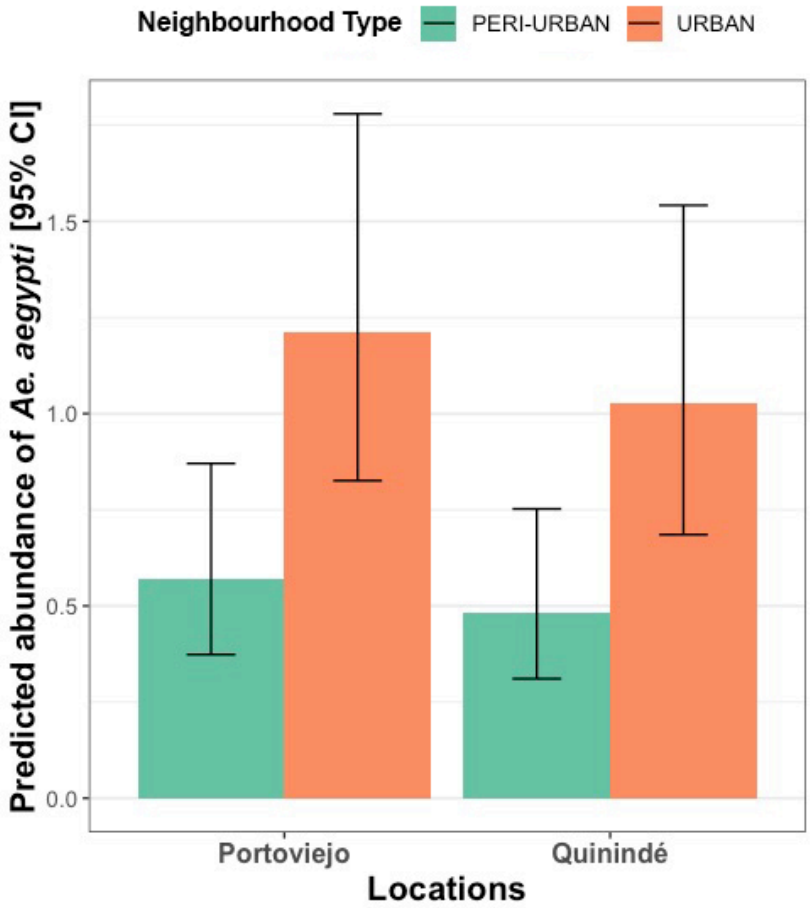

Figure 3. 5. Predicted *Ae. aegypti* female abundance according to neighbourhood type per canton. Height of columns indicate the estimated mean of *Ae. aegypti* females, while error bars indicate the 95% CI. Different colours of bar represent a different neighbourhood type.

There was no consistent difference in *Ae. aegypti* abundance between cities, with the relative difference depending on the mosquito trapping method used (Table 3.5). In collections made with indoor Prokopack aspirations, *Ae. aegypti* females were three times more abundant in Portoviejo than Quinindé (Figure 3.6, Table 3.6, GLHT Tukey: Z= -3.56, $p<0.01$), but there was no difference between cities in the number caught in outdoor aspirations (Figure 3.6, Table 3.6, GLHT Tukey: Z= 0.87, $p=0.95$). In Portoviejo, *Ae. aegypti* females were 6-fold higher in indoor versus outdoor Prokopack aspirations (Figure 3.6, Table 3.6, GLHT Tukey: Z= -6.73, $p<0.001$), with no difference between outdoor and indoor collections in Quinindé (Figure 3.6, Table 3.6, GLHT Tukey: Z= -1.40, $p=0.72$). The abundance of *Ae. aegypti* females in BGS traps was also similar in Portoviejo and Quinindé

(Figure 3.4, Table 3.6, GLHT Tukey: Z= 0.91, *p*= 0.94). BGS traps collected significantly more female *Ae. aegypti* than outdoor Prokopack aspirations in both cities (Figure 3.4, Table 3.6, **Portoviejo:** GLHT Tukey: Z= -3.90, *p*< 0.01; **Quinindé:** GLHT Tukey: Z= -4.07, *p*< 0.001); but were not significantly different from indoor Prokopack aspirations (Figure 3.4, Table 3.6, **Portoviejo:** GLHT Tukey: Z= 2.72, *p*= 0.07; **Quinindé:** GLHT Tukey: Z= -2.76, *p*= 0.06).

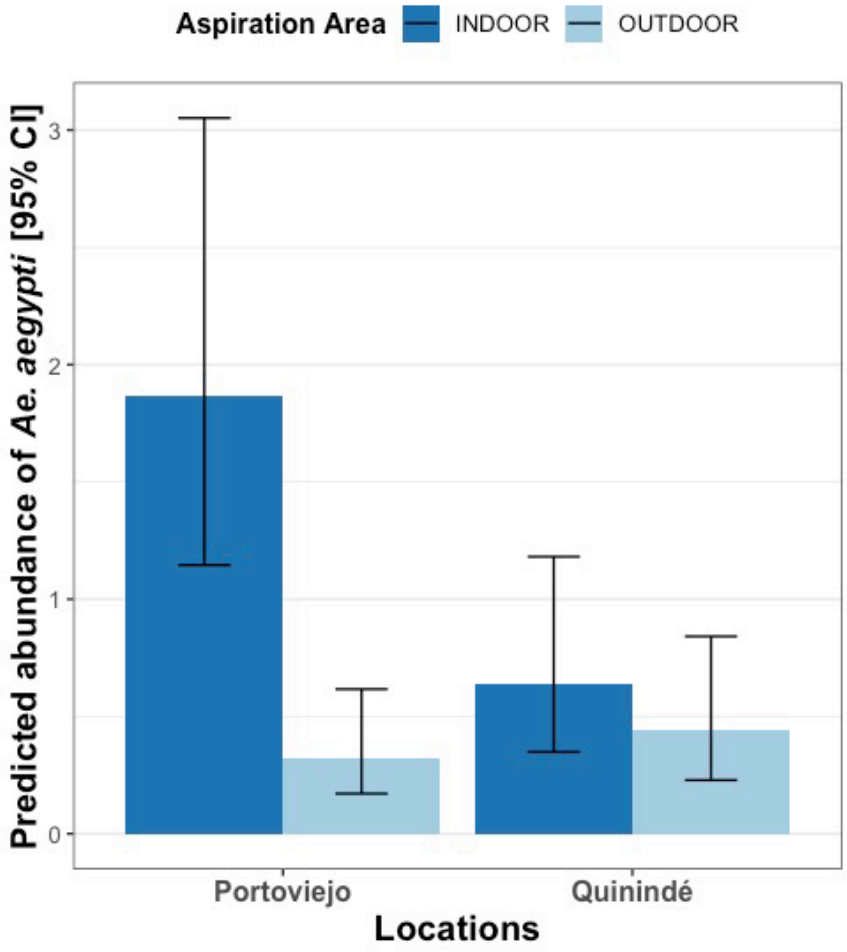

Figure 3. 6. Predicted *Ae. aegypti* female abundance in indoor or outdoor Prokopack aspiration collections, in different cities. Height of columns indicate the estimated mean of *Ae. aegypti* females, while the error bars indicate the 95% CI. Different colours of bar represent whether mosquitoes were collected in Prokopack aspiration made inside or outside of houses.

Finally, there was a negative association between the cumulative rainfall occurring in the third week before collection (28 to 22 days before the collection day) and the mean daily abundance of *Ae. aegypti* (Figure 3.7, Table 3.5).

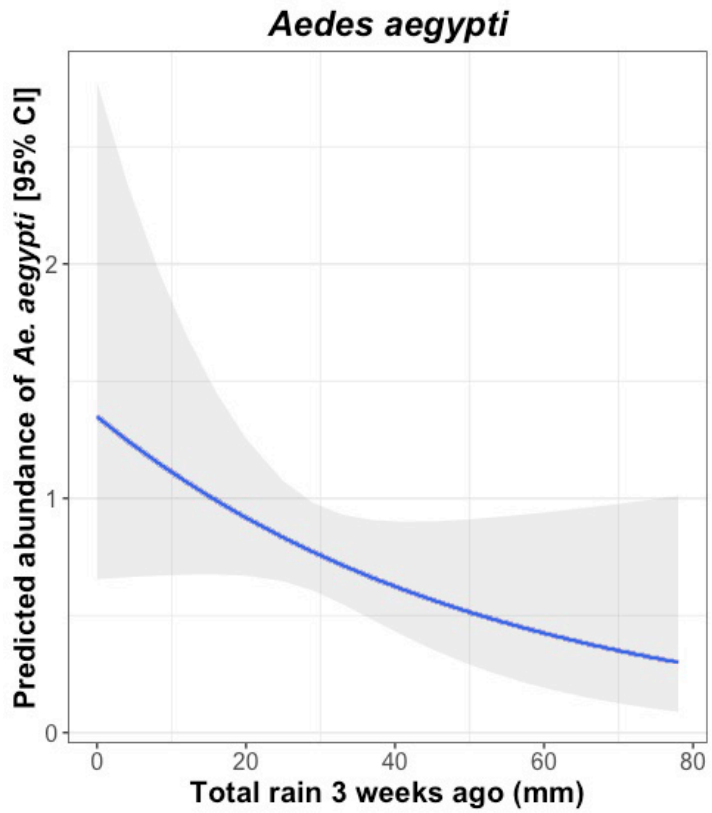

Figure 3. 7. Predicted association between *Ae. aegypti* female abundance according and the volume of rainfall falling 28 to 22 days before collection day. The blue line indicates the estimated mean of *Ae. aegypti* females, while the grey shaded area indicates the 95% CI.

Table 3. 5. Summary table of statistical significance of explanatory variables tested for association with *Ae. aegypti* female abundance. Significance values for each of the explanatory variables from the fitted models. Values of chi-square (X^2), degrees of freedom (df), and p-values for each of the covariates tested are shown. Bold values with an asterisk (*****) indicate significant terms. Fixed effects with a double S symbol (§) indicate the interaction term. "NA" indicates "not applicable" values for which single term significance was not possible because of their involvement in significant interaction terms. The letter "w" means week.

Explanatory variables	X^2	df	*p*-value
Month of collection	10.11	3	**0.02***
Canton	NA	NA	NA
Neighbourhood type	8.60	1	**<0.01***
Trap type	NA	NA	NA
Temperature	0.01	1	0.94
Rain 1w ago	0.92	1	0.34
Rain 2w ago	0.62	1	0.43
Rain 3w ago	5.07	1	**0.02***
Canton: Neighbourhood type §	0.05	1	0.82
Canton: Trap Type §	19.83	2	**<0.001***
Canton: Month §	3.77	3	0.29
Neighbourhood type: Trap type §	0.83	2	0.66
Temperature: Canton §	0.12	1	0.73

Table 3. 6. Estimated mean abundance of *Ae. aegypti* females. Mean values are given for each month of collection neighbourhood type, and canton and trap type combination, with the corresponding 95% CI of the lower and upper limits. Values for each of the three trapping methods, BG-Sentinel traps (BGS) and indoor Prokopack aspirations (PPK-IN) and outdoor (PPK-OUT) are given too.

Covariates	Covariate levels		Mean	95% CI	
				Lower lim.	Upper lim.
Month	November 2016		0.45	0.19	1.07
	January 2017		0.44	0.21	0.93
	March 2017		1.37	0.81	2.31
	April 2017		1.17	0.47	2.89
Neighbourhood type	Urban		1.12	0.82	1.53
	Peri-urban		0.53	0.37	0.75
Canton and Trap type	Portoviejo	BGS	0.97	0.56	1.68
		PPK-IN	1.87	1.15	3.05
		PPK-OUT	0.32	0.17	0.62
	Quinindé	BGS	1.30	0.75	2.27
		PPK-IN	0.64	0.35	1.18
		PPK-OUT	0.44	0.23	0.84

Restricting analysis just to the data set for which specific microclimatic measurements were made at the trapping point (BGS collections, January to April 2017), the abundance of *Ae. aegypti* females was significantly related to collection month and rainfall occurring 22-28 days before collections. However, there was no significant impact of local temperature or humidity at the trapping point (Table 3.7).

Table 3. 7. Summary table of significance of variables tested for microclimatic association with *Ae. aegypti* female abundance. Significance values for each of the explanatory variables from the fitted models. Values of chi-square (X^2), degrees of freedom (df), and p-values for each of the covariates tested are shown. Bold values with an asterisk (*****) indicate significant terms. Fixed effects with a double S symbol (§) indicate the interaction term. "NA" indicates "not applicable" values for which single term significance was not possible because of their involvement in significant interaction terms. The letter "w" means week.

Explanatory variables	X^2	df	*p*-value
Month of collection	12.84	2	<0.01*
Canton	2.12	1	0.15
Neighbourhood type	2.62	1	0.11
Humidity	0.75	1	0.39
Temperature	2.10	1	0.15
Rain 1w ago	0.33	1	0.56
Rain 2w ago	0.10	1	0.75
Rain 3w ago	8.68	1	<0.01*
Canton: Neighbourhood type §	0.16	1	0.69
Canton: Temperature §	0.83	1	0.36
Canton: Month §	5.60	1	0.06
Canton: Humidity §	1.71	2	0.19
Temperature: Humidity §	2.34	1	0.13

3.3.3. Arboviral detection in Ae. aegypti

RNA was extracted from a total of 213 pools of female *Ae. aegypti* containing 483 individual mosquitoes from the two study sites (Table 3.8). Detection of DENV, CHIKV and ZIKV through PCR using virus-specific primers was conducted on 89.66%

of the pools (N=208), with screening in remaining samples not possible due to RNA concentration from extracted products being too low or reverse transcription unsuccessful. All positive controls worked in all PCR runs.

Table 3. 8. Number of pools and mosquitoes analysed. Number of pools and individual female *Ae. aegypti* mosquitoes processed at each stage to test presence of DENV, CHIKV and ZIKV. Successful reverse-transcription was determined from a control PCR on the mosquito S7 gene.

Canton	Collected from the field		RNA successfully extracted		RNA converted into cDNA (PCR on S7)		PCR on DENV, ZIKV and CHIKV	
	# pools	# indiv.	# pools	# indiv.	# pools	# indiv.	# pools	# indiv.
Portoviejo	140	288	126	271	125	267	125	267
Quinindé	92	217	87	212	83	202	83	202
Total	232	505	213	483	208	469	208	469

None of the samples analysed by conventional PCR were positive for DENV- 2,3,4, CHIKV, or ZIKV. However, one pool containing 3 blood fed *Ae. aegypti* females was positive for DENV-1, corresponding to an overall individual mosquito infection rate of 0.0021 (CI 95%: 0.0001 – 0.0094). This positive result was corroborated by deep sequencing analysis, through which the full genome of this sample was obtained. The DENV-1 positive pool was collected in an urban neighbourhood of Portoviejo by indoor aspiration in April 2017.

3.3.4. Phylogenetic Analysis

A phylogenetic reconstruction of DENV-1 was performed using the full genome sequence from the DENV-1 positive sample found in this study and other published DENV-1 sequences from other sites in Ecuador, South America, and other regions of the world (Table 3.1, Figure 3.8a). Based on the tree with the highest likelihood (-37183.99), all sequences from South America were clustered together with 100% of support, and had their closest sequence from Côte d'Ivoire (Africa) (AF298807.1, 100% of support), followed by the cluster from Thailand and the Pacific Islands being the most distant sequences. The three full genome DENV-1 sequences from Ecuador (including the one from this study, Table 3.1) clustered

together with 100% of support (Figure 3.8). The two other DENV-1 sequences from Ecuador had been collected from patients in 2014 from Esmeraldas (MF797878.1 [293]) and Machala (KY474303.1 [162]). These Ecuadorian samples were predicted to be most closely related to another DENV-1 from Venezuela collected in 2015 (MH450312.1, 100% of support) (Figure 3.8). The outgroup sequence corresponding to DENV3 from Ecuador collected in 2000 was correctly placed at the root of the tree (FJ898457.1) (Figure 3.8). Most of the branches of the phylogenetic tree had strong support and sequences were clustered together by either being from the same location or by coming from similar years. The only exceptions to this were sequences from Argentina and Brazil, that despite coming from the same country and similar years (Argentina 1999 and 2000; Brazil 2010 and 2012), were cross clustered between the two countries in two different groups.

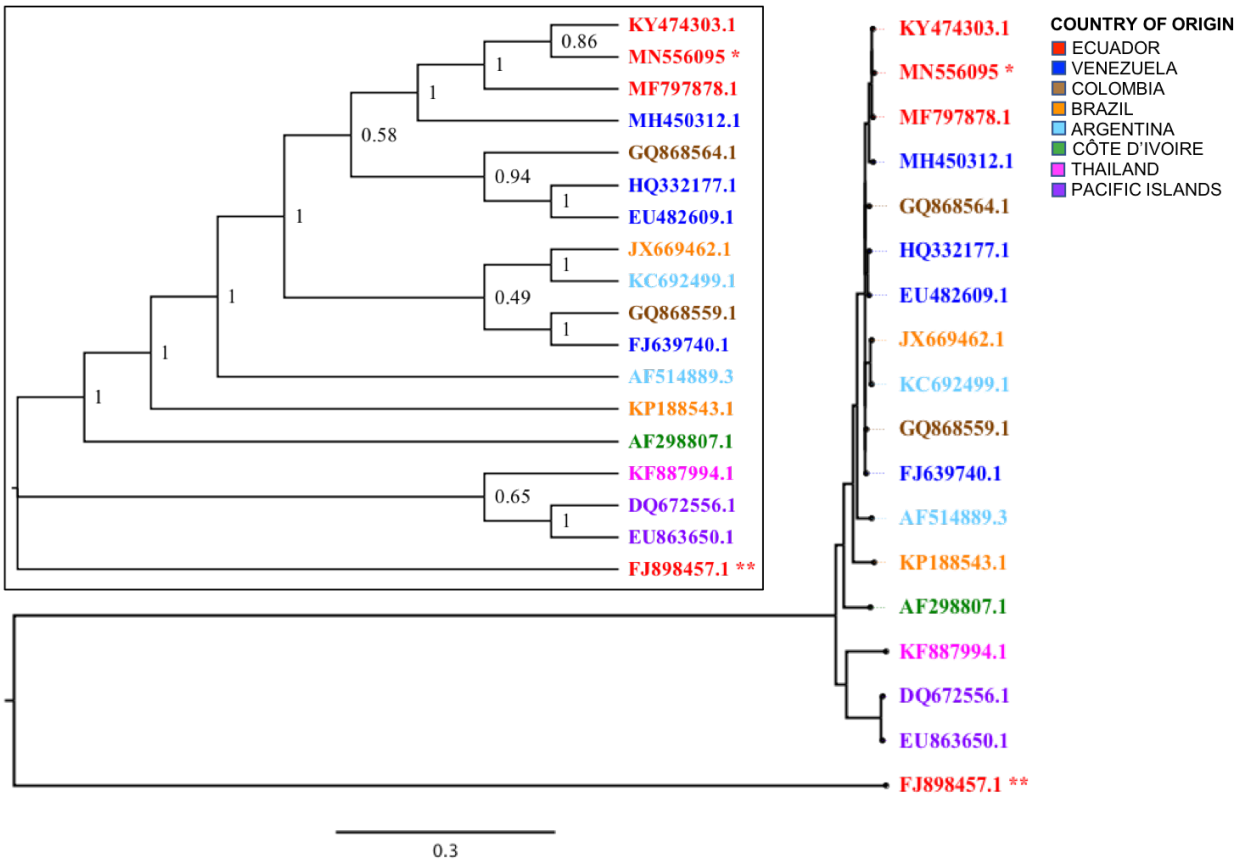

Figure 3. 8. Phylogenetic reconstruction tree of DENV-1. Phylogenetic tree obtained from molecular reconstruction using the Maximum Likelihood (ML) method from 350 bootstrap replicates. Labels at the tip of the branches indicate the accession numbers of each of the sequences from GenBank and different colours represent the countries from where the sequences were obtained. Sequence marked with (*) corresponds to the sequence obtained from this study; sequence marked with (**) corresponds to the outgroup sequence of DENV-3. (A) Topology-only tree shows the relative positions of each sequence and numbers next to the branches represent the proportion of bootstrap replicates where the associated taxa clustered together. (B) Default tree with branch lengths corresponding to the number of nucleotide substitutions per site.

3.4. DISCUSSION

Here surveillance of *Aedes* vector populations was conducted within two hotspots of arboviral transmission in coastal Ecuador with the aim of updating knowledge on vector ecology to guide appropriate vector control strategies. *Aedes aegypti* was present across the study period in both cities being more abundant during the wet and warm months (March and April) than in cooler months of November and

January. There was also substantial variation in *Ae. aegypti* abundance between neighbourhoods in association to their degree of urbanization. Vectors were two times more abundant in urban than in peri-urban neighbourhoods. *Aedes aegypti* was collected with all trapping methods, and notably found resting both inside houses and in the surrounding peri-domestic area with Prokopack aspirations. Although human cases on DENV and ZIKV were reported from both cities during the study period (see Chapter 4), only one infected pool of *Ae. aegypti* (derived from 3 blood females) was found, testing positive for DENV-1. Considerable heterogeneity in vector populations was detected between the two study sites, understanding vector dynamics could elucidate improvements for vector control in transmission hotspots.

Overall, *Ae. aegypti* abundance was higher in the warm and wet months of March and April, compared to the dry and cooler months of November and January. This matches observations from the southern coast of Ecuador, where *Ae. aegypti* populations peak during the wet and warm months of the year [177]. Although *Aedes* are present throughout the year in coastal Ecuador [177], it may be most effective to focus vector control activities in the months running before the peak period of vector abundance. Year-round vector surveillance over multiple year is needed would be valuable for confirming the repeatability of *Aedes* seasonality in coastal Ecuador, and planning vector control accordingly.

As expected, *Ae. aegypti* were also more abundant in urban than in peri-urban neighbourhoods. The ability of *Ae. aegyp*ti to adapt to urban environments is well known [118,294], with lower abundance in peri-urban areas likely due to the reduced availability of artificial container habitats for larvae [266,295]. Heterogeneity of living standards between urban and peri-urban neighbourhoods may also account for the variation in *Ae. aegypti* reported here. For instance, the lack of piped water in low-resource households, which are often associated with high densely populated areas within urban neighbourhoods, forces residents to bring water from elsewhere and store it in large containers around their home, creating permanent habitats for *Ae. aegypti* larvae [177,295]. Due to the limited flight range of *Ae. aegypti*, migration of people is the main method of spreading *Aedes*-borne diseases [237,238,296]. Migrants coming into urban centres from rural areas are generally employed in low paid casual work [297], forcing them to stay in socio-economically deprived neighbourhoods. Consequently, the higher

rates of human migration into urban than peri-urban neighbourhoods, and enhanced suitability of urban areas for *Aedes* vector populations likely gives rise to higher ABV transmission within poorer, more urbanized neighbourhoods. In addition, limited access to infrastructure in these neighbourhoods is further compounded by high population densities, providing ample blood feeding sources for *Aedes* [298]. Thus again, a more targeted approach of targeting vector control to high density (people and vectors) neighbourhoods may be more cost effective than a city-wide approach. However as *Aedes* vectors were also consistently found in peri-urban neighbourhoods, indicating these areas should not be ignored in surveillance and control activities.

Indoor aspiration using Prokopack [196] or other methods [212] are known to be highly efficient for sampling *Ae. aegypti* in urban areas. Additionally, aspiration methods have also been used to sample *Aedes* in the peri-domestic area of households; with abundance generally being higher indoors than outdoors [123,153,299,300]. This observation was repeated in Portoviejo where *Ae. aegypti* were six times more abundant in aspirations made inside than outside, but not in Quinindé, where abundance was similar at indoor and outdoor collections. While targeting both indoor and outdoor settings may be optimal, the notable differences in the relative abundance of *Ae. aegypti* resting in outdoor collections between cities highlights the importance of local vector ecology which can vary even between similar urban settings. For instance, it has been seen that spatial clustering of *Ae. aegypti* may be influenced by small-scale environmental determinants within and around households [272]. For that reason, investigating household conditions that promote *Ae. aegypti* proliferation can be used to target vector control activities and focus on hotspot areas with high mosquito productivity [301]. Therefore, risk assessments may be carried out through the use of indices such as the premise condition index (PCI), which quantitatively ranks favourable determinants for *Ae. aegypti* proliferation [302].

