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Spatio-Temporal distribution of mosquito species *Aedes albopictus*: the associated health risk and an assessment of the effectiveness of control interventions in Italy.

Thesis submitted for the degree of Doctor of Philosophy in
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Against God
And the statistics
I will get through

The Beast, by Teho Teardo and Blixa Bargeld

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Introduction

"In theory, there is no difference between theory and practice. But, in practice, there is."
attributed to Jan L. A. van de Snepscheut

In their tiny sizes, arthropod crawl or fly in huge numbers and diversity often unnoticed around us. How many of these little terrestrial companions have been splattered without us hearing a single desperate scream of agony would be forever unknown. However, not all of them go unnoticed, their lives being put under the microscope of science and curiosity. Unravelled by scientists, the connections between humans and some of this species could be deep and unfortunately at human disadvantage. Sometimes the discovery of an ecological or epidemiological link happens almost by chance and surrounded by incredulity. Take as an example the mosquito, at present known to be responsible for the transmission of deadly diseases such as Malaria or Yellow Fever, but initially few would have thought or investigate it as the vector of these dreadful health problems. Indeed, several species of mosquitoes feed on human and as unpleasant as it already is, it is even worse. These itchy bites are often the entry point for numerous pathogens and viruses as discovered by the remarkable work of pioneers such as Ross and many others. Their research and sacrifices ought to be never forgotten and are still strikingly relevant nowadays.
The study of arthropod that are competent in transmitting zoonotic disease or that impacts human health is the research field of Medical Entomology, a fascinating discipline that unravels the darkest secrets and beautiful complexity of nature. This thesis aims to contribute in this field by advancing the current knowledge about the mosquito species *Aedes albopictus*, its distribution and its impact on public health also providing an assessment of the commonest control strategies employed against it.

Aedes albopictus

Aedes albopictus is a mosquito species characterized by a day-time