Climate is known to be an important driver of *Ae. aegypti* population dynamics [303,304]. In contrast to previous studies, here it was found no impact of association between *Ae. aegypti* abundance and temperature and humidity. This finding could be due to a relative low variation of these climate variables over the sampling period. The only environmental variable of significance was lagged rainfall occurring 22 – 28 days before collection, which had a negative effect on

Ae. aegypti abundance. Rainfall may have mixed effects on the development of *Ae. aegypti* larvae. On one hand, rainfall ensures artificial container habitats are filled and available as aquatic habitats for larvae. This is of particular importance in places where containers are left unattended due to limited garbage collection services [268,302]. On the other hand, reduced rainfall during dry periods causes people to increase water storage particularly where potable water is scarce. Water storage containers are a major source of *Aedes* larval habitats, which may thus increase breeding sites when rainfall is limited [295,305–307]. I hypothesize the predicted negative association between *Ae. aegypti* abundance and lagged rainfall observed here is mediated through the larval stage. Specifically I propose that heavy rainfall occurring 3 weeks before adult collection could have washed larvae out of breeding sites [308,309]. .

Although CHIKV, DENV and ZIKV transmission was actively occurring in both cities during the sampling period (Chapter 4), only one pool of *Ae. aegypti* females was found to be infected. This pool was positive for DENV-1, and was derived from 3 blood fed *Ae. aegypti* females collected with indoor aspirations at an urban neighbourhood in Portoviejo canton. Pooling across all female *Ae. aegypti* collected across both sites, this corresponds to a predicted DENV infection rate of approximately 0.2%. Although low, this rate is not unusual for arboviruses in *Aedes*. Even during epidemic years, infection rates for DENV, CHIKV and ZIKV in *Ae. aegypti* range between 0.4% to 1.2% [310,311]. Finding positive samples of *Aedes* does give a clear indication of active transmission, risk [312]. However, it is possible that no infection in vectors can be detected even with high rates of transmission. For example, no CHIKV or ZIKV infected mosquitoes were found here despite considerable human disease incidence in both study sites (Chapter 4). Despite finding positive cases in people may be a better indicator of an active circulation of arboviruses, finding positive cases in vectors should trigger immediate action from local authorities.

Although infection rates in *Aedes* vectors may be too low to quantify fine-scale patterns of transmission, isolation of viral material from even a small numbers of vectors is useful for mapping viral evolution and origin. Such investigation may be particularly valuable in the context of new epidemics such as that of ZIKV in 2016, by revealing the source of viral incursion. Unfortunately no ZIKV infected mosquito samples were found here to shed light on the origin of Ecuadorian strains.

However, phylogenetic analysis of the DENV-1 isolated from *Ae. aegypti* here revealed it was most closely related to two other DENV-1 sequences collected from Ecuador in 2014. A previous study from Ecuador detected that DENV-1 has had two introductions between 2011 and 2013 [313]. The sequence analysed here indicates that dengue circulating in these coastal cities derived from this previous introduction. It is important to promote early detection of new introductions of *Aedes*-borne viruses (ABVs) in the country as they may signify different preventive or mitigation measures [313].

This study provides a useful update on the ecology of *Ae. aegypti* populations and ABV transmission in urban coastal Ecuador. However, it has a number of limitations which raise the need for further investigation. First, although this study was concentrated on the highest transmission period of the year (encompassing the rainy season of a ZIKV epidemic year, with active transmission of CHIKV and DENV), further surveillance into the dry season would be required to fully characterize seasonal dynamics and capture the extremes of environmental conditions which may impact *Ae. aegypti* populations. Year-round surveillance of *Ae. aegypti* populations over multiple years would be of great value to confirm their seasonality and underlying environmental drivers. Longer-term surveillance would also permit more robust analysis of micro and macro climatic effects that may only be detectable across longer time periods. Furthermore, future studies would benefit from concurrent entomological and epidemiological surveillance (human case incidence) across the year, to provide a stronger foundation for assessment of the potential impact of vector control on human infection and disease.

Results from this study contribute to improving knowledge of arbovirus transmission within coastal Ecuador, highlighting the need of permanent vector surveillance to understand local *Ae. aegypti* ecology. Findings show that there can be substantial heterogeneity in *Aedes* vector abundance and behaviour (indoor versus outdoor resting) within urban settings. Understanding the drivers of household and neighbourhood-level heterogeneity in *Ae. aegypti* abundance and associated human infection risk could pave the way for more targeted and efficient vector control implementation with urban settings as required to meet the ultimate objective of disease prevention and mitigation.

CHAPTER 4: ANNUAL INCIDENCE PATTERNS OF DENGUE, CHIKUNGUNYA AND ZIKA VIRUS IN COASTAL ECUADOR AND THEIR ASSOCIATION WITH CLIMATIC AND ENTOMOLOGICAL VARIABLES

4.1. BACKGROUND

Climate plays an important role in the seasonality of infectious diseases in human populations, mainly by modifying the interactions between hosts (humans, vectors and reservoirs) and pathogens [314,315]. For instance, seasonal transmission of infectious diseases that require physical proximity (e.g contact-borne, respiratory-aerosol borne), is driven by temporal variation in host behaviour (overcrowding) and immunity, which are often linked with climate [316–322]. In contrast, seasonality in vector-borne diseases is overwhelmingly driven by climatic variation that impacts the population dynamics and demography of arthropods, and replication rate of pathogens within them [323–327]. These impacts are primarily driven by seasonal variation in rainfall which impacts larval habitat availability of mosquito vectors [328] and temperature which has multiple impacts on vector development, behaviour [329–331] and pathogen replication rates [332]. The impacts of seasonal climatic variation may vary between diseases in accordance with the optimal environmental conditions for individual pathogens and their vectors. Quantitative analysis of seasonal drivers of infectious diseases is thus essential to improve the design of surveillance systems and assess whether different diseases can be targeted with common prevention and control measures [333]. Additionally in the face of global environmental changes, it is essential to increase capacity for epidemic prediction by quantifying the relationship between climatic factors and disease transmission [315].

A core requirement for modelling relationships between climate and disease transmission is having accurate data on infection cases. The most accurate method of case detection is active surveillance; whereby researchers actively search for infected individuals within a representative sample of the population regardless

of whether they show symptoms or not [138]. While active surveillance has the advantage of picking up both symptomatic and asymptomatic cases, it is logistically demanding and expensive; thus unlikely to be practical for widescale surveillance [138]. An alternative is passive surveillance [334,335] whereby cases are reported to health facilities, by patients experiencing symptoms and requesting diagnosis. Passive surveillance has the disadvantage of missing asymptomatic cases but can generate wide scale data. Passive surveillance is often the only practical method for collecting multi-year and multi-site data on disease incidence; especially in resource poor settings where there is limited infrastructure for community surveillance. Although passive surveillance may not accurately quantify the absolute magnitude of transmission, these data can provide a reliable representation of seasonal and inter-annual trends, especially for arboviruses that trigger acute short-term symptoms such as fever or rash.

Aedes-borne viruses (ABVs) often have seasonal transmission dynamics underpinned by intra-annual climatic variation [336–339]. Temperature plays a major role in the seasonal dynamics of ABVs [340]. For example temperature has been associated with seasonal increases in dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) virus incidence [337,340–342]. The impact of climate on DENV transmission has received extensive attention, with a wide range of empirical data, statistical and mathematical modelling approaches being used to estimate its respective effects on the virus, host and vector populations [303,304,336,338,343–345][346–348]. While this provides a useful framework for understanding how seasonal climatic variation can impact ABV transmission, accurate prediction of seasonal disease dynamics in a particular setting likely requires localized data reflecting both climate, population susceptibility and vector ecology. Additionally as ABVs may vary in their response to climate [349]; pathogen-specific analysis may be required to predict the seasonality of viruses even when they are transmitted by a common vector. Amongst ABVs, seasonality has been most widely studied in DENV [323], but there is poorer understanding of this phenomenon in more recently emerged ABVs like chikungunya (CHIKV) and Zika (ZIKV). Thus comprehensive understanding of ABV transmission requires consideration of both pathogen-specific variation, and conditions of the focal setting, which may present unique climatic and demographic characteristics.

Investigation of seasonality in ABVs is often based on analysis of how individual microclimatic variables impact *Aedes* vectors under laboratory and field conditions; and/or associations between large-scale climatic phenomena (e.g., El Niño Southern Oscillation - ENSO), *Aedes* vector populations and DENV incidence [336,350,351]. The most common climatic variables incorporated in models of *Aedes* population dynamics are temperature and rainfall [303,304]. Various estimates of temperature including minimum, maximum, mean and diurnal temperature range (DTR) have been associated with *Aedes* population growth rate, fertility, longevity, survival and behaviour of *Aedes* mosquitoes [246,331,352–354]. In particular, temperature has been shown to be positively correlated to the development rate of *Ae. aegypti* mosquitoes [329,355], which leads to a faster population growth [331]. In addition, the speed of which *Ae. aegypti* digests a bloodmeal also been seen to increase as temperature rises [356]. Temperature also impacts the developmental success of arboviruses within *Aedes*, with the extrinsic incubation period of DENV and ZIKV (EIP – time from when the virus is ingested until it can be transmitted by the mosquito in the next blood meal) decreasing at higher temperatures [324,342]. This effect may have strong epidemiological consequences as reducing the EIP means a substantially higher proportion of mosquitoes will survive long enough to become infectious [357–359]. Temperature can also affect the susceptibility of mosquito vectors to infection [338,360]. For example, temperature can trigger physiological changes in larval or adult mosquitoes that impact their immune responses, and that can modulate the infectiousness and replication rate of viruses in *Aedes* [361–365]. Given the crucial role of temperature for vectors, arboviruses and their interaction, it is likely to be a major predictor of seasonal and spatial patterns of disease incidence.

In addition to temperature, rainfall is hypothesized to be an important driver of arboviral transmission on account of its strong association with *Aedes* population dynamics [328]. The impact of rainfall on *Aedes* mosquito populations is most pronounced on larval stages because they require aquatic habitats for development [111,328]. In urban environments in South America, typical larval habitats include artificial containers such as plastic, metal and cement ground tanks, trays, tires and generally discarded material that collect rain water [307]. *Aedes* mosquitoes require about one week to develop from egg to pupae in aquatic

habitats before emerging as adults [111]. Larval development success depends on temperature [329] and rate of evaporation from aquatic habitats [366]. Thus, the availability of aquatic habitats for larval development, container types, source of water (rain or tap), and purpose of use are key limiting factor of mosquito population growth [295,305,307,367–369]. Although rainfall can enhance *Aedes* larval populations, too much water can also be detrimental by causing an "overflow effect" that drives larvae out of the breeding site [308,309]. At a larger scale, rainfall and drought patterns throughout the year affect the availability of water for larval growth and the proliferation of *Aedes* mosquito populations [345,370]. Human populations that have limited access to tap water tend to store water more frequently during times of drought [295,305,306]; which also increases the availability of *Aedes* breeding sites during extended periods of dry weather when water storage increases [295,305,306,371]. Thus through its impact on both human and mosquito resource use, seasonality in *Aedes* populations may be heavily influenced by rainfall.

Temperature and rainfall are often assumed to be proxies for disease transmission due to their association with *Aedes* abundance [336]. However, the value of *Aedes* population size as a predictor of arboviral incidence is uncertain given entomological and epidemiological data are often weakly correlated [197,372,373]. This is likely due to other non-entomological determinants including as host factors (population immunity, socioeconomic status and movements [177,238,298,374]) and other virus and vector specific factors [303,336]. While the effects of climate on arboviral transmission are undoubtedly mediated through vectors, a comprehensive understanding of these relationships requires direct analysis of epidemiological data.

To understand the impact of climatic variation and arboviral transmission in coastal Ecuador, here I analysed multi-year (2013-2018) data on arboviral incidence based on cases of DENV, CHIKV, and ZIKV reported in two cantons of the Ecuadorian coast. In addition to climatic factors, I also investigated relationships between reported incidence and *Aedes* abundance in these cantons based as estimated from entomological surveillance carried out between 2016-2017 as described in Chapter 3. Specific objectives were to: (i) Characterize and compare the annual incidence patterns of three *Aedes*-transmitted viruses (DENV, CHIKV, and ZIKV); (ii) evaluate associations between concurrent or lagged climatic

conditions on the weekly incidence of each ABV; and within the time period for which entomological and epidemiological data were available; and (iii) test for associations between adult *Aedes* vector abundance and recorded cases of dengue. This study will provide an understanding of the role of climate in seasonal and interannual variation in ABV transmission within these high burden settings.

4.2. METHODS

4.2.1. Study sites and data description

This study used epidemiological, climatological, and population data from the cantons of Portoviejo and Quinindé, located in the coastal region of Ecuador (as described in Chapter 1, study sites).

Epidemiological data consisted of the number of DENV, ZIKV, and CHIKV cases reported in each canton per epidemiological week (EW) starting from EW10 in 2013 up to EW 52 in 2018. The reported date corresponded to when the patient reported the onset of symptoms. Case reports were collected through passive surveillance procedures based on reporting at public and private health facilities (HF) in Portoviejo (71 HF) and in Quinindé (40 HF) [375]. "Epidemiological weeks" start on Mondays and refer to the week in the year when a case was reported, with the first "EW" ending at least four days into the new year, therefore starting between December 29th and January 4th, according to the definition from the World Health Organization [376]. Epidemiological data was obtained from the National Directorate of Epidemiological Surveillance from the Ministry of Health of Ecuador, through the SIVE-ALERTA monitoring system.

Daily climatological data for each of the study cantons over the study period were obtained from the National Institute of Meteorology and Hydrology of Ecuador (INAMHI; from one meteorological station located in each canton). In the case of the Quinindé canton, data were derived from the M0156 meteorological station located at 0.316 ° N, 79.469 ° W, at an altitude of 109 m.a.s.l. For the Portoviejo canton, data were obtained from the M1208 meteorological station (1.164 ° S, 80.390 ° W, altitude of 60 m.a.s.l.). Data obtained were daily records of mean, minimum and maximum temperature, and daily rainfall from 2013 to 2018.

Annual estimates of the populations size of each canton were derived from projections made by the National Institute of Statistics and Censuses of Ecuador (INEC), based on the last national census carried out in 2010, and adjusted to the local conditions of each canton until 2020 [166]. In this study, annual population estimates from 2013 to 2018 were used for the cantons of Quinindé and Portoviejo.

Finally, entomological data were obtained from the two study sites during six months of the wet season from November 2016 to April 2017. Sampling was conducted for three consecutive days in each canton at houses from urban and peri-urban neighbourhoods. Indoor and outdoor Prokopack aspirations as well as BG-Sentinel (BGS) traps were used to collect mosquitoes and weekly aggregated data was used for the purpose of these analyses. Full details of entomological sampling are given in Chapter 3.

4.2.2. Data analysis

Climatological, epidemiological and population data was compiled for statistical analyses. All statistical analyses and data manipulation were done in R 3.6.2 [377] and RStudio 1.1.419. Daily climate data were used to calculate weekly mean values using the *dplyr* package [378]. The allocation of dates with the corresponding EW number was carried out using the package *epical* [379]. Additionally, daily precipitation values were summed to obtain the accumulated precipitation (mm) within each EW during the study period for each canton.

To calculate the weekly incidence of DENV, ZIKV and CHIKV, weekly counts of cases reported in the cantons of Portoviejo and Quinindé were divided by the estimated annual population size as projected by INEC. Dengue transmission occurred annually in both cantons, thus data on incidence was available for all years between 2013-2018. In contrast, outside the CHIKV and ZIKV outbreak years (2015 and 2016, respectively), <150 cases were detected between the two cantons. Seasonal dynamics could not be reliably inferred from sporadic cases in those low transmission years, thus analysis of CHIKV and ZIKV incidence was limited to outbreak years. Plots for visualizing weekly trends in incidence were created using the package *ggplot2* [288].

Generalized Additive Models (GAM) from the *gamm4* package [380] were used to test for associations between weekly arboviral incidence and climatological variables, epidemiological week, year of occurrence, and cantons. GAMs were used as arbovirus incidence was expected to be seasonal, and follow similar seasonal patterns between years (for DENV). Therefore, epidemiological week was incorporated into models as a smoothing function using a t2 tensor and a cubic regression cyclical spline method, which assumes continuous periodicity between consecutive years (i.e. continuity of the incidence pattern between December of the precedent year and January of the following year) [381]. In these analyses, associations between incidence and current and lagged climatological variables were investigated; with the latter intended to capture delays between the ecological impact of climate variable on mosquito vectors, and the subsequent infection and reporting process. Specifically, current evidence suggests it takes approximately one week between the time people are infected and the development of symptoms that would trigger reporting to a clinic [111]. Additionally, there may be further lags between environmental conditions and epidemiological processes depending on which part of the *Aedes* life cycle they affect. For example, DENV has an extrinsic incubation period of approximately 1.5 weeks [111]; meaning that infected vectors would have been alive for at least 2 weeks at the adult stage before transmitting the pathogen, as they blood feed after three days of emergence [111]. Furthermore, several environmental variables may have their greatest impact on the larval stages of *Aedes* (occurring 1-2 weeks before adult emergence). To capture delayed impacts arising from environmental conditions at the time of *Aedes* larval development, adult emergence and infection; five different lags of weekly cumulative rainfall before case reporting were included in models. Temperature data (minimum, maximum and average per week) for both the EW of case reporting and one week before were also incorporated. Only a one-week lag was considered for temperature data, as this period is thought to capture the week when the infected mosquito bit the person.

As data for the three arboviruses spanned different years, separate models were constructed for DENV, CHIKV and ZIKV. Additionally, data for ZIKV was only available for Portoviejo, as there were too few cases reported from Quinindé during 2016 for analysis (n =13). A total of 6,425 CHIKV cases were recorded in

Portoviejo and Quinindé in 2015. However, only 1,387 of these case records could be used in analysis because the rest were not recorded in a manner where the EW could be assigned. Similarly from a total of 828 cases of ZIKV in Portoviejo, only 402 were included in analysis as the remaining could not be assigned to a specific EW.

Before fitting the models, I tested for collinearity between predictor variables (a measure of correlation between variables). This was evaluated by calculating the variance inflation factor (VIF) using the *corvif* function from Ieno and Zuur [382], and conducting a visual diagnosis for patterns using a scatterplot matrix. Variables with a VIF value of less than 3 were retained in the models, and those with VIF values between 3 and 5 were only kept if the Pearson's correlation index between a pairwise comparison of each variable was not 0.8 or above. If visual inspection of the scatterplots showed a non-linear relationship, any of the variables compared was dropped from the model. Model selection was carried out through backward stepwise elimination of terms from a maximal model. At each step, predictor variables with the highest p-value were dropped, one by one, until the *p*-value of the remaining predictor variables were all <0.05. Predicted relationships between environmental variables and incidence were plotted from model output using the *ggplot2* package [288].

Finally to address the third objective, analyses were performed on the subset of epidemiological data for which mosquito surveillance data (as described in Chapter 3) was also available. Here, the aim was to test for associations between weekly DENV incidence and mean *Aedes* abundance as estimated for that same week, and one and two weeks before a DENV case was reported. First, weekly estimates of female *Ae. aegypti* abundance were obtained from collections made in Portoviejo and Quinindé in November 2016, and January, March & April 2017. Generalized Linear Mixed Models (GLMM) were used to estimate the mean weekly abundance of female *Aedes* for each of the collection weeks. Here, female *Aedes* abundance was modelled as a function of EW of collection, canton, and mosquito collection method (all fixed effects), with collection day, household ID and trap ID included as random effects. In scenarios where the 3 consecutive days of mosquito collections did not fall in the same epidemiological week, the assigned EW was that corresponding to at least two of the three days of mosquito sampling.

The response variable was fit to a negative binomial distribution to account for overdispersion.

Mean values of *Aedes* abundance obtained from these models were tested for association with weekly DENV incidence. Three separate Generalized Linear Models (GLM) were used to model DENV incidence as a function of female *Aedes* abundance during the same week of collection, and one and two weeks after the entomological surveillance took place. Each of the three models included canton and female *Aedes* abundance as estimated from each of the three trapping methods (BGS trap, indoor and outdoor Prokopack aspirations) as fixed effects.

Model selection in all sets of models were made through backwards step-wise elimination using the *drop1* function from *stats* package [377]. General Linear Hypotheses tests (GLHT – *Post Hoc* Tukey tests for GLM) using the package *multcomp* were carried out to test for statistical differences between cantons if and when resulted significant in the final model [286].

4.3. RESULTS

4.3.1. Characterization of arboviral incidence

Between the study period of 2013 – 2018, a total of 16,944 cases of DENV, CHIKV and ZIKV were reported in the study sites (Table 4.1). Of all reported ABV cases during this period, the majority were DENV (55.97%;N= 9,484), which was reported on all years between 2013-2018 (Figure 4.1). Most of the CHIKV and ZIKV cases were reported during the outbreak years of 2015 and 2016 respectively, with CHIKV representing 38.34% (N= 6,496), and ZIKV 5.69% (N= 964) of total ABV cases (Table 4.1, Figure 4.2).

Across the study period, the highest annual incidence of DENV was reported in 2015 which accounted for 44.84% (N= 4,253) of all DENV cases (Table 4.1; for ease of visualization, the 2015 incidence data is shown separately; Figure 4.2). During the same year, the CHIKV outbreak occurred and accounted for 98.91% (N= 6,425) of CHIKV cases reported. During the outbreak year in 2016, 87.45% of all ZIKV cases reported during the study occurred.

Table 4. 1. Reported cases of Zika, dengue and chikungunya in Portoviejo and Quinindé between 2013-2018.

	ZIKA		DENGUE		CHIKUNGUNYA	
YEAR	Quinindé	Portoviejo	Quinindé	Portoviejo	Quinindé	Portoviejo
2013	0	0	33	593	0	0
2014	0	0	95	653	0	0
2015	0	0	905	3348	725	5700
2016	15	828	692	1640	54	13
2017	11	110	87	1116	0	4
2018	0	0	25	297	0	0
TOTAL	26	938	1837	7647	779	5717

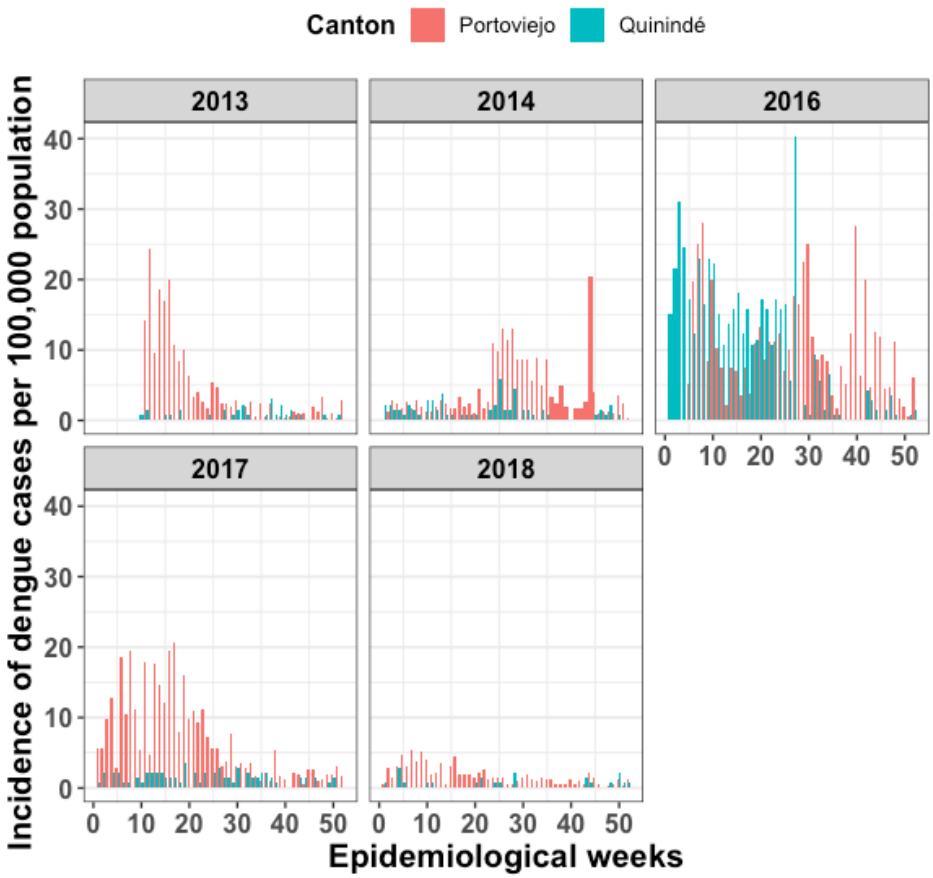

Figure 4. 1. Weekly reported dengue incidence estimated from cases reported between 2013-2018. Incidence is shown from 2013 to 2018, with the exception of 2015.

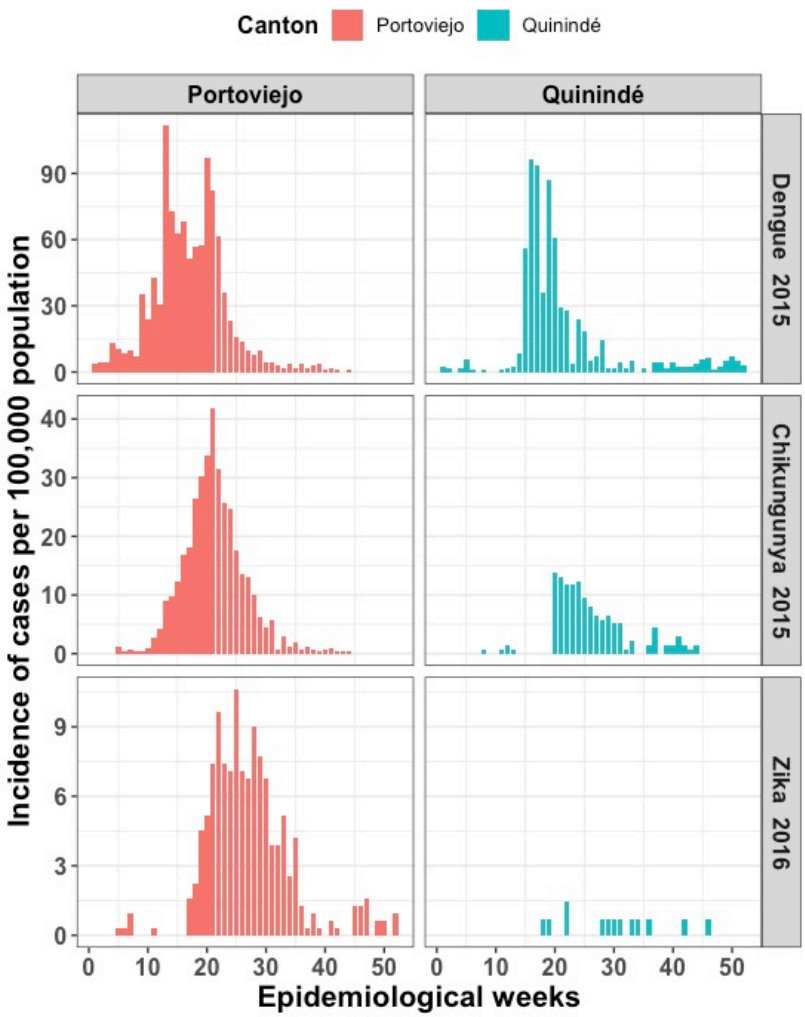

Figure 4. 2. Weekly reported incidence of dengue, chikungunya and Zika estimated from cases reported during the major outbreak years since 2013. Chikungunya and dengue outbreaks occurred in 2015, while Zika outbreak occurred in 2016.

DENV transmission was evident within all months of the year, with peaks occurring during first half of some years (2013, 2015 and 2017), the second half of others (2014), and no visually discernible peaks in others (2016 and 2018) (Figure 4.1). In 2015, CHIKV transmission peaked in the first half of the year, whereas ZIKV incidence peaked around the middle of 2016 (Figure 4.1).

4.3.2. Temporal and climatological influence on arboviral incidence

4.3.2.1. Dengue incidence

Statistical analysis was conducted to identify potential climatic drivers of seasonal variation in DENV incidence. Preliminary analysis indicated there was no strong collinearity between the eight environmental variables considered, as defined by having a VIF of 3-5 or above [382] (Table 4.2). Only “mean temperature” showed evidence of possible collinearity with “maximum temperature” as the VIF value of the former was 3.76; and the scatterplot matrix of these 2 variables showed a linear relationship, with a Pearson’s correlation coefficient of 0.7 (Figure 4.3, [382]). However, both variables were kept in the full model because the VIF value was less than 5 and the Pearson's correlation did not exceed the 0.8 threshold as explained in the Methods section.

Table 4. 2. Collinearity analyses for dengue incidence models. The variance inflation factor (VIF) values are shown for each explanatory variable. Values between 3 and 5 indicate possible collinearity [382], and values below 3 indicate no collinearity.

Explanatory variables	Variance inflation factor (VIF)
Mean temperature (1 week lag)	3.78
Minimum temperature (1 week lag)	1.59
Maximum temperature (1 week lag)	2.59
Cumulative weekly rainfall (1 week lag)	1.58
Cumulative weekly rainfall (2 weeks lag)	1.73
Cumulative weekly rainfall (3 weeks lag)	1.77
Cumulative weekly rainfall (4 weeks lag)	1.70
Cumulative weekly rainfall (5 weeks lag)	1.50

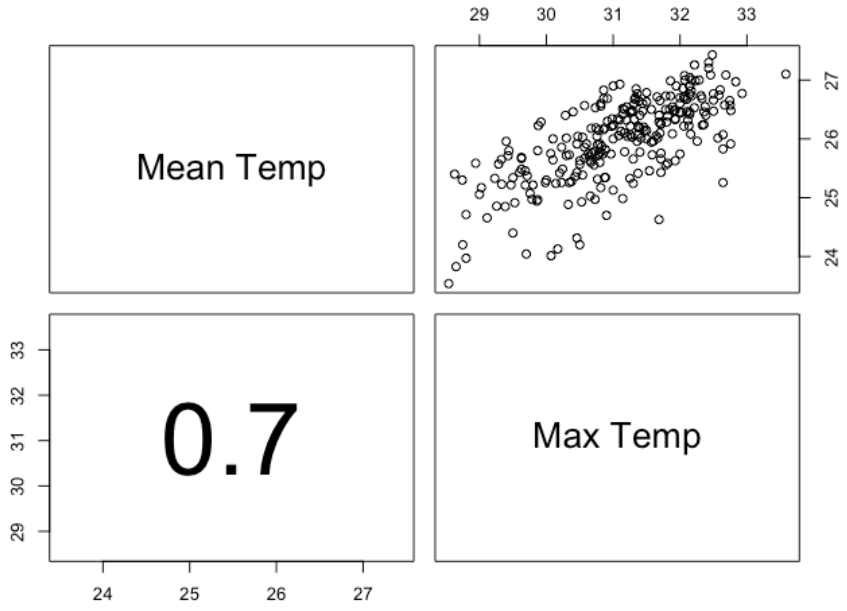

Figure 4. 3. Visualization for collinearity for dengue virus models. A scatterplot matrix displaying potential patterns of correlation between "mean temperature" and "maximum temperature", both measured in °C. Upper left and lower right panes indicate the name of the variables, upper right pane shows a scatterplot of the raw data, and lower left the Pearson's correlation coefficient.

Variation in DENV incidence was best explained in a final model that included year, canton, maximum temperature, and two lagged cumulative rainfall variables representing rainfall occurring 1 and 2 weeks before case reporting (Table 4.3). Intra-annual variation in DENV incidence was largely driven by the significant increase in 2015 relative to all other years (Table 4.3); however pairwise post-hoc analysis indicated that DENV incidence was significantly different between all study years except for 2013 and 2014 (Figure 4.4, Table 4.4). DENV incidence was significantly different between the two study areas (Table 4.3, Figure 4.5), being about 1.75 times higher in Portoviejo compared to Quinindé. The final model also confirmed strong seasonality in DENV transmission, as reflected by the significance of the temporal smoothing term for epidemiological week (X^2= 438.7, effective degrees of freedom (edf)= 2.94, p= < 0.001; Figure 4.4 and 4.5). This indicates significant within-year (seasonal) variability in DENV incidence, following a general pattern of increase in the first half of the year, before peaking around the 16th epidemiological week. Weekly DENV incidence was also positively associated with the mean weekly maximum temperature (Figure 4.6), and cumulative precipitation one and two weeks before case reporting (Figure 4.7).

Table 4. 3. Summary table of statistical significance of explanatory variables tested for association with dengue incidence. Significance values for each of the explanatory variables from the fitted models. Values of chi-square (X^2), degrees of freedom (df), and *p*-values for each of the predictors tested are shown. Bold values with an asterisk (*****) indicate significant terms.

Explanatory variables	X^2	df	*p*-value
Year	4701.86	5	< **0.001***
Canton	593.66	1	< **0.001***
Mean temperature (1 week lag)	0.005	1	0.95
Minimum temperature (1 week lag)	1.09	1	0.30
Maximum temperature (1 week lag)	37.48	1	< **0.001***
Cumulative weekly rainfall (1 week lag)	8.82	1	< **0.01***
Cumulative weekly rainfall (2 weeks lag)	88.98	1	< **0.001***
Cumulative weekly rainfall (3 weeks lag)	2.91	1	0.09
Cumulative weekly rainfall (4 weeks lag)	2.60	1	0.11
Cumulative weekly rainfall (5 weeks lag)	2.05	1	0.15

Table 4. 4. Summary table of statistical significance of the pairwise Post-Hoc test of the "year" category for association with dengue incidence.
Significance values for each of the pairwise comparison between each level of the "year" explanatory variable. Z-values and *p*-values for each of pairwise comparison are shown. Bold values with an asterisk (*) indicate significant levels.

Explanatory variables	***Z*-value**	***p*-value**
2013 – 2014	-1.56	0.61
2013 – 2015	36.83	< **0.001***
2013 – 2016	21.17	< **0.001***
2013 – 2017	6.52	< **0.001***
2013 – 2018	-13.64	< **0.001***
2014 – 2015	42.68	< **0.001***
2014 – 2016	24.93	< **0.001***
2014 – 2017	8.80	< **0.001***
2014 – 2018	-12.76	< **0.001***
2015 – 2016	-24.79	< **0.001***
2015 – 2017	-40.12	< **0.001***
2015 – 2018	-44.39	< **0.001***
2016 – 2017	-18.46	< **0.001***
2016 – 2018	-32.11	< **0.001***
2017 – 2018	-20.15	< **0.001***

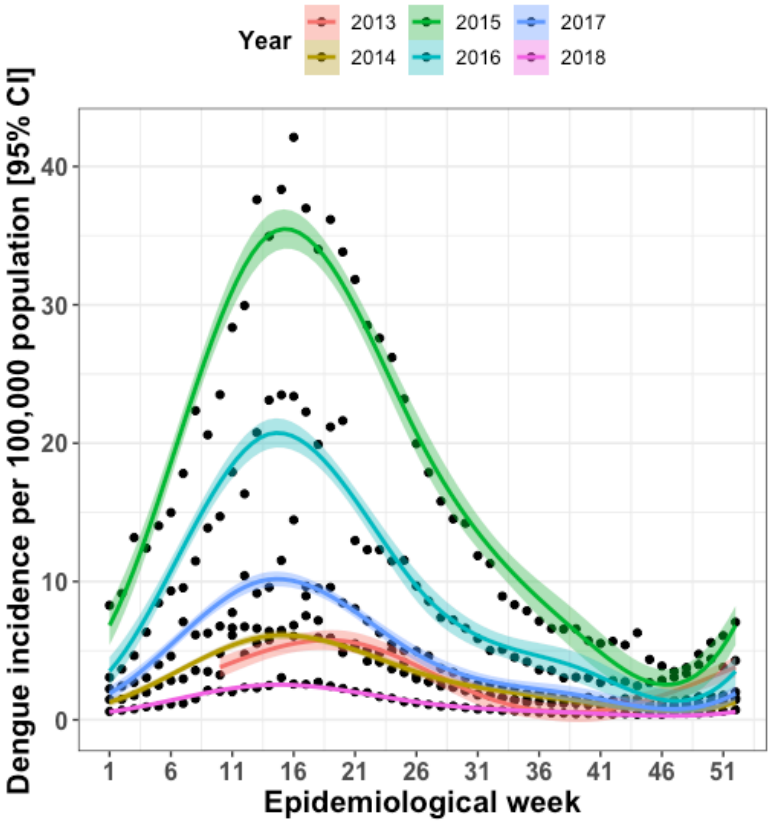

Figure 4. 4. Interannual variation of dengue incidence. Predicted mean weekly incidence of dengue virus in two cantons in Coastal Ecuador between 2013-2018, which are represented by the black dots. The seasonal smoothing function predicted by the GAM for each of the two cantons is represented by the solid lines. Shaded areas around the solid lines indicate the 95% confidence intervals.

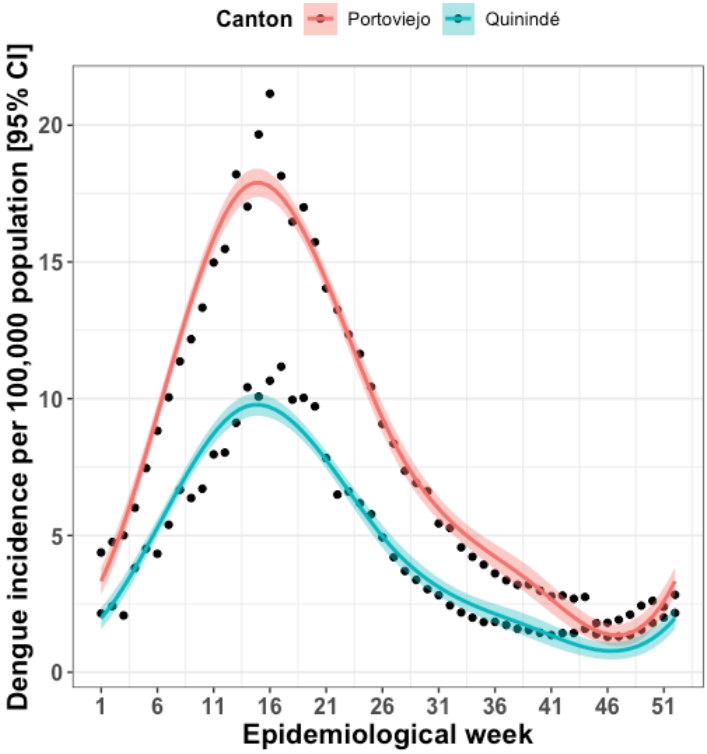

Figure 4. 5. Within year (seasonal) variation of dengue incidence. Predicted mean weekly incidence of dengue virus in two cantons in Coastal Ecuador between 2013-2018, which are represented by the black dots. The seasonal smoothing function predicted by the GAM for each of the two cantons is represented by the solid lines. Shaded areas around the solid lines indicate the 95% confidence intervals.

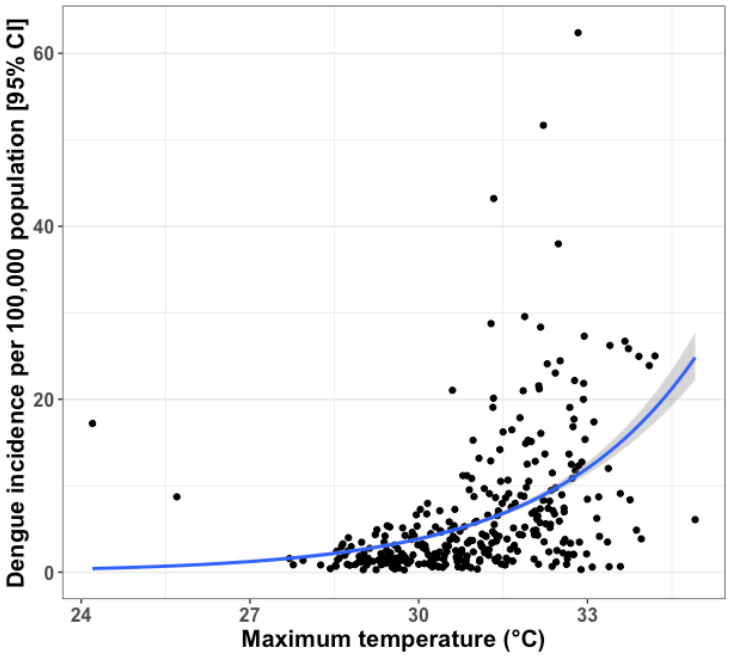

Figure 4. 6. Predicted association between maximum temperature and weekly dengue incidence in two cantons in Coastal Ecuador between 2013-2018. X-axis corresponds to the mean weekly values of maximum temperature (C°), and Y-axis represents the reported dengue incidence per 100,000 population. Black dots indicate the fitted values, and the blue line represents the predicted relationship. Shaded area around the blue line indicates the 95% confidence intervals for the prediction.

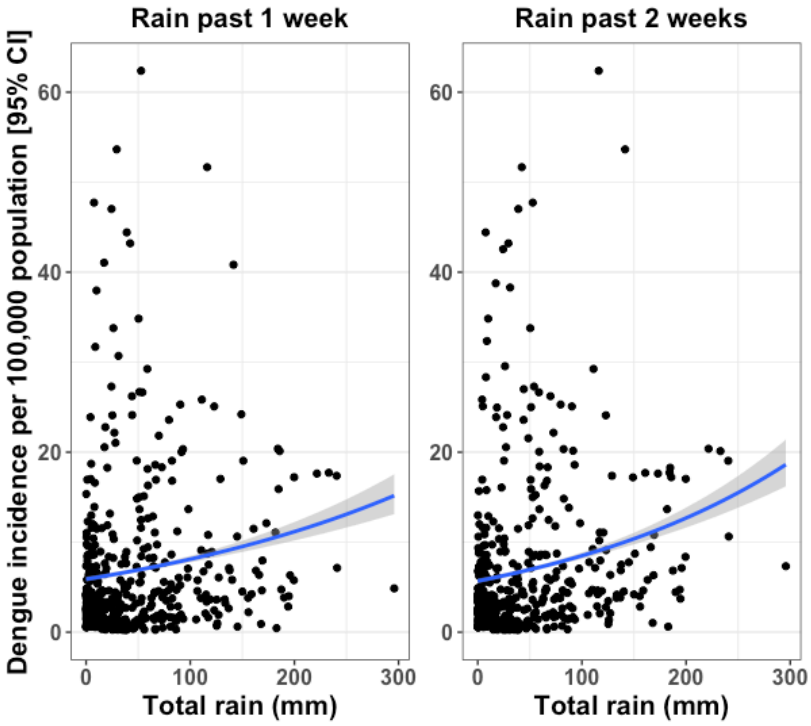

Figure 4. 7. Effect of rainfall on dengue incidence in two cantons in Coastal Ecuador between 2013-2018. X-axis shows the accumulated weekly rainfall recorded in mm, and Y-axis represents the dengue incidence per 100,000 population. Past rain corresponds to accumulated rainfall recorded over an entire week, with "Rain past 1 week" corresponding to the 7 days before case reporting and "Rain past 2 weeks" corresponding to 8-14 days before case reporting. Thus, left and right panes correspond to the effect of one week lag and two weeks lag, respectively, on the incidence of dengue. Fitted values are represented by the black dots and the blue lines represent the predicted relationships. Shaded areas around the blue lines indicate the 95% confidence intervals.

4.3.2.1. Chikungunya incidence

There was no evidence of strong collinearity between the 8 environmental variables tested for association with CHIKV incidence (2015 only, Table 4.5). The highest Pearson's correlation coefficient was of 0.7 between "mean temperature" and "maximum temperature". The scatterplot matrix did not show any non-linear correlation between these two variables (Figure 4.8). Both variables were kept in the full model because the VIF value was less than 3 and the Pearson's correlation did not exceed 0.8, as explained in the Methods section.

Table 4. 5. Collinearity analyses for chikungunya virus models. The variance inflation factor (VIF) values are shown for each explanatory variable. Values between 3 and 5 indicate possible collinearity [382], and values below 3 indicate no collinearity.

Explanatory variables	Variance inflation factor (VIF)
Mean temperature (1 week lag)	2.44
Minimum temperature (1 week lag)	1.33
Maximum temperature (1 week lag)	2.01
Cumulative weekly rainfall (1 week lag)	1.25
Cumulative weekly rainfall (2 weeks lag)	1.43
Cumulative weekly rainfall (3 weeks lag)	1.41
Cumulative weekly rainfall (4 weeks lag)	1.30
Cumulative weekly rainfall (5 weeks lag)	1.47

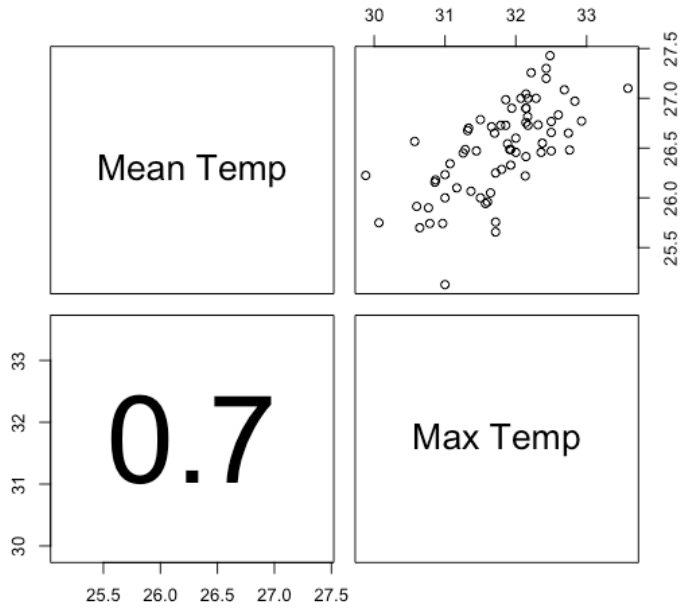

Figure 4. 8. Visualization for collinearity for chikungunya virus models. A scatterplot matrix displaying potential patterns of correlation between "mean temperature" and "maximum temperature", both measured in °C. Upper left and lower right panes correspond to the name of the variables, upper right pane shows a scatterplot of the raw data, and lower left the Pearson's correlation coefficient.

Seasonal variation in CHIKV incidence was best explained in a model that included canton, weekly mean and maximum temperature (Table 4.6). CHIKV incidence was higher in Portoviejo than in Quinindé (Table 4.6, Figure 4.9), corresponding to a difference of ~2.8 times during the peak week of transmission (~EW 21). There was also strong seasonality in CHIKV transmission as reflected by the significance of the temporal smoothing term of epidemiological weeks (X^2= 1516, edf= 2.94, p= < 0.001) (Figure 4.9). This seasonality was reflected by a single peak in incidence occurring at around the 21st epidemiological week; slightly later than the predicted peak for DENV (EW 16). Weekly CHIKV incidence was also positively associated with mean and maximum weekly temperatures (Figure 4.10).

Table 4. 6. Summary table of statistical significance of explanatory variables tested for association with chikungunya incidence. Significance values for each of the explanatory variables from the fitted models. Values of chi-square (X^2), degrees of freedom (df), and *p*-values for each of the predictors tested are shown. Bold values with an asterisk (*****) indicate significant terms.

Explanatory variables	X^2	df	*p*-value
Canton	88.38	1	**< 0.001***
Mean temperature (1 week lag)	11.20	1	**< 0.001***
Minimum temperature (1 week lag)	0.60	1	0.44
Maximum temperature (1 week lag)	7	1	**< 0.01***
Cumulative weekly rainfall (1 week lag)	0.01	1	0.93
Cumulative weekly rainfall (2 weeks lag)	0.50	1	0.48
Cumulative weekly rainfall (3 weeks lag)	0.03	1	0.85
Cumulative weekly rainfall (4 weeks lag)	0.05	1	0.82
Cumulative weekly rainfall (5 weeks lag)	2.88	1	0.09

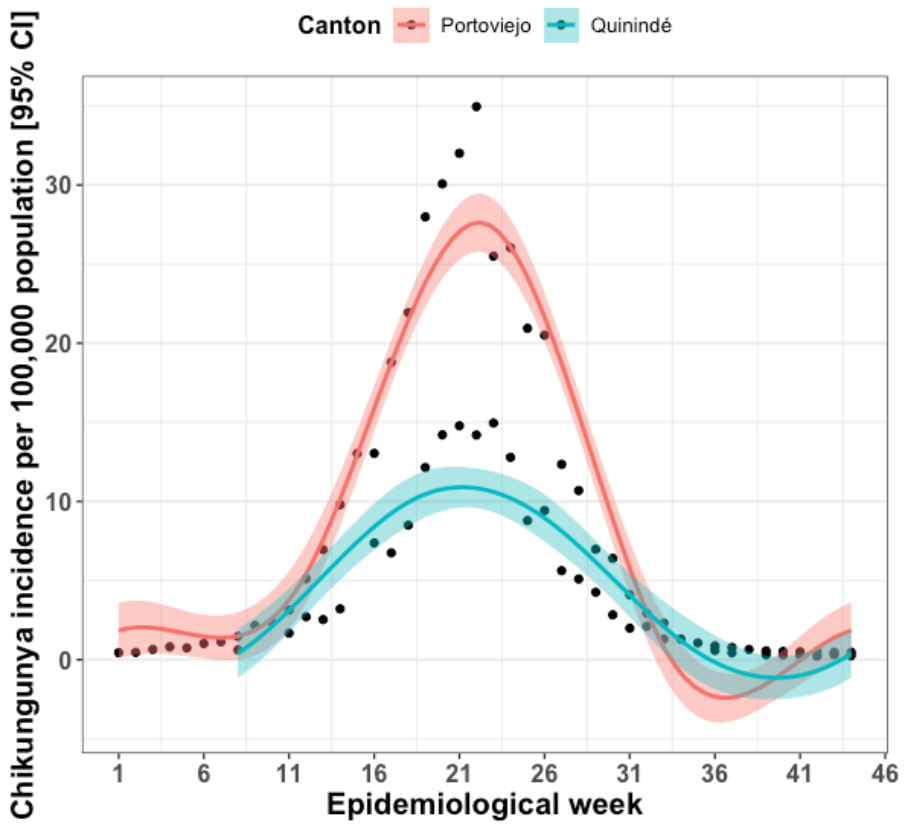

Figure 4. 9. Within year (seasonal) variation of chikungunya incidence. Predicted mean weekly incidence of chikungunya virus in two cantons in Coastal Ecuador in 2015, which are represented by the black dots. The seasonal smoothing function predicted by the GAM for each of the two cantons is represented by the blue lines. Shaded areas around the blue lines indicate the 95% confidence intervals.

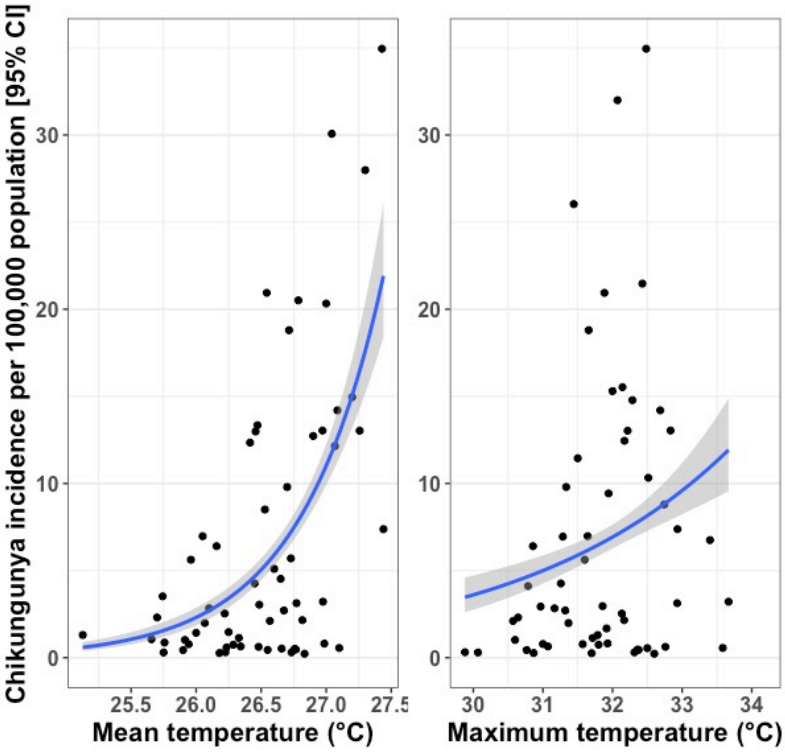

Figure 4. 10. Effect of temperature on chikungunya incidence in two cantons in Coastal Ecuador in 2015. X-axis corresponds to the recorded temperature (C°), and Y-axis represents the chikungunya incidence per 100,000 population. Left and right panes correspond to the effect of weekly mean temperature (C°) and mean weekly values of maximum temperature (C°), respectively, on the incidence of chikungunya. Fitted values are represented by the black dots and the blue lines represent the predicted linear relationships using a Poisson distribution. Shaded areas around the blue lines indicate the 95% confidence intervals.

4.3.2.1. Zika incidence

Preliminary analysis indicated possible collinearity between the 3 temperature variables used in the analysis of ZIKV incidence (analysis included only data from Portoviejo in 2016). The initial VIF test estimated values as high as 19.2, 17.38 and 6.58 for mean, minimum and maximum temperature, respectively. The Pearson's correlation coefficient between "mean temperature" and "minimum temperature" reached the threshold of 0.8 that indicates redundancy due to collinearity. Consequently, "minimum temperature" was dropped from the

analysis. After dropping “minimum temperature” variable, the VIF values of the other two temperature variables fell within accepted range (Table 4.7). The VIF value of “mean temperature” was slightly higher than 3, but after visualizing in the scatterplot matrix no obvious pattern was observed and thus it was retained (Figure 4.11).

Table 4. 7. Collinearity analyses for Zika virus models. The variance inflation factor (VIF) values are shown for each explanatory variable. Values between 3 and 5 indicate possible collinearity [382], and values below 3 indicate no collinearity. The VIF value for "Minimum temperature" term is shown before being dropped from the terms chosen for building the model. The rest of the VIF values shown are those after dropping "Minimum temperature" variable.

Explanatory variables	Variance inflation factor (VIF)
Mean temperature (1 week lag)	3.11
Minimum temperature (1 week lag)	17.38
Maximum temperature (1 week lag)	2.33
Cumulative weekly rainfall (1 week lag)	1.30
Cumulative weekly rainfall (2 weeks lag)	1.82
Cumulative weekly rainfall (3 weeks lag)	1.35
Cumulative weekly rainfall (4 weeks lag)	1.37
Cumulative weekly rainfall (5 weeks lag)	1.29

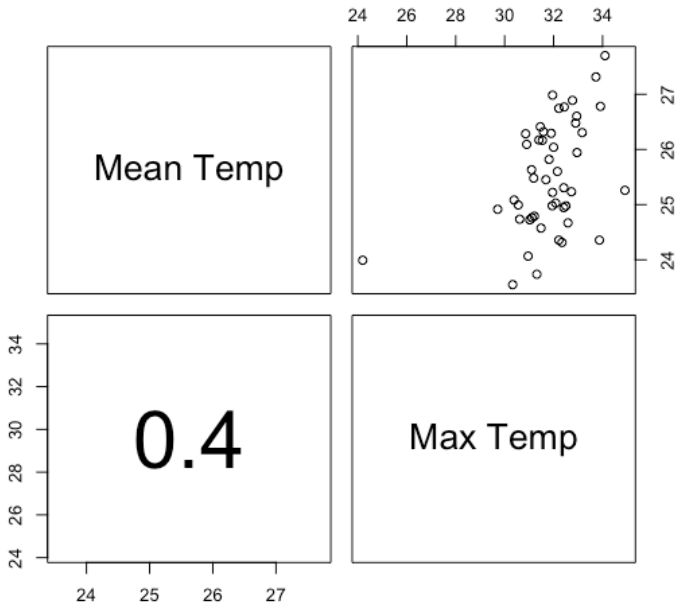

Figure 4. 11. Visualization for collinearity for Zika virus models. A scatterplot matrix displaying potential patterns of correlation between "mean temperature" and "maximum temperature" is presented. Upper left and lower right panes correspond to the name of the variables, upper right pane correspond to a scatterplot of the raw data and lower left pane shows the correlation coefficient. X and Y axis correspond to the units of the variables, which in this case is measured in °C.

Weekly variation in ZIKV incidence (from Portoviejo in 2016) was best explained in a model that included mean temperature and cumulative rainfall during 2 and 5 weeks before cases were reported (Table 4.8). In 2016, ZIKV transmission was highly seasonal as reflected by the significance of the temporal smoothing term of epidemiological weeks (X^2= 90.62, edf= 2.80, p= < 0.01, Figure 4.12). ZIKV incidence rose from near zero at the start of the year to reach a maximum at the 26th epidemiological week. In contrast to DENV and CHIKV, ZIKV incidence was negatively associated with mean temperature (Figure 4.13) and with rainfall from 2 and 5 weeks before (Figure 4.14).

Table 4. 8. Summary table of statistical significance of explanatory variables tested for association with Zika incidence. Significance values for each of the explanatory variables from the fitted models. Values of chi-square (X^2), degrees of freedom (df), and *p*-values for each of the predictors tested are shown. Bold values with an asterisk (*****) indicate significant terms.

Explanatory variables	X^2	df	*p*-value
Mean temperature (1 week lag)	7.36	1	**<0.01***
Maximum temperature (1 week lag)	0.47	1	0.49
Cumulative weekly rainfall (1 week lag)	0.94	1	0.33
Cumulative weekly rainfall (2 weeks lag)	4.48	1	**0.03***
Cumulative weekly rainfall (3 weeks lag)	0.18	1	0.67
Cumulative weekly rainfall (4 weeks lag)	1.10	1	0.29
Cumulative weekly rainfall (5 weeks lag)	4.08	1	**0.04***

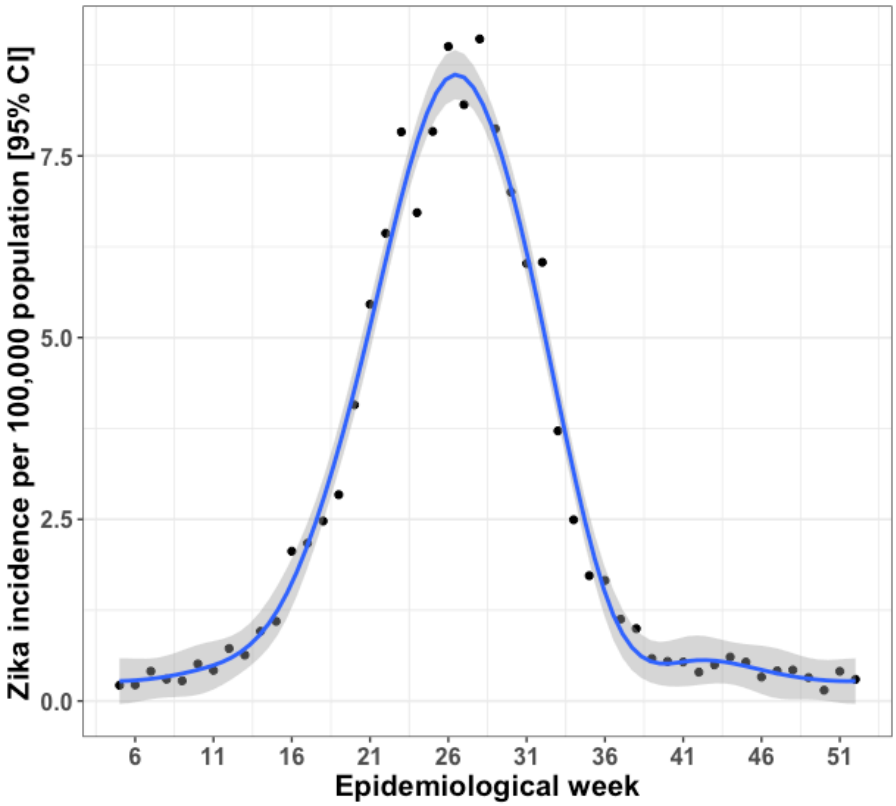

Figure 4. 12. Within year (seasonal) variation of Zika incidence. Predicted mean weekly incidence of Zika virus in Portoviejo during 2016, which is represented by the black dots. The seasonal smoothing function predicted by the GAM is represented by the blue line. Shaded area around the blue line indicates the 95% confidence intervals.

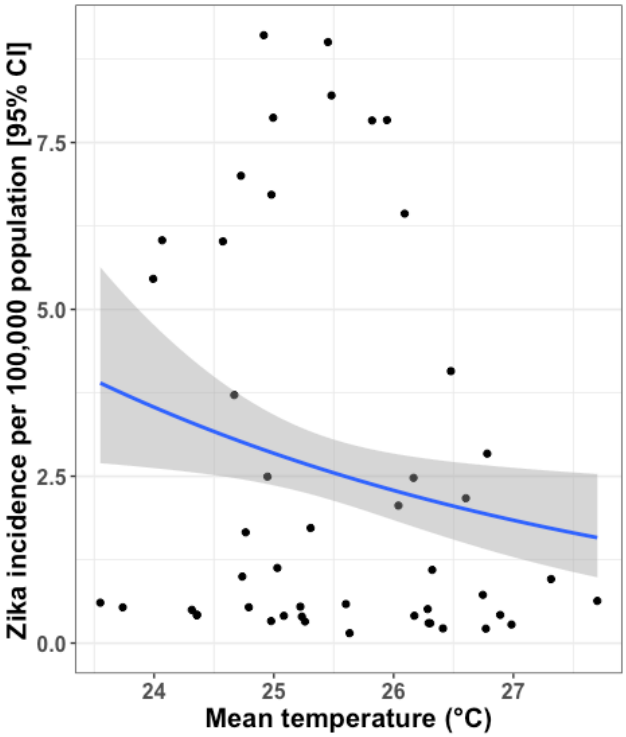

Figure 4. 13. Effect of temperature on Zika incidence in Portoviejo in Coastal Ecuador in 2016. X-axis corresponds to the mean recorded temperature (C°), and Y-axis represents the Zika incidence per 100,000 population. Fitted values are represented by the black dots and the blue lines represent the predicted relationships. Shaded areas around the blue lines indicate the 95% confidence intervals.

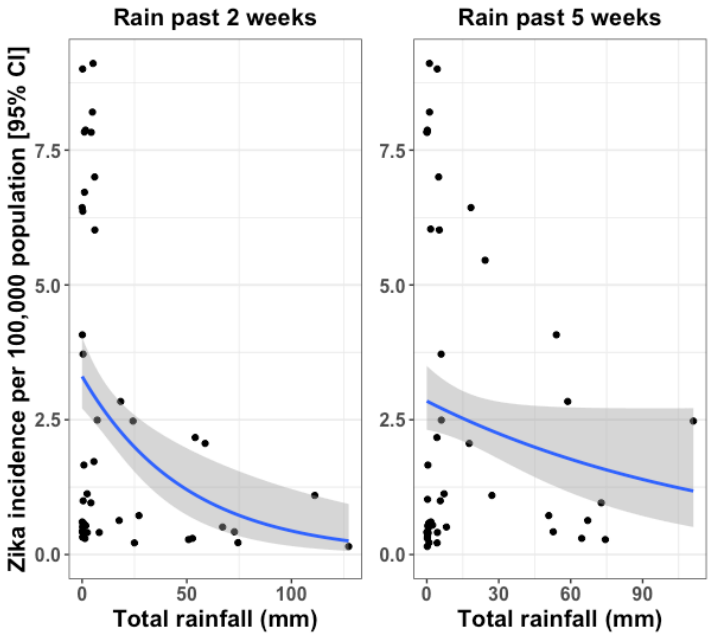

Figure 4. 14. Effect of rainfall on Zika incidence in Portoviejo in Coastal Ecuador in 2016. The X-axis corresponds to the accumulated weekly rainfall recorded in mm, and Y-axis represents the Zika incidence per 100,000 population. Left and right panes correspond to the effect of two and five week lags, respectively, on the incidence of Zika. Fitted values are represented by the black dots and the blue lines represent the predicted relationships. Shaded areas around the blue lines indicate the 95% confidence intervals.

4.3.3. Association between Aedes vector abundance and dengue incidence

Statistical analyses were performed to determine associations between mean *Ae. aegypti* abundance as estimated for each study site and DENV incidence on the concurrent week, and one and two weeks afterwards. These analyses were performed just on the subset of incidence data for which temporally linked entomological data were available. Between the study months where entomological and epidemiological data were available (November 2016 – April 2017); DENV incidence was significantly associated with canton and *Aedes* abundance (Table 4.9); however the nature of the association varied somewhat between mosquito sampling methods. The mean abundance of *Ae. aegypti* in BGS traps and in outdoor Prokopack aspirations were positively associated with concurrent DENV incidence (same week), and outdoor Prokopack aspirations were positively correlated also with DENV incidence one week afterwards (Figure 4.15).

In contrast, there was a negative association between *Ae. aegypti* abundance in indoor Prokopack collections and concurrent DENV incidence and one week afterwards (Figure 4.15). There was no significant association between *Ae. aegypti* abundance and DENV incidence two weeks afterwards.

Table 4. 9. Summary table of statistical significance of explanatory variables tested for association with dengue incidence. Analysis based on a subset of incidence data corresponding to the timing of *Aedes* vector surveillance carried out in each canton between November 2016 and April 2017. Values of chi-square (X^2), degrees of freedom (df), and *p*-values for each of the predictors tested are shown. Bold values with an asterisk (*) indicate significant terms. "NA" indicates "not applicable" values for which single term significance was not possible because of their involvement in significant interaction terms.

Lag periods	Explanatory variables	X^2	df	*p*-value
0 week lag	Canton	51.85	1	**< 0.001***
	BG-Sentinel trap	6.10	1	**< 0.05***
	Indoor Prokopack aspiration	29.14	1	**< 0.001***
	Outdoor Prokopack aspiration	26.40	1	**< 0.001***
1 week lag	Canton	29.42	1	**< 0.001***
	BG-Sentinel trap	1.95	1	0.16
	Indoor Prokopack aspiration	16.65	1	**< 0.001***
	Outdoor Prokopack aspiration	23.90	1	**< 0.001***
2 week lag	Canton	6.98	1	**< 0.01***
	BG-Sentinel trap	0.17	1	0.68
	Indoor Prokopack aspiration	0.08	1	0.78
	Outdoor Prokopack aspiration	0.17	1	0.68

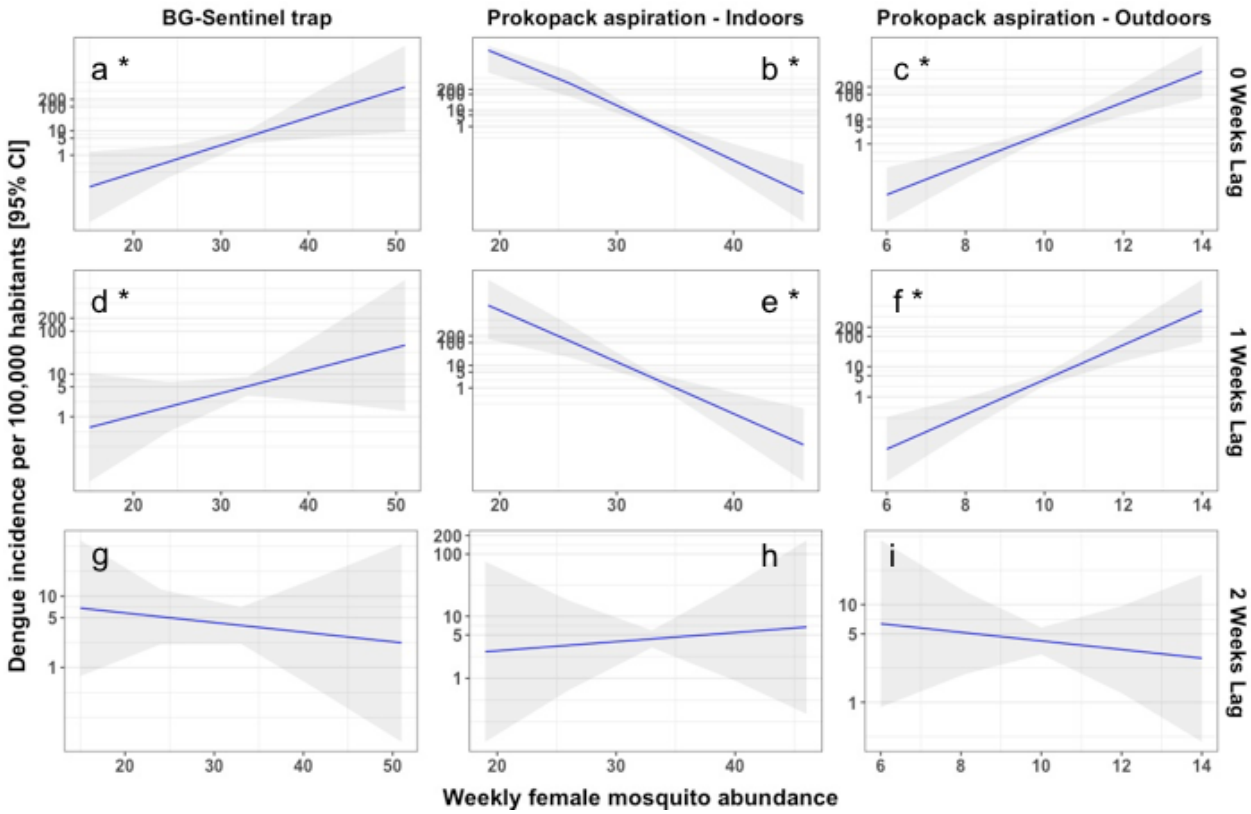

Figure 4. 15. Effect of female *Aedes* abundance on dengue incidence during 3 lag periods. Predicted mean incidence of dengue virus in Portoviejo and Quinindé during 2016 and 2017 given by female *Aedes* abundance. Columns represent the trapping method used to collect *Aedes* female mosquitoes, and rows represent the lag periods. Asterisks (*) next to the pane label indicate significant relationships. The trend of the relationship is represented by the solid blue line and shaded areas around the blue lines indicate the 95% confidence intervals.

4.4. DISCUSSION

In this study, an extensive dataset of clinical records of DENV, CHIKV and ZIKV was used to elucidate the incidence and seasonal dynamics of these ABVs within two major hotspots of transmission on the Ecuadorian coastal region. In the two study sites, DENV transmission was persistent over all years between 2013 and 2018, with significant CHIKV and ZIKV transmission occurring only in their respective 'outbreak' years of 2015 and 2016. The transmission of all three ABVs was highly seasonal, with most cases concentrated in the middle months of the year. Peak incidence varied somewhat between ABVS, occurring earliest for DENV (EW 16,

end of April), followed by CHIKV (EW 21, early June) and then ZIKV (EW 26, mid July). The incidence of DENV and CHIKV was higher in Portoviejo than Quinindé, with no comparison possible for ZIKV as it was only reported in Portoviejo. The importance of environmental predictors varied somewhat between ABVs. Weekly maximum temperature (one week lag from reporting) was positively associated with DENV and CHIKV, whereas ZIKV incidence was negatively associated with it. Cumulative rainfall occurring 1 and 2 weeks before reporting was positively associated with DENV, whereas lagged rainfall (2 and 5 weeks before reporting) had a negative association with ZIKV. DENV incidence was positively associated with the mean abundance of *Ae. aegypti* caught in BGS traps and resting in peri-domestic areas, however negatively associated with abundance in indoor Prokopack collections for the concurrent week and one week lag. These findings elucidate the role of temperature and rainfall in the dynamics of ABV transmission, and demonstrates that the predicted impact of environmental variables can vary between DENV, CHIKV and ZIKV even though they are transmitted by the same mosquito vector.

Arboviruses transmitted by *Ae. aegypti* have been recognized as a major public health problem in South America since the conquest of the Americas and the introduction of yellow fever virus. Despite attempts to eradicate *Ae. aegypti* was part of a PAHO-led continental yellow fever control programme in 1947 [383], the vector persisted in some countries and started spreading throughout from the continent from the 1960's with a subsequent increase in DENV outbreaks [23]. DENV transmission has rapidly accelerated in recent years, with cumulative cases in the Americas between 2011-2020 (16.5 million) doubling from those reported in 2001-2010 (7.8 million cases [157]). In Ecuador, the first DENV outbreak following *Ae. aegypti* reinfestation occurred in the 1970's, with a major outbreak in 1988 [384]. Since then, DENV transmission has been reported every year with major outbreaks occurring every 3-5 years at the regional level [385]. Studies from Ecuador and other South American settings indicate that major climatological events (e.g. El Nino) shape inter-annual DENV transmission [345,350,351], but with significant additional contributions from socio-economic and other local environmental variables, which make it hard to predict in which years major outbreaks may occur [177]. During the study period (2013-2018), the Americas as a whole experienced high DENV cases in 2013, 2015 and 2016 [157]. However in

our study sites in coastal Ecuador, a marked increase in DENV incidence was detectable only in 2015; with notable transmission still present in all other years. This persistence confirms that coastal Ecuador is one of the highest burden settings for DENV in South America, with reported cases in 2015 in Portoviejo (1068.42 cases per 100,000 population) and Quinindé (660.95 cases per 100,000 population) ranking 4th and 10th respectively within 50 countries and territories in the Americas that year [157]. It has also been noted in other countries in the Americas that DENV incidence drastically decreased after the regional ZIKV epidemics, probably due to cross-protection generated by ZIKV infection decreasing susceptibility to DENV infection in local populations [386,387].

In contrast to DENV, CHIKV transmission was more limited in this setting and mostly occurred in 2015. CHIKV epidemics in the Americas started in late 2013, with most countries experiencing a major outbreak in either 2014 or 2015, followed by extended scattered transmission until 2017 [52]. In the 2015 outbreak, the reported incidence of CHIKV in these study sites (1,853.88 cases per 100,000 people in Portoviejo; 529.49 in Quinindé) were considerably higher than the Ecuadorian average (179.67 cases per 100,000 population [52]); with these 2 cities ranking 5th and 14th compared to other countries and territories in the Americas [52]. Furthermore, the incidence of CHIKV is likely to have been significantly underreported as it is often misdiagnosed as DENV. For example, a study in Machala, southern Ecuador, based on active sero-surveillance found that 43.1% of those diagnosed with DENV actually had CHIKV, with a further 11.5% that were diagnosed as DENV only being positive for both diseases [162]. If this is also the case for these two study sites, incidence of CHIKV could be much higher than officially. Furthermore, active sero-surveillance of DENV and CHIKV conducted in Quinindé [388] revealed that seroprevalence of DENV was at 97% in people of 60 years old or more, being consistent of permanent exposure to DENV since the 1980's. In contrast, CHIKV seroprevalence averaged at 27% for all ages, with a peak of 42% in 9 years old children. The lack of a drastic peak of CHIKV seroprevalence in a specific group age was also consistent to the exposure to the virus on a single outbreak season.

ZIKV emerged in the Americas in 2015, causing major outbreaks in Brazil and Colombia. By the last week of 2015, it had arrived in Ecuador leading to an outbreak that peaked in later parts of 2016 and first half of 2017. There was

marked differences ZIKV transmission between the 2 study sites here, with only 13 reported cases in Quinindé compared to 388 in Portoviejo. The annual incidence of ZIKV in 2017 in Portoviejo (124.93 per 100,000 population) was still higher than Ecuador overall (2017, 19.15 per 100.000 population) but ranking only 32nd place among 52 other countries and territories. The lower intensity of ZIKV transmission may have been due to considerable under-reporting. Between 29% to 82% of ZIKV-infected individuals may be asymptomatic [389], thus would not be picked up by the passive surveillance system. Furthermore, the apparent difference between Portoviejo and Quinindé may have been affected differences in surveillance resulting from the occurrence of a 7.8 Richter scale earthquake in Manabí province, where Portoviejo is located, on the 16th of April of 2016. The Ecuadorian government redirected resources including medical teams to Manabí to mitigate this crisis. Residents of Portoviejo may thus have had more access to health care and diagnosis during this time than Quinindé.

Seasonality in DENV, CHIKV and ZIKV transmission has been previously documented [340], and associated with climatic variation that impacts *Ae. aegypti* vector populations and the proportion of susceptible human population exposed to infected bites [340]. The finding in this study of a single annual peak in DENV incidence matches reports from other South American where incidence peaks once either in the first or second half of the year [343,385,390]. The predicted timing of the DENV peak in our settings (EW 16, end of April) corresponds with the general peak observed between April and May at national level [344,345,391]. Notably this study found that the predicted timing of the seasonal peak varies between ABVs (DENV>CHIKV>ZIKV). This contrasts with modelling studies that predicted all three ABVs should have similar seasonal patterns of transmission, as they share a common mosquito vector species [340,346–348]. Several factors may account for the apparent difference in seasonal dynamics of ABVs observed here. First, the later peaks observed for CHIKV and ZIKV may be a result of the timing at which these arboviruses arrived in Ecuador rather than their innate biological seasonality after establishment. The first cases of CHIKV in Ecuador were reported at the end of the preceding 'outbreak' year (December 2014), and the first cases of ZIKV were reported at the beginning of the first 'outbreak' year (January 2016). Then, CHIKV cases peaked in the 22nd EW (mid May, 2015), while ZIKV cases peaked in the 25th EW (early June, 2016) and had high plateau-like incidence during the first

half of the year in 2017 [224]. Therefore, the apparent delay of the high peaks of CHIKV and ZIKV may have been due to the extra time needed to increase from very low numbers at introduction, in contrast to DENV which was endemically circulating. Models on future outbreak scenarios of ZIKV have successfully been able to reproduce the one and two year seasonal outbreaks observed in the region, and have concluded that further outbreaks would not continue in consecutive years due to the lack of susceptible human populations [392]. Other possible reasons for observing different peaking times of incidence among the three ABVs might be true differences of viral responses to temperature, or limitations in statistical power due to the lower sample sizes from having only one epidemic year of ZIKV and CHIKV, versus five years of data from DENV. Further investigation on these possible reasons may be needed to understand whether climate really impacts the seasonality of the three ABV, or whether peaking times are shaped by transmission dynamics along the year.

In this study, DENV incidence was positively associated with weekly mean values of maximum temperature one week before case reporting, and weekly cumulative rainfall falling one and two weeks previously. This matches findings from Machala, southwestern Ecuador, where lagged temperature and rainfall were found to be positively associated with DENV incidence [344,345]. Another study in northwest Ecuador found that DENV incidence had a positive association with minimum temperature, but in interaction with rainfall [391]. In the present study, interactions between rainfall and temperature were not tested, thus similar interaction effects cannot be ruled out here. Generally, increases in temperature have been associated with increased DENV transmission worldwide [31,324,337,393]. Temperature in the study sites was usually above 22°C with relatively small variation in the diurnal temperature, which has been seen to increase DENV transmission when compared to lower temperatures [337,338]. Rainfall has also been positively associated with DENV transmission [31,345], as linked to its effects on *Ae. aegypti* populations [328]. However, rainfall may also have negative effects on vector populations due to the direct effect on the suitability of the breeding site [308,309], or due to an interaction with social or other climatic variables [177] (See Chapter 3). In this study, a positive effect of both lagged temperature and rainfall were observed to be positively associated with DENV incidence, showing no association with larval stages of *Aedes*

mosquitoes but rather adult, host-seeking stages. Ideally, investigating the impact of climate on disease transmission should serve to help health authorities to prepare in advance to an epidemic [394]. Results from the present study indicate climate variables can also predict DENV incidence up to two weeks in advance from the reported cases, given a relatively short time window for response. However if these temperature and rainfall patterns are consistent between years, then the repeatability can be predicted.

Possibly due to the more limited occurrence CHIKV and ZIKV outbreaks in the Americas region, there have only been a few investigations of how their incidence is related to seasonal climatic variation [348,395,396]. These studies have found that extrinsic incubation period is reduced at higher temperatures, which may be more likely under climate change scenarios [342,364]. Other studies have focussed on modelling the predicted impacts of climate on outbreak size and speed. Huber et al. (2018) estimated that transmission speed and final epidemic size of DENV, CHIKV, and ZIKV increase with warmer temperatures, and are favoured under low temperature variability regimes [340]. Results from this study show positive associations between DENV and CHIKV incidence and temperature and rainfall. However, ZIKV incidence was negatively associated with the two variables. Previous studies of vector-pathogen interactions in terms of vector competence analyses and extrinsic incubation period [341,397] and may shed light on why the differential impact of environmental factors on ABVs suggested here. For example, *Ae. aegypti* appears to be more susceptible to CHIKV infection when reared at low temperatures [397], indicating CHIKV vectorial capacity could decrease at higher temperatures. However the opposite result was observed here, with CHIKV incidence being highest during the hottest periods of the year. This could be explained by a stronger influence of temperature on the extrinsic incubation period of CHIKV than on *Aedes* susceptibility [364]. Thus, a combination of intermediate temperatures (24°C - 28°C) may provide the best trade-off in terms of maximizing infection susceptibility and the EIP in vectors.

ZIKV was a notable outlier amongst the ABVs investigated here, being the only one where the predicted association with temperature (weekly mean) and rainfall (cumulative weekly values, lagged by 2 and 5 weeks) was negative. One possible explanation for this difference could be that ZIKV has a lower thermal tolerance in mosquitoes than CHIKV or ZIKV. However, a previous study based on

experimental infection under lab conditions reported that the extrinsic incubation period of ZIKV reduced as temperature increases [342], suggesting that vector competence and thus transmission should rise with temperature in contrast to what was found here. There may be another biological explanation for the apparently contradictory temperature effects found for ZIKV here, including that it is an artefact of the reporting process. On account of the recent arrival of ZIKV into Ecuador relative to the start of the study, transmission was still expanding during the warmer months and did not plateau until later in the year (mid June/July) when temperatures were cooling down. Such a delay in dynamics due to the later introduction of ZIKV in the study area may also account for the apparently negative association with rainfall. Testing this hypothesis would require observation of ZIKV dynamics across further years. However given the rapid collapse of ZIKV transmission in South America after the 2016-17, no further data is available to support this. While the mechanisms remain unclear, these results highlight that ABVs may have different seasonal dynamics in the same setting despite sharing a common vector species. Thus ABV-specific models may be required for reliable forecasting of risk different settings and time periods.

Although *Ae. aegypti* vector density is frequently assumed to be a proxy for DENV transmission risk (e.g. [336]), most vector indices are poor predictors of arboviral incidence [155]. The lack of concordance between vector density and human infection risk may be due to biases in *Aedes* sampling methods [197,373], which capture the abundance of different life stages but not direct biting rates of humans (as discussed in Chapter 2). Given the high expense and logistics involved with epidemiological monitoring in human populations, there would be great value in finding appropriate entomological indicators of risk. Here, a positive association was between the abundance of *Ae. aegypti* collected in BGS traps and outdoor Prokopack aspirators and weekly DENV incidence. However, the nature of this association was negative for collections made using Prokopack aspirations inside houses (concurrent week or one week before DENV reporting). Despite entomological indices have mixed associations with epidemiological outcomes, it has been found that adult stages indices have better predictive associations with arbovirus incidence [197]. The association between *Ae. aegypti* abundance and DENV incidence differed among trapping methods. Assuming DENV incidence should increase with *Aedes* abundance, a possible explanation could be that most

of the transmission was happening at the outdoor area; with BGS and outdoor Prokopack collections providing a better representation of this. However, another explanation could be that during epidemic times, people tend to control *Aedes* abundance at the indoor area. Thus the fraction of the *Aedes* population that remains outside could be reflecting the incidence of DENV. The findings of the associations between vector populations and DENV incidence are somewhat unexpected given the relatively limited timeframes from which mosquito data was collected. For corroborating the results, further studies with extended periods of vector monitoring and analyses of their associations with DENV incidence at wider geographical and temporal scales would be needed.

This study provides insights into the seasonality and environmental dependency of arboviral transmission in coastal Ecuador and the value for planning surveillance and control activities. DENV incidence was heterogeneous across years, locations, and seasonal timing within a year, highlighting the need to tailor predictions to the local context. Even within the same country, DENV incidence intensity varied significantly between the two high transmission settings investigated here. While these geographical differences in transmission were consistent across all three ABVs (e.g always higher in Portoviejo than Quinindé), seasonality was not; with DENV peaking earlier than CHIKV, followed by ZIKV. There was also notable differences in the environmental correlates of incidence between ABVs, with DENV and CHIKV having positive association with temperature and rainfall variables, and ZIKV being negatively associated. While it cannot yet be concluded whether this is a real biological effect or signature of the timing of invasion, it highlights that arboviral-specific analysis may be needed, with caution required before generalizing results from DENV to other *Aedes*-transmitted viruses in the same setting. A notable limitation in this analysis of CHIKV and ZIKV dynamics is that they occurred on only one outbreak year, with ZIKV data only available for one site. Expansion of analysis to include information from other areas in Ecuador and South America is required to assess the generalizability of these results.

CHAPTER 5: GENERAL DISCUSSION

5.1. OVERVIEW

Aedes-borne virus (ABV) diseases have greatly affected human populations in the last few decades. Despite the development of a vaccine enabling the control of yellow fever virus (YFV) in the Americas, other ABVs have emerged in the continent causing serious outbreaks. Dengue virus (DENV) is endemic in many central and South American countries, with global incidence increasing in the last few decades and now infecting approximately 390 million people per year, with 9 deaths. Since 2013, chikungunya (CHIKV) and Zika virus (ZIKV) have emerged in the Americas infecting a total of 3.5 million people across the continent. Currently, there are no vaccines that can be applied to prevent from DENV, CHIKV or ZIKV, thus making control of *Ae. aegypti* vector populations the main strategy to suppress transmission.

Entomological and epidemiological surveillance are mandatory to guide effective vector control and public health response. Therefore understanding the ecology of *Ae. aegypti* populations and drivers of arboviral transmission dynamics is essential to develop effective strategies. In this study, I investigated the ecology of *Aedes* vectors and the epidemiology of three major arboviruses that they transmit within two hotspots of transmission on the Ecuadorian coast. As the study coincided with the tail end of the first major ZIKV epidemic in the country, initial aims were to investigate the transmission of this new arbovirus in relation to endemic DENV and CHIKV. The primary focus was on understanding the environmental drivers of *Aedes* population dynamics, behaviour and transmission potential in these settings, and the seasonality of disease incidence in people. It is envisioned that results will have implications for improving vector surveillance (Chapter 2), understanding vector ecology and control (Chapter 3), and identifying when communities are at greatest risk of infection (Chapter 4). Knowledge of the spatial temporal drivers of *Aedes* vector abundance and behaviour (such as biting and resting behaviour), and arboviral infection rates in female *Ae. aegypti* are required to estimate where and when people are at greatest risk of exposure to infected bites, and where control should be targeted. Additionally, knowledge of the environmental and entomological drivers of arboviral disease incidence can

help guide health system preparedness and guide the timing of seasonal interventions. Here I briefly review key findings with respect to understanding of arboviral transmission in Ecuador.

5.2. PRINCIPAL FINDINGS

5.2.1. Mosquito Electrocuting Trap for Aedes surveillance

A potentially significant contribution arising from this work is demonstration of proof-of-principle that the Mosquito Electrocuting Trap (MET) could be used to directly estimate human biting rates by *Aedes* vectors. The human biting rate is crucial predictor of the transmission of vector-borne diseases [184]. Despite the importance of the human biting rate to vector-borne disease transmission, currently there is no way to directly measure this for ABVs.

Due to the lack of chemoprophylaxis measures against DENV, CHIKV, and ZIKV, the Human Landing Catch (HLC) technique used for other vector-borne diseases like malaria is not permissible for *Aedes* vectors. A variety of indirect methods are used to provide indices of adult *Aedes* vectors including passive surveillance traps that use artificial odours to attract mosquitoes such as the BG-Sentinel (BGS) trap [398]. This method is often considered the standard approach to capture host-seeking *Aedes* spp. However, none of the current surveillance methods for adult *Aedes* mosquitoes reliably correlate with human infection risk [155,197]. By providing an equivalent measure to the HLC by collecting mosquitoes just before the land on a person but while preventing human exposure, the MET could provide a safe solution. Due to ethical implications of the HLC, no direct comparison of the MET and HLC was possible in this study. However the MET was compared with the most widely used indirect method for measuring host seeking *Aedes* – the BG-Sentinel (BGS) trap. Results shown in Chapter 2 revealed that the MET tended to outperform the BGS trap when used in peri-domestic settings in Quinindé, although not significantly. Additionally, the MET provided a consistent representation of *Ae. aegypti* female diel biting activity compared to the BGS. To confirm that the MET can be used to estimate the EIR of arboviruses, it is also necessary to confirm that viral infection rates can be measured in mosquitoes sampled by this method. I attempted to do so here by screening all *Ae. aegypti* females caught in METs (n=118) for DENV, CHIKV, and ZIKV but probably due to

characteristically low viral infection rates, no infected mosquito pools caught were detected. This is unlikely to be due to the sampling method, as no infection was found in *Aedes* caught in the BGS (n=118) either. Further analyses based on larger sample sizes of mosquitoes is needed to confirm that arboviral infection can be detected in *Aedes* caught in METs, however it is concluded that the MET is a promising tool for surveillance of *Ae. aegypti* behavioural patterns and infectivity rates.

5.2.2. Implications of Aedes ecology for vector control in the study area

Knowledge of the spatial distribution, biting and resting behaviour and temporal dynamics of adult *Ae. aegypti* females are important to identify where and when people are at higher risk of infectious bites. Although the two study sites investigated here are important hotspots of ABV transmission in Ecuador (Chapter 4), there is limited up-to-date information on the local ecology of *Ae. aegypti* in these settings. Results of the 6-month period of entomological surveillance conducted here indicate that *Ae. aegypti* ecology differed between the two study sites, showing different behavioural patterns and overall abundance. For instance, female *Ae. aegypti* were six times more abundant in indoor than outdoor resting collections (Prokopack) in Portoviejo than in Quinindé, where abundance was similar in outdoor and indoor collections. Also, there was significant variation in *Ae. aegypti* abundance at the neighbourhood levels within each canton that was associated with the degree of 'urbanization'. Overall, female *Ae. aegypti* were two times more abundant in urban than in peri-urban neighbourhoods. This heterogeneity in vector abundance between neighbourhoods and cities has direct implications for vector control measures. For instance, urban areas should be prioritized over peri-urban neighbourhoods, and vector control strategies applied within each location should take into account local heterogeneity as showed in this work, where indoor and outdoor *Ae. aegypti* abundance varied between study sites. Thus, it is essential to carry out small scale surveillance and determine whether ecological trends vary across time and space.

5.2.3. Viral infection rates in mosquitoes and phylogeny

Measurement of infection rates in *Ae. aegypti* females can provide confirmation of active circulation of an ABV in a specific location [150,276–278]. In addition,

analysis of ABV samples from infected vectors can reveal the arrival of new ABVs and strains, and their routes and means of virus introduction [282]. *Ae. aegypti* females collected during entomological surveillance here were screened for ABV presence, with viral isolate analysed using phylogenetic techniques to establish evolutionary relationships. Contrary to initial expectations, no ZIKV infection was found in any *Ae. aegypti* sample, despite the occurrence of a sizeable outbreak during the collection period. Similarly CHIKV was not detected, and only one pool containing three adult female *Ae. aegypti* was positive to DENV-1. This sample was most closely related to other DENV-1 samples collected in Ecuador in 2014, suggesting that no apparent new DENV-1 introductions had occurred in the study area since then. The low infection rates in these *Ae. aegypti* populations highlights the difficulty of using *Aedes* infection rates as epidemiological indicators. Data on disease incidence in people indicates all three arboviruses were in relatively high circulation throughout the study period, yet almost no evidence of infection was found in mosquitoes.

5.2.4. Seasonality and environmental drivers of arboviral incidence in humans

Analyses of human cases of DENV, CHIKV and ZIKV between 2013 – 2018 (Chapter 4) showed that arboviral disease incidence differed between the two study sites, being always higher in Portoviejo than in Quinindé. There was also heterogeneity in seasonality between the three arboviruses, reflected by 3-6 weeks differences in the timing of their seasonal peaks. In addition, interannual variation in DENV incidence revealed significant heterogeneity between all years, with a notable increase of incidence in 2015. This is currently unknown, but may have been caused by El Niño Southern Oscillation (ENSO) which occurred in 2014-2016, which was also observed in Venezuela by Vincenti-Gonzalez et *al*. (2018) [350].

Analysis of seasonal variation in arboviral disease incidence revealed potential environmental drivers of transmission. Notably, the environmental factors that were associated with weekly reported incidence varied somewhat between the arboviruses considered, despite their common vector species. Lagged rainfall and temperature were studied at the micro and the macro scale in relation to the effects on ecological and behavioural patterns of *Ae. aegypti* as well as on the incidence of DENV, CHIKV and ZIKV. Environmental variables had differing impacts

on mosquito vector populations and infection incidence in people. For example, *Ae. aegypti* abundance was found to be negatively correlated with past cumulative rainfall recorded during the 22-28 precedent days before the adult mosquito collection took place, and temperature was not significantly related to adult *Aedes* abundance (Chapter 3). In contrast, the incidence of DENV and CHIKV were positively related to past temperature (both arboviruses) and past rainfall (only DENV), while ZIKV incidence was negatively correlated with these environmental variables (Chapter 4). This difference in environmental predictors between ABVs could potentially be a product of the timing of the arrival of ZIKV to the country or be a potential direct effect of climate on the host-pathogen interaction. Therefore, a close investigation should take place on how climate may influence the transmission dynamics of ABV separately.

5.3. IMPLICATIONS OF THE FINDINGS

This study represents an advancement in the knowledge of *Ae. aegypti* ecology in Ecuador and could serve to guide policies for vector control and disease prevention. The study focused on two urban hotspots of *Aedes*-borne diseases in Ecuador where up-to-date information on vector populations is scarce. Despite most studies of *Ae. aegypti* ecology and transmission potential have been published from the Southern Coastal Ecuador, El Oro Province (e.g., [162,170,403–406,171,177,344,345,399–402], among others), others from few settings from the Coastal region and the Galapagos [164,391,407–413], and others using country-level data [414,415], to my knowledge this work represents the first description of the influence of environmental drivers on vector ecology and ABV transmission in this region outside of El Oro Province. It is essential to understand ABV transmission along the Coastal region because it constitutes the bulk of ABV disease cases in Ecuador [416]. The whole Coastal region is hyperendemic, with urban settings in Ecuador being amongst some of the highest transmission settings in South America [416]. By providing data from these two study sites, I hope to contribute to the evidence base and strengthen insights into how transmission can be most effectively suppressed in this setting.

The need for safer methods for estimation of human exposure to infected mosquito bites motivated the development of the MET for malaria vectors. The use of this trap for *Aedes* surveillance could be an enormous step forward by

providing safer, more direct way to measure human exposure to arboviral infection. Given the lack of accurate surveillance methods of *Ae. aegypti*, results here show a promising application of the MET in local surveillance systems. For instance, the use of the MET to characterize the biting behaviour of local mosquito populations and to determine the EIR could provide an otherwise intractable method to assess human exposure and transmission. Moreover, the implementation of the MET as an additional surveillance tool together with other surveillance traps could serve to expand understanding on targeted mosquito populations (e.g., from resting to host-seeking mosquitoes), and to calibrate existing surveillance tools in relation to the MET (e.g. calibration of the BGS traps in relation to the MET).

This work also revealed the importance of conducting entomological and epidemiological surveillance at fine scale, as spatial and temporal heterogeneity of both *Ae. aegypti* ecology and arboviral disease transmission were detected. Specifically, crucial aspects of *Aedes* vector ecology and demography can vary both between and within cities (neighbourhood level) in a way that influences the expected impact of interventions. For example, although female *Ae. aegypti* are often assumed to be largely indoor resting [123]; this study revealed considerable variation in the endophily of *Ae. aegypti* between the two study sites. Specifically, most female *Ae. aegypti* (80%) were captured resting indoors in Portoviejo, a relatively equal proportion were found in indoor and outdoor resting collections in Quinindé. This could impact the choice of optimal vector control intervention at each site. For instance, control activities in Portoviejo should possibly focus on targeting mosquitoes just indoors (e.g. indoor residual spraying), whereas at Quinindé, there could be added value from supplementary methods targeting vectors in the peri-domestic area too (e.g., outdoor space spraying). However the success of both these approaches will depend on the insecticide resistance status of *Aedes* vector populations [147], which was not considered here. Actions from the Ministry of Health (MoH) should be focalized to specific local conditions of vector populations and applied accordingly. Despite the difficulty of tailoring vector control strategies to small areas such as neighbourhoods, where feasible this could provide efficient and cost effective control.

It remains unknown why the resting behaviour of *Ae. aegypti* females appeared to vary between sites. Studies of *Anopheles* malaria vectors in Africa indicate that

resting behaviour may be impacted by household characteristics such as livestock presence and climatic variation [417]. It is possible similar household or environmental variables may have impacted *Ae. aegypti* resting behaviour here too. I did not observe obvious differences in living styles and house construction; thus, a deeper analysis would be needed to understand the underlying reasons that may be responsible for this apparent difference in *Aedes* behaviour. Further investigation will be required to determine the reason why *Ae. aegypti* resting behaviour appeared to vary between the two study sites, and whether this variation has an impact on epidemiological outcomes, such as arboviral incidence in human and mosquito populations, and the effectiveness of interventions.

In addition to *Aedes* vector ecology, there was also considerable heterogeneity in arboviral incidence between the two study sites as described in Chapter 4. Across all 3 arboviruses considered, incidence was much higher in Portoviejo than in Quinindé. However, general arbovirus-specific patterns of seasonality were similar between ABVs but slightly differed in the peaking timing. between arboviruses. Such results suggest that MoH epidemiological surveillance has to encompass the whole period in which ABVs occur by improving testing capacity to avoid misdiagnosis due to overlapping dynamics.

5.4. IMPORTANCE OF COMMUNITY ENGAGEMENT

Inclusion of citizen participation is crucial to tackle transmission of ABVs. In particular, water storage practices need to be either reduced or method-improved in order to avoid breeding sites for mosquitoes. Poor waste management may result in the proliferation of unintentional water breeding containers. Thus waste disposal systems also need to be improved to improve ABV control in these settings. Successful environmental management fundamentally relies upon community understanding and participation, and support from local authorities (e.g. council, MoH, etc., [150]). Scientific findings on their own may have limited impact unless they are effectively communicated and coordinated with all stakeholders, including communities themselves.

With the aim of enhancing engagement and reinforcing understanding of arboviruses, prevention and control, I tried to incorporate community empowerment within my PhD research by designing and conducting a series of

public engagement activities within each of the cities I worked in at the end of my field work (July- August, 2017). These activities were funded by a supplementary public engagement grant I obtained from the Wellcome Trust (Grant ref: MC_PC_15081), and later written up as a case study of public engagement practice on the MESH – Community Engangement Network website (https://bit.ly/3kNoOJJ, Appendix 2). In brief, educational activities were developed in conjunction with collaborator Lucía Chávez from "Sarawarmi Laboratorio de Ideas", that were directed to teenagers, high school students and elderly people. Participants were included in workshops focussing on common community problems (e.g. poor waste management or water storage practices). Participants were provided with opportunity to explore and learn about ABVs in their community, the role of mosquito vectors, and different scenarios of water storage and waste management practices. They also learned about basic facts of the mosquito life cycle, how to distinguish an *Ae. aegypti* from other mosquitoes, and the transmission of ABVs in their communities. For full details of these activities, please see Appendix 2. Widening these activities and establishing a long term and sustained education programme would be ideal to get people involved in vector control activities and understand the problem of ABV transmission within their communities.

5.5. LIMITATIONS OF THE STUDY

In addition to study-specific issues described in Chapter 2-4, this study had some broad general limitations. The first was the relatively short period of study possible for different examples. For example , the testing of the novel MET as a surveillance tool for *Aedes* vectors was only conducted over 12 days on the same month in only one urban neighbourhood. Although accurate observations were made in relation to the BGS traps and to previous literature (i.e., observations regarding diel activity and biting activity per hour), a more comprehensive understanding of biting behaviour could have been made if the study had been conducted over a longer period of time, and included more study sites. Similarly, entomological surveillance presented in Chapter 3 only encompassed the rainy season of one year, and by the inclusion of only two study sites. Vector surveillance should be conducted throughout the entire year to encompass the full range of seasonal environmental extreme, and over multiple years. Although entomological surveillance encompassed the period where *Ae. aegypti*

populations and ABVs are likely to be highest in coastal Ecuador, it is also important to also understand vector dynamics when mosquito populations are low. A second limitation is that insights are drawn from only two distinct study sites. Inclusion of more study sites would allow a wider comparison between local vector populations and would clarify the potential effects of macro and micro environmental factors over vector population dynamics. In particular, it has been observed that incidence of ABVs changes geographically within the Coastal region every year [416]. Therefore by having more study sites, a better and comprehensive understanding of vector populations within and between sites can be achieved. Finally, an inherent limitation shown in Chapter 4 was the reliance on passive surveillance data to infer ABV transmission dynamics. As has been recognized for all three arboviruses studied, a substantial proportion of infections may be asymptomatic [389,418,419]. Such infections would not be picked up by the passive surveillance system, and could mean substantial amounts of transmission were being missed. In the present study, the magnitude or under-reporting and misdiagnosis is unknown. To confirm predictions based on passive surveillance here, it is recommended a more focussed programme of active surveillance be carried out from time to time.

5.6. PERSPECTIVES ON FURTHER WORK

As discussed above, a wider understanding of the relationship between *Aedes* ecology and its effects on arboviral transmission is needed. Therefore, it is important that studies include more scenarios where the potential heterogeneity of mosquito populations could be displayed and identified. For instance, it should be prioritized to evaluate the MET against BGS traps at different seasons and locations, as well as its assessment against the HLC under controlled conditions (i.e., with uninfected *Ae. aegypti*). Findings presented in Chapter 3 and 4 indicate substantial heterogeneity in *Aedes* ecology and arboviral disease incidence between sites. This may be explained by variation in *Aedes* biting rates; with the latter providing a much more accurate indicator of epidemiological outcomes than any existing *Aedes* index. I recommend further work be conducted to assess relationships between *Aedes* catches in METs with human infection and disease, to assess the utility of this trapping method for epidemiological prediction.

Finally, by including active surveillance to detect active cases of arboviral diseases as recommended, it is suggested that similar analyses are conducted in other arboviral transmission hotspots in Ecuador. Seasonal and interannual variability should be compared to the two study sites to assess consistency of the results found, and ideally, this should be accompanied by entomological surveillance in order to assess potential relationships at fine-scale. Field and laboratory work should then be coupled with mathematical and statistical modelling that can use such information to elaborate predictions of risk that can guide actions of disease prevention and mitigation. Also, it would be important to continue and expand community participation work in ABV vulnerable settings to increase engagement and make citizens actors of their own solutions.

I hope that this work will help contribute to tackling burden of ABVs diseases in Ecuador, particularly the unacceptable burden on the poorest and most economically vulnerable citizens and their families. With this work, I am to highlight the great need for effective disease surveillance control systems to deal with persistent problems like DENV as well as new pathogens like ZIKV, and coronavirus disease (COVID-19). Ideally, these improvements would move us closer to WHO's vision of "Health for all" [420].

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APPENDIX 1

The following article is a summary of the study from Chapter 2, which was published for science engagement at the BugBitten blog at:

https://blogs.biomedcentral.com/bugbitten/2020/02/28/electrocuting-mosquitoes-a-new-hope-for-monitoring-dengue-vectors/

7.1. ELECTROCUTING MOSQUITOES: A NEW HOPE FOR MONITORING DENGUE VECTORS?

The Mosquito Electrocuting Trap (MET) was tested for the first time as an alternative method to collect and monitor mosquito populations that transmit dengue virus. Potentially, this could improve the ability to determine exposure risk to infected bites and increase the effectiveness of disease prevention programmes.

As many of us may have experienced, mosquito bites are quite annoying and their buzz is not pleasant either. Besides this, probably the most important reason why there is so much attention on these little insects is because females of some mosquito species are capable of transmitting pathogens, many of which affect humans. Pathogens present in the salivary glands of the mosquito are transferred to the human host when the female gets a bloodmeal. Malaria, for instance, a disease caused by *Plasmodium* parasites and transmitted by mosquitoes from the genus *Anopheles*, affect hundreds of millions of people each year. While other mosquitoes, such as those from the genus *Aedes,* are responsible for transmitting pathogenic viruses, such as yellow fever, dengue, Zika and chikungunya virus. Nowadays, dengue virus has probably become the most common *Aedes*-borne virus as its incidence has increased dramatically in the last decades, placing about half of the global population at risk.

To prevent transmission of the aforementioned diseases, several approaches have been developed to control mosquito populations. However, in order to make these strategies effective enough, scientists need to understand where and when people are at highest risk to mosquito bites. The most accurate strategy is by trapping

mosquitoes at different times and places, using the human landing catch (HCL) technique. This consists of people exposing their own unprotected skin and trapping mosquitoes using a mouth or a hand aspirator. The advantage of this strategy relies on that it uses natural human odours and other visual and olfactory clues that mosquitoes use to find their next bloodmeal.

Probably, the most obvious problem with the HLC is that participants are directly exposed to mosquito bites that are potentially infected. Some malaria parasites have developed resistance to these drugs, making the HLC risky to study *Anopheles* mosquitoes. It has also been harder to study *Aedes* mosquitoes using HLC as there is no way of prevention from *Aedes*-borne viruses.

In 2015, the Mosquito Electrocuting Trap (MET) was developed to trap malaria-carrying mosquitoes as an exposure-free alternative to the HLC. The MET consists of four squared electrified wired surfaces that are assembled around the legs of the participant while they sit on a chair and the rest of the body is covered by mosquito net. Mosquitoes can be collected and studied after they receive an electrical shock when they try to get through the wired surfaces.

In a study published last month, we tested the MET for the first time on *Aedes* mosquitoes from Ecuador. We compared its performance against the BG-sentinel (BGS) trap, which is the golden trapping method used for *Aedes* surveillance that is baited with artificial odours. In this 12-day study, we used two BGS traps and two METs that were deployed at the outdoor area of four properties, in the city of Quinindé-Ecuador. All traps ran from 7am to 7pm and were swapped each day between each trapping type, so by the end of the study, six full days of trapping were done by each trap type at all houses. Additionally, as attractiveness of mosquitoes towards people may vary from person to person, we alternated participants from the METs each hour of collection, thus avoiding any bias caused by this. Finally, we measured microclimate conditions at each trapping station with data loggers.

Impressively, we found as many *Aedes* mosquitoes with the METs as we did with the BGS traps and we could record the same mosquito species with both trapping methods. We found that *Culex quinquefasciatus* was the most abundant mosquito

species, followed by *Aedes aegypti*, *Aedes angustivittatus*, *Limatus durhami* and *Psorophora ferox*.

With the MET, we were also able to precisely record the biting activity time of *Ae. aegypti* and the other very common mosquito *Cx. quinquefasciatus*, which has been incriminated as vector of West Nile virus. We found that females of both species have higher biting activity during early morning and late afternoon and also found that biting activity is negatively associated with temperature.

Despite that we did not find any infected mosquito, we recognize that infection rates of arboviruses in *Aedes* mosquitoes tend to be very low. We, therefore, could confirm that the primary advantage of the MET is to be able to accurately estimate the biting rates of mosquitoes and potentially estimate the entomological inoculation rates (rate of infected bites) when infected mosquitoes are found. An enormous advantage of the MET is that it could be used to calibrate other trapping methods and be used in combination with other traps when a large scale mosquito surveillance is planned.

APPENDIX 2

The following text is a summary of the public engagement work that was carried out in Portoviejo and Quinindé in July and August of 2017, to increase public awareness on *Aedes*-borne diseases and the mosquito vector *Aedes aegypti*. This summary served as the basis for the article published for the MESH – Community Engagement Network website at: https://bit.ly/3kNoOJJ.

8.1. PROJECT REPORT FOR "WORLD MOSQUITO DAY COMMUNITY FESTIVAL TO RAISE AWARENESS OF MOSQUITO VECTORS IN LOCAL COMMUNITIES"

8.1.1. Project Overview (DISCURSIVE) [1 paragraph]

The project was delivered in the cities of Portoviejo and Quinindé, in the Coastal region of Ecuador at the conclusion of an 8-month study of the mosquito vectors of Zika, dengue and chikungunya in these areas. A high number of Zika cases occurred in both these settings during the 2016-2017 South American epidemic, in addition to persistent high rates of dengue and chikungunya viruses. Mosquito vector control is currently the only option for interrupting transmission of these diseases, and our research focused on identifying where and when people were at greatest risk of exposure. These public engagement events were designed to inform local residents about the causes and risks of Zika, dengue and chikungunya in their community, and how they can protect themselves from mosquito bites by taking simple measures at home. We aimed to empower the people by improving their understanding of mosquito vectors and their role in disease transmission. This was accomplished through conducting a series of half-day community festivals centred around "World Mosquito Day" on August 20th, 2017. The aim was to disseminate and reinforce public health messages about mosquito-borne diseases (Zika, dengue, chikungunya) and locally-relevant information on mosquito vectors through a mixture of artistic performances, displays and participatory activities. These festival events were supplemented with a series of workshops running before the event targeted at community groups at particular risk, including schoolchildren, youth groups, and the elderly. Before and after each workshop activity, participants were invited to carry out short, anonymous surveys to give

feedback on their understanding of the causes of Zika and how to prevent mosquito bites, and to give feedback on the events. These surveys will be used for assessment of the Public Engagement event, and are currently being analyzed.

8.1.2. Project Lead and Partners [1-2 short paragraphs]

This project was established as an extension of a research project on mosquito vector ecology, behaviour and transmission with four hotspots of Zika transmission in Ecuador and Colombia as funded by the MRC Zika Rapid Response Initiative (MC_PC_15081). The research project was led by Professor Heather Ferguson and Leonardo Ortega-López at the University of Glasgow, Dr. Renato León at the Universidad San Francisco de Quito (Ecuador) and Dr's Felio Bello and Alexandra Segura at the Universidad Antonio Narino in Colombia.

The public engagement events described here were led by Leonardo Ortega-López, currently a PhD student at the University of Glasgow. Leonardo led the planning and organization of these events including recruiting a team for development of content (information booklets, banners, promotional materials) and performers including a theatre group and musicians to participate in festival events and workshops. He also liased with all local partners to plan the workshops and invite participation at the main Mosquito Festival events. In the run up, Leonardo also took part in radio and television interviews to advertise the event and provide information on Zika and mosquito vectors.

The City Council of Quinindé helped recruit a local traditional music band to play at the Festival, and with publicity through the council radio station. The Junta Parroquial "Abdón Calderón" also helped with the organisation of the event at the central park of Calderón (Portoviejo). All the offices from the Ministry of Health helped by providing personnel to staff information booths at the festivals to talk with local residents, and material for displays related to vector-borne diseases. The community group "House of the Youth" (transl. from Spanish) and the Rights Protection Council of Quinindé helped by providing access to venues for holding workshops at their facilities, and with logistic organization. The School Management District of Quinindé granted the permissions to each of the invited schools to attend the event. The Neighbourhood Federation of Quinindé helped

delivering the invitations to each of the neighbourhood leaders of Quinindé. The Geriatric Centre "Santa Gema Galgani" in Calderón (Portoviejo) helped with the facilities of the Centre to hold the workshop for the elderly.

8.1.3. Ambitions [1 paragraph]

Our vision was to improve people's understanding of the causes of Zika, dengue and chikungynya viruses, the risks of mosquito exposure in their communities and how they can protect themselves from bites and therefore mosquito-borne diseases. Originally, activities were envisioned to consist solely of the half-day festival events at each location. However in liaising with local communities and stakeholders from government during the planning, we realized there was interest in having our research team spend additional time with local school and community groups to tell them about our research and how to protect themselves from mosquito-borne diseases. Thus we identified opportunities to conduct additional complementary workshops with some of these target groups in the weeks or days before festival events. These workshops consisted of 2 hour sessions with interactive activities related to mosquitoes and arbovirus transmission. They were aimed to reinforce key messages on the topic and activities were assessed by anonymous evaluations before and after them once the workshops had finished.

8.1.4. Approach [1-2 paragraphs]

The project was designed to involve people from different age groups in local communities, and tailor activities to those representative of different groups at risk, e.g (1) the general public (through attendance at Mosquito Festival), (2) school groups through information displays and presentations from the study team before the festival, (3) to disadvantaged youths through involvement in a participatory theatre performance which was performed at a Festival to highlight the risk posed by mosquitoes, and two dedicated workshops, and (4) the elderly through a workshop with members of senior care home. Whilst all residents of arbovirus-endemic cities can be at risk of infection, some groups may be more at risk of infection and/or be harder to reach for disseminating information. This was the rationale for targeting the engagement both at the general community and some specific groups.

For all of the mentioned activities, with the exception of the Mosquito Festival and the workshops with the elderly, we conducted short anonymized written surveys with participants before and after each event to evaluate both the existing knowledge level of knowledge on mosquito-borne diseases and how it had been influenced by workshop attendance. The aim of conducting them was to assess the effectivity of our activities and get feedback from the participants. Analysis of these surveys is ongoing.

The main milestones identified for this project were: 1) The write up of the detailed plan for each of the activities to be implemented; 2) approach and acceptance of collaboration with external partners from the local communities, including publicity of the events; 3) carrying out the events and workshops and 4) the accomplishment of the post-events assessment (still underway).

The publicity strategy was led by Fibios Comunicación Ambiental Cía. Ltda. (https://www.fibios.org/) and consisted of radio and TV interviews with the support from the local media, and direct communications from the local external partners. In addition, distribution of flyers and posters around the cities was carried out. Finally, we created a Facebook fan page for these events (https://www.facebook.com/PilasConElZancudo/) to advertise and inform the public about these activities.

8.1.5. Evaluation and Lessons Learnt [1-2 paragraphs]

We were able to involve around 400 people in our workshops and Mosquito Festival events. We could conduct the surveys to most of these participants, except for the elderly group since we failed to design an appropriate survey adjusted to their physical conditions (i.e. a survey short enough and adapted to their physical abilities, so that they could be interviewed instead of filling out the questionnaires themselves). The surveys have not been analysed yet and are currently being evaluated.

The main lesson learnt was that it is either necessary to dedicate more time to plan and request a bigger budget to effectively cover the range of groups or that

a focus on less groups with more intensive evaluation of activities is necessary. This could improve the design of the activities and the posterior survey analysis.

8.1.6. Advice for someone wanting to do something similar [Very Brief 3 or so bullet points]

- Always plan for unexpected costs within your budget, and assign more to this if the target audience and area are somewhat unknown.

- Try to maximize the probabilities of attaining the aim of the project by having a narrower focus and deliver it completely. Otherwise, aiming for bigger objectives with limited time and money is risky.

- Plan well in advance involving local people since they give the most helpful advice to work within their communities adapted to their needs and adjusted to their own culture.

- Design the aim of your project adjusting it to the needs of the target audience. By this way, you ensure that people become interested in your activities, participate in proposed event(s), and most importantly, get benefits from them.

APPENDIX 3

From the following page, the paper published from Chapter 2 is attached. Original citation:

Ortega-López, L.D., Pondeville, E., Kohl, A. *et al*. The mosquito electrocuting trap as an exposure-free method for measuring human-biting rates by *Aedes* mosquito vectors. *Parasites Vectors* **13,** 31 (2020). https://doi.org/10.1186/s13071-020-3887-8 (Appendix 3).

Parasites & Vectors

METHODOLOGY

Open Access

The mosquito electrocuting trap as an exposure-free method for measuring human-biting rates by *Aedes* mosquito vectors

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Abstract

Background: Entomological monitoring of *Aedes* vectors has largely relied on surveillance of larvae, pupae and non-host-seeking adults, which have been poorly correlated with human disease incidence. Exposure to mosquito-borne diseases can be more directly estimated using human landing catches (HLC), although this method is not recommended for *Aedes*-borne arboviruses. We evaluated a new method previously tested with malaria vectors, the mosquito electrocuting trap (MET) as an exposure-free alternative for measuring landing rates of *Aedes* mosquitoes on people. Aims were to (i) compare the MET to the BG-sentinel (BGS) trap gold standard approach for sampling host-seeking *Aedes* vectors; and (ii) characterize the diel activity of *Aedes* vectors and their association with microclimatic conditions.

Methods: The study was conducted over 12 days in Quinindé (Ecuador) in May 2017. Mosquito sampling stations were set up in the peridomestic area of four houses. On each day of sampling, each house was allocated either a MET or a BGS trap, which were rotated amongst the four houses daily in a Latin square design. Mosquito abundance and microclimatic conditions were recorded hourly at each sampling station between 7:00–19:00 h to assess variation between vector abundance, trapping methods, and environmental conditions. All *Aedes aegypti* females were tested for the presence of Zika (ZIKV), dengue (DENV) and chikungunya (CHIKV) viruses.

Results: A higher number of *Ae. aegypti* females were found in MET than in BGS collections, although no statistically significant differences in mean *Ae. aegypti* abundance between trapping methods were found. Both trapping methods indicated female *Ae. aegypti* had bimodal patterns of host-seeking, being highest during early morning and late afternoon hours. Mean *Ae. aegypti* daily abundance was negatively associated with daily temperature. No infection by ZIKV, DENV or CHIKV was detected in any *Aedes* mosquitoes caught by either trapping method.

Conclusion: We conclude the MET performs at least as well as the BGS standard and offers the additional advantage of direct measurement of *per capita* human-biting rates. If detection of arboviruses can be confirmed in MET-collected *Aedes* in future studies, this surveillance method could provide a valuable tool for surveillance and prediction on human arboviral exposure risk.

Keywords: Zika, Dengue, Chikungunya, Arbovirus, Host-seeking, *Aedes aegypti*, Mosquito electrocuting trap, BG sentinel trap, Vector surveillance, Ecuador

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Background

Mosquito-borne viruses (arboviruses) are an important cause of diseases in humans and animals. In 2017, estimates suggested that mosquitoes were responsible for approximately 137 million human arboviral infections with dengue (DENV), chikungunya (CHIKV) and Zika virus (ZIKV) being the most important [1]. Arbovirus transmission to humans depends on multiple factors that involve spatial movement and immunity of human populations [2–4], socio-economic factors and access to basic services (especially water) [5, 6], and the ecology and distribution of the mosquito vectors that transmit them [7–9]. These factors combine to determine the distribution and intensity of arboviral transmission and generate often complex and highly heterogeneous patterns of exposure and infection [10, 11]. As safe and effective vaccines for DENV, CHIKV and ZIKV are not yet available [12–14], control of the *Aedes* mosquito vectors remains a primary strategy for reducing transmission [15–17].

Knowledge of where and when humans are at greatest risk of exposure to infected mosquito bites is vital for prediction of transmission intensity and effective deployment of vector control [18–20]. In the case of malaria, this information is used to estimate a time or site-specific "Entomological Inoculation Rate" (EIR); defined as the number of infected mosquito bites a person is expected to receive. This metric is usually derived from conducting human landing catches (HLCs); a method in which a participant collects and counts the number of mosquito vectors landing on them over a given sampling period, then the sample is tested for the presence of a pathogen [21]. By providing a direct estimate of human exposure, the HLC provides sensitive predictions of malaria transmission [19, 22–24]. However, this method raises ethical concerns due to the requirement for human participants to expose themselves to potentially infectious mosquito bites [25]. In the case of malaria, this risk can be minimized by providing participants with prophylaxis [26]. However, such remediation is not possible for arboviruses where often no prophylaxis is available, and therefore HLCs are not recommended for the surveillance *of Aedes*-borne arboviruses [27, 28].

Standard entomological monitoring for *Aedes* vectors is usually based on "exposure-free" surveillance of larvae or non-biting adults. This includes surveys of larvae or pupae in water containers [29, 30], and collection of adult mosquitoes resting inside and/or around houses to indirectly estimate human-vector contact rates [29, 31]. While such surveillance methods are useful for confirming vector abundance and distribution, they are poor predictors of epidemiological outcomes such as disease incidence and outbreak potential [32, 33]. Consequently, there is a need for vector sampling methods that can provide more reliable entomological indicators of arboviral transmission.

Human exposure to arboviral infection is likely best assessed by surveillance of "host-seeking" (human-biting) *Aedes* mosquitoes. Several methods have used to sample host-seeking *Aedes* including a variety of fan-operated traps that use visual attraction cues (e.g. Fay [34], the Fay-Prince trap [35], the black cylinder suction trap [36], duplex cone trap [37]) and lure-based traps. For the latter, artificial odours and attractants have been developed and tested for use in traps such as kairomone blends [38, 39], BG-Lure® cartridges [40, 41] and carbon dioxide (CO_2) [42]. Additionally, other trapping methods have been developed that use live hosts as lures (e.g. animal-baited traps [43] and human-baited traps [44, 45]). Only a few studies have directly compared such alternative trapping methods against the HLC with most being outperformed by the latter [44, 45]. Out of all these methods, the BG-sentinel (BGS) trap has been demonstrated as one of the most effective and logistically feasible [46, 47], and thus often considered a gold standard for *Aedes* surveillance [48, 49]. In a range of trap evaluation studies, the BGS outperformed other methods for *Aedes* vectors except for HLC [50]. Despite these advantages of the BGS, its ability to accurately reflect the biting rates experienced by one person remains unclear. Consequently, there is still a need for a safe alternative for direct assessment of human biting rates.

Recently, a new mosquito electrocuting trap (MET) was developed as an exposure-free alternative to the HLC for sampling malaria vectors [51–53]. This trap was built on previous work using electrified nets and grids to trap tsetse flies [54, 55] and mosquitoes [56, 57] attracted to hosts or their odours. Similar to the HLC, this sampling method also uses human participants to lure mosquito vectors and trap them. However, the MET provides participants with full protection from mosquito bites so that no exposure is required. The MET consists of four squared-shaped electrocuting surfaces that are assembled around the legs of a host, with the rest of their body being protected by netting. Host-seeking mosquitoes are attracted towards the host by odour and heat cues as normal but are intercepted and killed before landing. In previous trials in Tanzania, the MET matched the performance of the HLC for sampling malaria vectors in rural and urban settings [51–53]. This trap has also been used to assess host preference by baiting with human and livestock hosts [53], although it has not yet been evaluated for sampling *Aedes* vectors. If successful in this context, the MET could significantly improve ability to monitor and predict arboviral transmission by facilitating an exposure-free direct estimation of EIR.

This study reports the first evaluation of METs for sampling host-seeking *Aedes* vectors in a hotspot of DENV and ZIKV transmission in coastal region of Ecuador. This region is endemic for such arboviral diseases and has accounted for most of the cases reported in Ecuador. For instance, during the CHIKV outbreak in 2015, a total of 33,625 cases were reported in Ecuador, from which 96.02% was reported in the coastal region [58]. A similar pattern occurred during the ZIKV outbreak in 2016 and 2017, where approximately 98.49% of the cases were reported in this region from a total of 5303 cases [59, 60]. DENV has been reported every year in high numbers and considering 2016 and 2017, 84.78% of cases came from the coastal region from a total of 25,537 cases [60, 61].

The objectives of this study were to: (i) evaluate the performance of the MET relative to the BGS trap for sampling host-seeking *Ae. aegypti* and other mosquitoes in the study area; and (ii) use the MET to characterize the biting time of *Ae. aegypti* and other relevant mosquito species and their association with microclimatic conditions.

In addition, we took the opportunity to test for the presence of arboviruses in the collected *Aedes* females by both trapping methods to investigate arboviral transmission in the local area.

Methods

Location and time of the study

This study was conducted in the neighbourhood of "Los Higuerones" (0°19′34″N, 79°28′02″W, 78 meters above sea level), located in the city of Quinindé (Rosa Zárate) (Ecuador). This neighbourhood is located in an urban setting dominated by small, closely packed houses (Fig. 1c), bordering the eastern side with the Blanco River (Fig. 1d). Quinindé is located in the Province of Esmeraldas, the northernmost province in the coastal region of Ecuador. During the 2015 outbreak of CHIKV, this province accounted with the highest disease burden in the country, with a total of 10,477 cases [58]. While for DENV, during 2016, Quinindé alone accounted for 52% of the cases within Esmeraldas Province, with a total of 689 cases out of a total of 1319. In 2017, the number of DENV cases in Quinindé was much lower compared with 2016, where only 87 cases were reported out of 334 in the Province of Esmeraldas. Although there is a permanent incidence of arbovirus cases along the year, a higher incidence is usually reported during the first half of the year [6].

The study was carried out across 12 days in May 2017 (4th–12th, and 16th–18th). On each day of the study, mosquito sampling was conducted over 12 h, from 7:00–19:00 h. Mosquito sampling was conducted within the peridomestic area (garden/yard) of four households (Fig. 1d). These houses were selected on the basis of being physically accessible, and having residents present and willing to participate during an initial tour of the area with a local guide. Houses were separated by approximately 90 m from one another.

Trapping methods

Over the study period, host-seeking mosquitoes were sampled by two different methods as described below.

BG-Sentinel trap (BGS)

The BG-Sentinel® trap (BioGents, Regensburg, Germany) is a white, cylinder-shaped trap made of plastic with a gauze cloth covering the top and a hollow black cylinder in the top centre of the trap (Fig. 2a). The trap operates with a 12 V battery that powers an internal fan that produces inwards artificial air currents. In this study, each trap was baited with two BG-Lure® cartridges and a 1.4 l cooler bottle filled with dry ice in order to maximize the attractiveness of traps to *Aedes*; as it is known that CO_2 increases the catch efficiency of BGS traps [46, 47, 62]. Mosquitoes are attracted towards the baited traps and then sucked through the hollow black cylinder into an internal mesh bag that can be easily removed for subsequent processing.

Mosquito electrocuting trap (MET)

The METs used here consisted of four 30 × 30 cm panels which are assembled into a box around the lower legs of a seated person (Fig. 2b). Each panel is made up of stainless-steel electrified wires set within a PVC frame. The wires are positioned 5 mm apart, which is close enough so that mosquitoes could not pass through without making contact. Wires are vertically arranged in parallel, alternating positive with negative. When mosquitoes try to go through, contact is made and the voltage between wires kills them.

Mosquitoes attracted towards the volunteer were intercepted and killed on contact with these panels. The MET is powered by two 12 V batteries connected in series to a power source giving a power output of approximately 6 W (10 mA, 600 V). As an additional safety feature, a protective inner panel made from wide non-conductive plastic grid was fit into each frame preventing accidental contact between users and the electrified wires.

As an additional accessory to the MET, a retractable aluminium frame was built to cover the rest of the volunteer's body with untreated mosquito-proof netting. Thus, volunteers were completely protected from mosquito bites during their participation in trapping. A plastic tarpaulin was erected over the MET station at a height of 2 m to protect users from direct rain and sunlight. Each MET was also set up on top of a white plastic sheet to isolate it from the ground and make it easier to see and

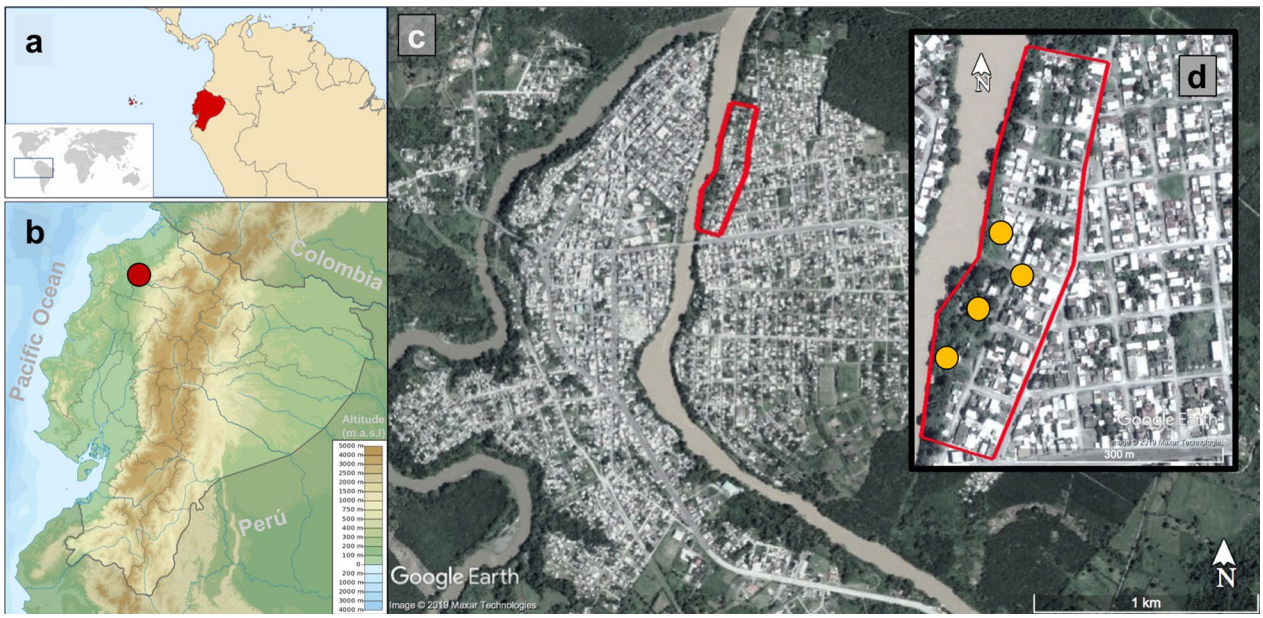

Fig. 1 View of the urban area of the city of Quinindé. **a** Location of Ecuador in the Americas highlighted in red (taken from [96]). **b** Location of the city of Quinindé in the Pacific Coastal region, spotted by the red circle. **c** City of Quinindé showing Los Higuerones neighbourhood enclosed by the red line. **d** Enlarged view of Los Higuerones with the houses sampled spotted by the orange circles

collect shocked mosquitoes that fell onto the ground after touching the MET.

Experimental design

Every day of the study, four traps (two METs and two BGS traps) were set up in the peridomestic area of the four households (one trap per household) at the ground level under shade conditions. Traps were rotated among households each day, so that a different trapping method was used every consecutive day in each house. At the end of the study, this resulted in 6 days of trapping being conducted with each of the 2 methods at all houses.

MET collections were carried out by members of the research team, who were all adult men (30–50 years-old). During each hour of the collection period, one member sat within the MET for 45 min, with the trap being turned off for the remaining 15 min to allow volunteers to take a break. Members of the study team took turns sitting in the trap so that different collectors lured every hour. During the 15 min period when traps were turned

Fig. 2 Trapping methods used in this study. **a** Typical set-up of a BGS trap. **b** Set-up of a MET with a technician luring mosquitoes

off, mosquitoes were recovered from trap surfaces and the ground below using a pair of forceps, counted and placed in empty 15 ml falcon tubes; which were labelled with a unique code linked to the date, household ID, trap ID, hour period and collector ID. Tubes were stored in a cooler box of 45 l capacity filled with dry ice to kill, preserve and transport the specimens.

Each BGS was baited with two BG-Lure® cartridges on each day of sampling; with lures exchanged between the two BGS traps each day to minimize bias due to differential lure efficiency. BGS traps were further baited with carbon dioxide by adding one 1.2 l Coleman® polyethylene cooler bottle filled with dry ice. Dry ice containers were topped up every day. Like the MET, BGS sampling was conducted for 45 min of each sampling hour, with mosquito collection bags being checked and emptied during 15 min break periods. Mosquitoes from BGS collection bags were emptied into pre-labelled plastic bags and transferred into a cooler box with dry ice to kill and preserve the mosquitoes.

Temperature and relative humidity data were collected every 10 min at each mosquito sampling point using TinyTag® Plus 2 TGP-4500 (Gemini Co., Chichester, UK) data loggers. Data loggers at the BGS sampling stations were tied and hung inside each of the traps, and loggers at MET sampling points were placed on top of the bottom border of the netting frame, next to the MET.

Morphological analysis

Mosquitoes collected in the field were transported to the Medical Entomology and Tropical Medicine Laboratory of the San Francisco de Quito University (LEMMT-USFQ) in cooler boxes filled with dry ice. At LEMMT-USFQ, mosquitoes were morphologically identified using taxonomic keys [63–65], counted and sorted into different cryo-vials according to date, household, trap type, hour of collection, species, sex and physiological status of females (blood-fed/gravid and non-blood-fed). All female *Ae. aegypti* specimens were retained for subsequent molecular analysis to test for the presence of ZIKV, DENV and CHIKV. These *Ae. aegypti* samples were grouped into pools of a maximum of 5 individuals.

Molecular detection of arboviruses

All pools of female *Ae. aegypti* specimens were screened for the presence of CHIKV, DENV and ZIKV. Details on the RNA extraction, reverse-transcription and PCR procedures are given in Additional file 1: Text S1, Table S1 and Table S2.

Data analysis

Statistical analyses were performed in R 3.5.0 and R Studio 1.1.419. Generalized linear mixed models (GLMM) were used to investigate variation in the abundance of host-seeking mosquitoes (per day and per hour) using the package *lme4* in R [66]. As mosquito abundance data were overdispersed, all models were fitted with a negative binomial distribution. For all response variables of interest as described below, model selection was carried out through a process of backward stepwise elimination from a maximal model using likelihood ratio tests (LRT) [67].

Statistical analysis was performed for *Ae. aegypti* and *Culex quinquefasciatus* as the latter was the only other mosquito species found in high abundance in the study area. *Culex quinquefasciatus* is a nuisance biting mosquito and also a known vector of West Nile virus (WNV) [68].

The BGS traps functioned continuously across all days and sampling hours. However, the METs stopped running during some sampling hours; generally, under conditions of very high humidity due to rainfall which resulted in dampness on the traps and some temporary short circuiting (e.g. observed as plumes of smoke at the bottom junction with the frames). When these malfunctions occurred, the damaged traps were turned off and repaired. This resulted in variation in the total number of hours sampled with each trapping method (MET: 229 h; BGS: 270 h). This variation in sampling effort was accounted for in the statistical analysis. Days having less than 9 h were excluded from the analysis.

Four models were built to assess the variation in the abundance of each mosquito species and sex combination, respectively. For each of these four response variables, a maximal model was constructed that included the fixed explanatory variables of sampling effort (total number of hours of collection), trap type (MET or BGS), daily mean relative humidity (%RH), and daily mean temperature (°C). In addition, the interaction between daily mean temperature with relative humidity was also included. Sampling day (1 through 12), household ID, trap ID and attractant ID (BG-Lure cartridge ID or MET volunteers ID) were included as random effects.

Mosquito biting activity was assessed through analysis of variation in the mean number of females (*Ae. aegypti* and *Cx. quinquefasciatus*) caught per hour. Here, each mosquito species was analysed separately. Each model included the explanatory variables trap type (MET or BGS), sampling hour, mean temperature (°C) per hour, mean relative humidity (%RH) per hour, and the interaction between hourly temperature and relative humidity. Sampling hour was defined as a continuous variable recoding the first hour of trapping (7:00–8:00 h) into 1, and increasing "hour" by one digit for each subsequent hour until 12 h (17:00–18:00 h). Sampling hour was fit both as a linear and quadratic term, with the latter being used to test for peaks in biting time as have been

previously reported for these mosquito species [69]. In addition, sampling day, trap ID, cluster ID, household ID (nested within cluster ID) and attractant ID (BG-Lure cartridge ID or MET volunteer ID) were fitted as random effects.

Results

Mosquito species and abundance

During the 12 day-experiment, a total of five mosquito species were collected by both trapping methods (Table 1). *Culex quinquefasciatus* was the most abundant species (78.6%) followed by *Ae. aegypti* (15.63%), and small numbers of *Aedes angustivittatus* (2.69%), *Limatus durhami* (2.33%) and *Psorophora ferox* (0.15%). A small proportion of mosquitoes could not be identified (0.51%, Table 1). Overall, more mosquitoes were collected with the BGS trap (60.77%) than with the MET (39.23%), but the numbers of *Ae. aegypti* were relatively similar (Table 1).

In the BGS traps, some non-target insects including house flies, butterflies, crane flies, and many fruit flies were caught. No insect taxa other than mosquitoes shown in Table 1 were caught in MET collections.

The mean daily abundance of *Ae. aegypti* was approximately 2 females and 3 males for the BGS trap, and 4 females and 4 males for the MET, but no significant differences between trapping methods were found (Table 2, Fig. 3a, b). The only significant predictor of daily abundance of females *Ae. aegypti* was temperature, which exhibited a negative association (Table 2, Fig. 4a). Similarly, the mean daily abundance of *Cx. quinquefasciatus* females did not significantly differ between trapping methods (Table 2, Fig. 3c, d); however, confidence intervals (especially for males) around estimates were very large, indicating that larger sample sizes may be required to robustly test if there were differences between trap types. The number of female *Cx. quinquefasciatus* per day varied between 16–207, with variation being even more pronounced for males where a high of 576 was caught on one day. The daily abundance of female *Cx. quinquefasciatus* was negatively associated with daily temperature (Table 2, Fig. 4b) and positively associated with the number of hours sampled in a day, while no significant differences were found in *Cx. quinquefasciatus* regarding any covariate (Table 2).

Mosquito biting activity

Hourly mosquito catches recorded for BGS and METs were used to characterize the biting activity of female *Ae. aegypti* and *Cx. quinquefasciatus.* Variation in the hourly biting activity of female *Ae. aegypti* was best explained by a quadratic association between hourly mosquito abundance and time (Table 3), with activity being highest in the early morning and late afternoon, and little activity during the middle of the day (Fig. 5a). After taking this hourly variation in biting rates into account, there was no additional impact of trapping method on the number of female *Ae. aegypti* collected per hour (Table 3, Fig. 6). Variation in the hourly biting activity of *Ae. aegypti* was also significantly associated with an interaction between temperature and relative humidity (Table 3). This interaction arose because the number of *Ae. aegypti* caught per hour was negatively associated with temperature under conditions of low relative humidity; but the strength of this association was lower as humidity increased (Table 3, Fig. 7), although temperature and humidity were strongly associated (Additional file 2: Figure S1).

The biting activity of female *Cx. quinquefasciatus* also varied significantly across the sampling day. As with *Ae. aegypti*, this pattern was characterized as a quadratic relationship in which mosquito activity peaked during the early morning and late afternoon (Table 3, Fig. 5b). Accounting for this activity pattern, there was no difference in the number of *Cx. quinquefasciatus* caught per hour in different trapping methods (Table 3, Fig. 6b), and no association with temperature or humidity.

Table 1 Abundance of mosquito species collected by MET and BGS traps

Species	Mosquito electrocuting trap (MET)				BG-Sentinel (BGS) trap				Grand total
	♂	♀ Unfed	♀ Fed	Total	♂	♀ Unfed	♀ Fed	Total	
Aedes aegypti	100	99	19	218	93	91	27	211	429
Culex quinquefasciatus	496	238	44	778	960	345	77	1382	2160
Aedes angustivittatus	4	38	6	48	0	24	2	26	74
Limatus durhami	0	22	0	22	0	42	0	42	64
Psorophora ferox	0	1	2	3	0	1	0	1	4
Unknown	0	5	3	8	0	5	1	6	14
Total				1077				1668	2745

Notes: Mosquito species abundances are split by sex and feeding status of females. The total sampling effort with the two METs was 229 h, while for BGS traps was 270 h over the 12 days of sampling

Table 2 Summary for the terms tested from mosquito daily abundance

Explanatory variable	*Aedes aegypti*						*Culex quinquefasciatus*					
	Males ♂			Females ♀			Males ♂			Females ♀		
	χ^2	*df*	*P*	χ^2	*df*	*P*	χ^2	*df*	*P*	χ^2	*df*	*P*
Sampling effort	3.38	1	0.07	1.95	1	0.16	0.31	1	0.58	15.91	1	< 0.001*
Trap type	2.18	1	0.14	0.60	1	0.44	0.95	1	0.33	1.5	1	0.22
Temperature	0.22	1	0.64	4.62	1	0.03*	0.06	1	0.8	6.86	1	< 0.01*
Relative humidity	1.14	1	0.29	2.17	1	0.14	1.23	1	0.27	1.1	1	0.29
Temperature × Humidity[a]	2.22	1	0.14	1.24	1	0.26	1.07	1	0.3	1.27	1	0.26

*Significant values

[a] Fixed effect indicating interaction term

Notes: Chi-square (χ^2), degrees of freedom (*df*) and *P*-values (*P*) are provided for each sex within species

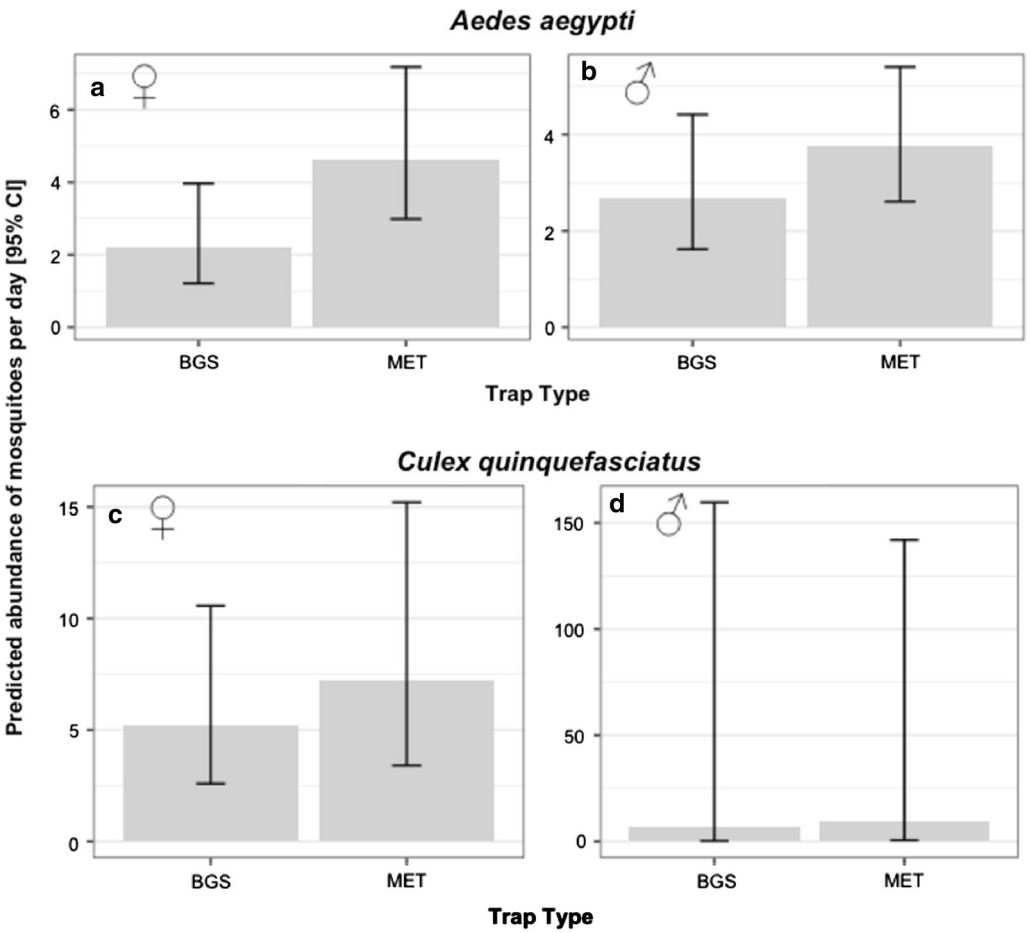

Fig. 3 Predicted mean daily abundance of mosquitoes caught with different trapping methods. **a**, **b** Data for *Ae. aegypti*. **c**, **d** Data for *Cx. quinquefasciatus*. **a**, **c** Data for females (♀). **b**, **d** Data for males (♂). Error bars indicate the 95% confidence intervals (CI)

Molecular screening for ZIKV, DENV and CHIKV

Aedes aegypti females were tested for ZIKV, DENV 1-4 and CHIKV and none of the samples were found positive. For a detailed description on the molecular results, please see Additional file 1: Text S2 and Additional files 3, 4, 5, 6, 7, 8, 9, 10: Figures S2–S9. In Additional files 4, 5, 6, 7, 8, 9, 10: Figures S3–S9, asterisk indicates the samples that had a weak band at the corresponding

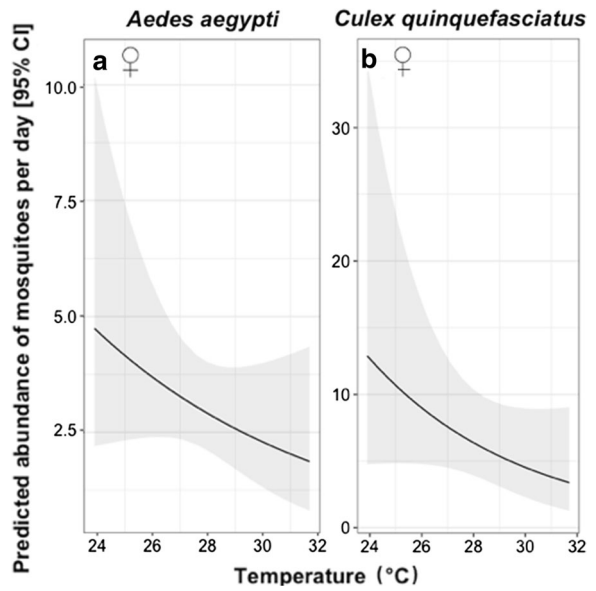

Fig. 4 Predicted relationship between mean temperature and number of female mosquitoes collected. **a** *Ae. aegypti* females. **b** *Cx. quinquefasciatus* females. The solid line indicates the mean predicted abundance and the shaded area indicates the 95% confidence intervals (CI)

expected size, and ^ indicates the samples that showed a size close to the expected one. The red dashed line is positioned at the corresponding expected size for each PCR run.

Discussion

Identifying an accurate method to predict the exposure of humans to infected mosquito vectors has been an enormous challenge for *Aedes*-borne pathogens [70, 71]. Here, we present the MET as a potential alternative for safe measurement of *Aedes* landing rates on humans. When tested in Ecuador, the MET provided similar estimates of *Ae. aegypti* abundance and biting activity as the current gold standard, the BGS sentinel method. While the BGS uses artificial odour baits and carbon dioxide (CO_2) to lure mosquitoes into a standardized trap, the MET directly estimates the number of *Aedes* host-seeking within the immediate vicinity of a real host. The MET can also be used to measure biting rates on a range of different host species (e.g. [53]), which currently cannot be performed with the BGS and other methods. The standardization provided by the BGS makes it easy and effective to use in widescale surveillance [48, 50], although a limitation is that non-biogenic CO_2 sources are not always available [72]. However, the degree to which BGS collections accurately reflect *per capita* human biting rates is unclear. For example, BGS trapping efficiency may vary with the type and number of lures used, rate of CO_2 released (quantity per time), location and colour of the trap (e.g. BGS 1 and BGS 2) [38, 46, 73], making it difficult to infer how different variants translate into exposure experienced by one person in that environment. An advantage of the MET is that it is more directly analogous to the human landing catch in sampling mosquitoes in the process of host-seeking on a person and also estimate variability in attraction between individuals. This could also be seen in the total catches of the other mosquito species when compared to the total numbers trapped by the BGS. The MET could thus provide a useful supplementary surveillance method for estimation and validation of human-biting rates and the associated entomological inoculation rate (EIR).

By facilitating a safe and more direct estimation of the EIR for *Aedes*-borne viruses, the MET could provide robust and precise entomological indicators of transmission intensity [51–53]. Such indicators are much needed to understand heterogeneity in transmission [33, 74, 75] and evaluate the efficiency of vector control interventions. However, this relies on the assumption that the

Table 3 Summary for the terms tested for association with female mosquito hourly abundance

Explanatory variable	*Aedes aegypti* females ♀			*Culex quinquefasciatus* females ♀		
	χ^2	*df*	*P*	χ^2	*df*	*P*
Trap type	0.60	1	0.44	7e-04	1	0.98
Time (linear)	na	na	na	na	na	na
Time (quadratic)	8.70	1	< 0.01*	142.1	1	< 0.001*
Temperature	na	na	na	2.07	1	0.15
Relative humidity	na	na	na	0.09	1	0.77
Temperature × Humidity[a]	6.60	1	0.01*	0.09	1	0.76

*Significant values

[a] Fixed effect indicating interaction term

Notes: Chi-square (χ^2), degrees of freedom (*df*) and *P*-values are provided for females of each species. "na" indicates "not applicable" values for which single term significance was not possible because of their involvement in significant higher order terms

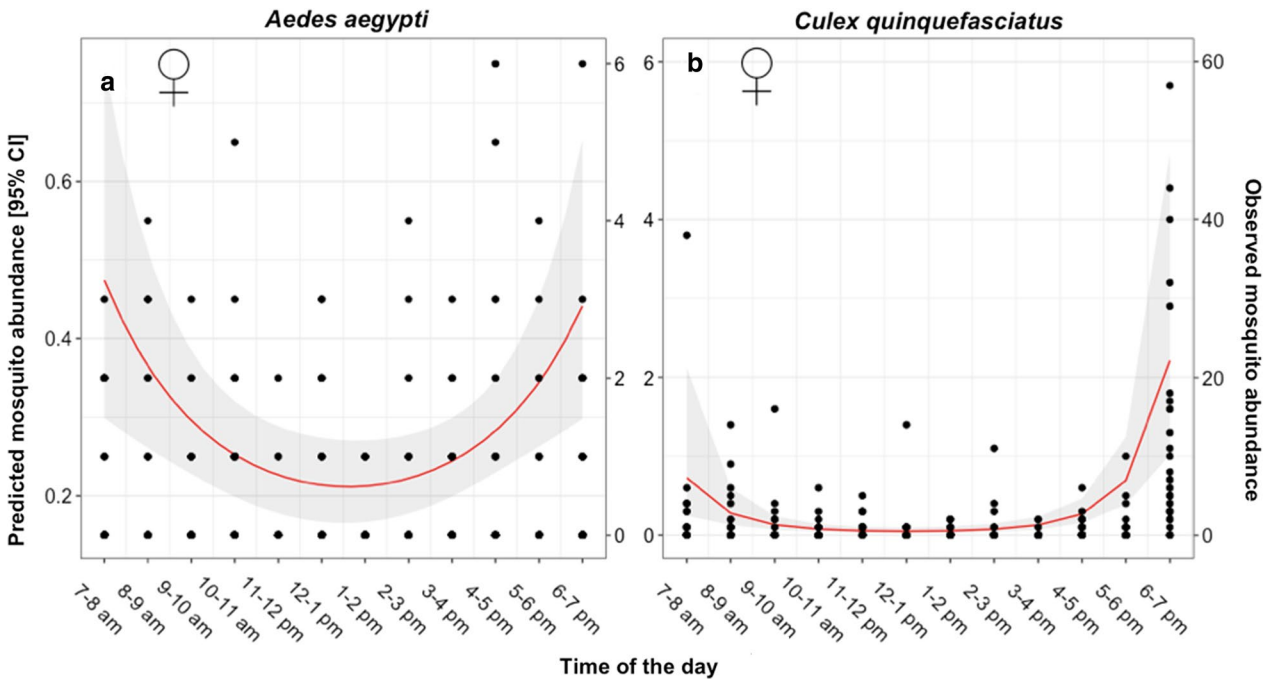

Fig. 5 Predicted abundance of biting mosquitoes between 7:00–19:00 h. **a** *Ae. aegypti* females. **b** *Cx. quinquefasciatus* females. Dots represent the observed values which correspond to the right Y-axes. The red line corresponds to the predicted mosquito abundance and the shaded area to the 95% confidence intervals (CI); both correspond to the left Y-axes

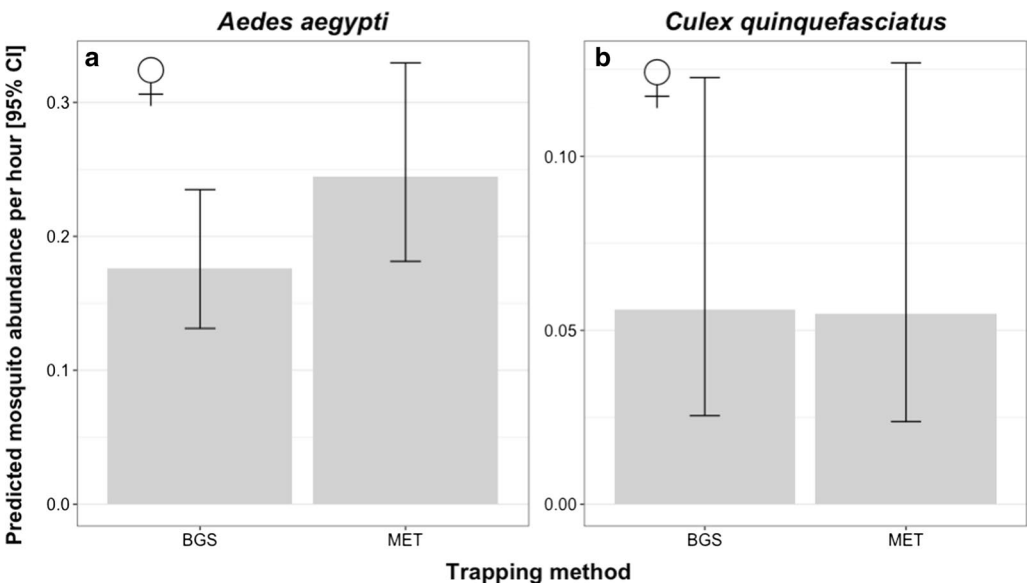

Fig. 6 Predicted hourly abundance of mosquitoes using different trapping methods. **a** *Ae. aegypti*. **b** *Cx. quinquefasciatus*. The error bars indicate the 95% confidence intervals (CI)

MET accurately reflects the true *Aedes* exposure of one person per unit of time. Estimates of human exposure to the malaria vector *An. gambiae* (*s.l.*) from the MET were similar to those of the human landing catch in some studies [53, 76], whereas in others mosquito abundance was underestimated by the MET compared to the HLC [52].

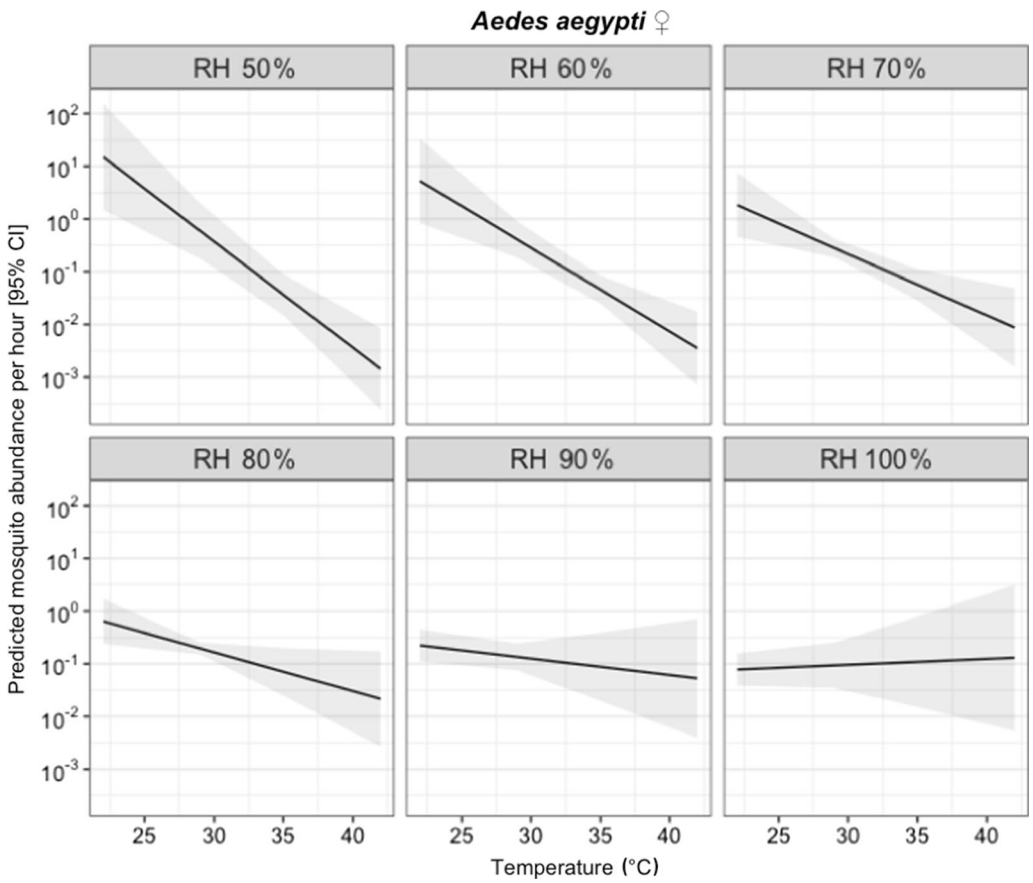

Fig. 7 Predicted relationship between the hourly abundance of *Ae. aegypti* females and mean temperature (°C) under different relative humidity (RH) conditions. The black line represents the predicted abundance of *Ae. aegypti* in that hour, with the shaded area representing the 95% confidence intervals (CI)

Here, it was not possible to directly compare the MET to the HLC because of ethical restrictions in using the latter in an area of high arboviral transmission. However, we speculate that one factor that could cause the MET to underestimate *Aedes* vectors biting rates is the area of the body protected. Whereas African *Anopheles* vectors generally prefer feeding on the lower legs and feet [77–79]; it is not clear if *Aedes* prefer to bite on specific parts of the body [80, 81]. As a next step in validating this approach, we recommend the MET to be directly compared to the HLC under controlled conditions with uninfected *Aedes* vectors (e.g. semi-field experiments), ideally using a defined *Ae. aegypti* strain and appropriate experimental design to act as a reference standard for future comparison.

Both the MET and BGS trap sampled a similar composition of mosquito species in the study period. However, estimates of the mean daily and hourly abundance of *Ae. aegypti* and *Cx. quinquefasciatus* were slightly but not statistically higher in MET than in BGS collections. The relatively short period of this (12 sampling days) may have limited power to detect for minor to moderate differences between trapping methods. We thus conclude the MET is at least as good as the BGS gold standard for sampling host-seeking *Aedes* vectors in this setting, but also recommend further longer-term comparisons over a wider range of seasons, sites and participants to evaluate whether the MET outperforms the BGS. If we assume that MET is equivalent to HLC, these results are also consistent to those shown by Kröckel et al. [50], who also observed that HLC captured more mosquitoes, although not statistically different from the BGS.

Mosquito collections conducted here were also used to test for associations between *Aedes* host-seeking activity and microclimatic conditions. The impact of temperature and humidity on the life history, physiology, behaviour and ecology of *Ae. aegypti* has been extensively investigated under laboratory conditions [82–85]. However, relatively little is known about how microclimate impacts the diel host-seeking behaviour of wild *Aedes*. In general, the host-seeking activity *Ae. aegypti* and *Cx. quinquefasciatus* was higher on days when mean temperatures

were lower (across the range of 25–30 °C). Additionally, the hourly biting rates of *Aedes* were negatively associated with temperature but only under conditions of low humidity. As mean hourly temperatures were strongly negatively correlated with relative humidity (Additional file 2: Figure S1), these results indicate that *Ae. aegypti* biting activity is highest during relatively cool and humid hours of the day. These microclimatic associations may account for the observed biting activity of *Ae. aegypti* and *Cx. quinquefasciatus*. A comprehensive review [69] of *Ae. aegypti* biting behaviour indicates that bimodal and trimodal activity patterns are often reported, with evidence of specific adaptations to other ecological features (e.g. artificial light availability) [69]. Such variability seems to be common and related to optimal humidity and temperature conditions available during such hours [86, 87].

A key feature of any method for estimating EIR is its ability to estimate human-biting rates and infection rates in mosquitoes. While the results here presented indicate that the MET could be used to estimate the human-biting rates, the infection rates could not be measured as none of the *Aedes* mosquitoes collected with either trapping method were positive for arboviruses. Reported rates of arboviruses in *Aedes* vectors are generally very low (0.1–10%) even in high transmission areas (e.g. [88–95]). Thus, failure to detect arboviruses within the relatively small sample size of vectors tested here (e.g. 207 individuals tested in 122 pools) is not unexpected.

Although promising, the MET has a number of limitations relative to the BGS for sampling host-seeking *Aedes*. First, although both trapping methods require a power supply, the current version of the MET requires two 12 V batteries compared to the one required by the BGS), requires human participants and the trap itself is heavier, which is more labour-intensive than using BGS. Also, as the METs used here are still research prototypes produced on a bespoke basis without a licensed manufacturer, their production cost is currently more expensive than BGS traps (approximately £650 *vs* £170 per trap, respectively). In addition, some technical problems were experienced including a tendency to short circuit under conditions of high air humidity. These limitations are expected to be improved if manufactured at scale as manufacturing costs would fall and technical improvements should make the MET suitable for humid environments. The primary advantage of the MET is, therefore, its potential ability to directly estimate the EIR for arboviral infections. This advantage could be leveraged to calibrate other existing trapping methods that are less labour intensive and more feasible to be deployed at large scale. Additionally, the MET could be used in combination with other trapping methods to identify hotspots of transmission before large scale deployment with other traps is carried out.

Conclusions

Here, we evaluated the MET as a tool for estimating human biting rates of the arboviral vector *Ae. aegypti* in a high transmission setting in coastal Ecuador. The MET performed at least as well as the current BG-Sentinel trap gold standard for estimating the mean abundance per hour of host-seeking *Aedes* and provided a realistic representation of hourly activity patterns. We conclude that MET is a promising tool for *Ae. aegypti* and other mosquito species surveillance, which could uniquely enable a relatively direct estimate of the arboviral entomological inoculation rate experienced by communities.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13071-020-3887-8.

Additional file 1: Text 1: Additional Methods. **Table S1.** Primers used for detection of arboviruses by RT-PCR. **Table S2.** Positive control DNA sequences used as PCR positive controls. **Text 2.** Additional Results.

Additional file 2: Figure S1. Relationship between observed temperature (°C) and relative humidity (%). Red dots represent individual observations per hour recorded.

Additional file 3: Figure S2. Visualization of the PCR products of S7 gene on agarose gels. All samples were positive, except 920-1.

Additional file 4: Figure S3. Visualization of the first PCR products of ZIKV on agarose gels. Expected size of positive fragments: 76 bp. ZIKV+: positive control.

Additional file 5: Figure S4. Visualization of the first PCR products of DENV 1–3 on agarose gels. Expected size of positive fragments: 63 bp. DENV1+: positive control.

Additional file 6: Figure S5. Visualization of the first PCR products of DENV 4 on agarose gels. Expected size of positive fragments: 63 bp. DENV4+: positive control.

Additional file 7: Figure S6. Visualization of the second PCR products of ZIKV on agarose gels. Expected size of positive fragments: 76 bp. ZIKV+: positive control.

Additional file 8: Figure S7. Visualization of the second PCR products of DENV 4 on agarose gels. All samples were negative. Expected size of positive fragments: 63 bp. DENV4+: positive control.

Additional file 9: Figure S8. Visualization of the second PCR products of DENV 1-3 on agarose gels. Expected size of positive fragments: 63 bp. DENV1+: positive control.

Additional file 10: Figure S9. Visualization of the individual PCR products of DENV1, DENV2 and DENV3. Samples from Figure S8 are shown for individual runs for each of the three DENV isotypes. Expected size for DENV1 run was 71 bp, 199 bp for the DENV2 run, and 167 bp for the DENV3 run. DENV1+, DENV2+ and DENV3+: positive controls.

Abbreviations

HLC: human landing catches; EIR: entomological inoculation rate; MET: mosquito electrocuting trap; BGS: BG-sentinel trap; ZIKV: Zika virus; DENV: dengue virus; CHIKV: chikungunya virus; WNV: West Nile virus; GLMM: generalized linear mixed models; LRT: likelihood ratio test; PCR: polymerase chain reaction.

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Authors' contributions

LDOL, HMF, RL and AK conceptualized the project. NM developed and built the mosquito electrocuting traps (METs). LDOL, MPB, ST and SS conducted the field work of mosquito collections under the supervision of NM, RL and HMF. MPB and LDOL carried out the morphological analyses. LDOL and FA carried out the molecular analyses under permanent supervision of EP and AK. LDOL carried out the statistical analyses and wrote the manuscript under the guidance of HMF. AK, EP, HMF, MPB, NM and RL edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data supporting the conclusions of this article are included within the article and its additional files. The dataset generated and analysed during this study is publicly available in the Open Science Framework repository at https://osf.io/zwbs8.

Ethics approval and consent to participate

Ethical approval for this research was granted by the MVLS College Ethics Committee of the University of Glasgow (Project No.: 200150175), and by the Ethics Committee of Research on Human Beings of the San Francisco de Quito University (2016-146M). Prior to the study, the objectives of this research and risks and benefits for taking part in this were explained to the participants in MET collections and their written informed consent was obtained. Oral informed consent was also obtained from the heads of households where mosquito collections were performed. The purpose and objectives of the study was explained to householders before requesting permission for the study team to collect mosquitoes within their properties.The Government of Ecuador, through the Ministry of Environment (MAE), granted the permits to carry out the present study under the Framework Agreement on Access to Genetic Resources No. MAE-DNB-CM-2016-0052. Transportation of samples from the study site in Quinindé (Rosa Zárate) to the LEMMT-USFQ in Quito was authorized by MAE through the document No. MAE-DPAE-2017-1163-O. Transfer of samples from the LEMMT-USFQ in Ecuador to the MRC-University of Glasgow CVR in the UK, was firstly established by a Material Transfer Agreement signed between Universidad San Francisco de Quito and the University of Glasgow on the 1st of September of 2017. The exportation of biological samples from Ecuador was authorized by MAE under the document 076-17-EXP-IC-FAU-DNB/MA. Finally, a certification of no-need of importation permit to Scotland was issued through a letter from the Animal Health and Welfare Division of the Agriculture and Rural Economy Directorate of the Scottish Government on the 14th of September of 2017.

Consent for publication

Individuals pictured in the photographs of this article granted their oral consent of the photographs in which they appear to be published.

Competing interests

The authors declare that they have no competing interests.

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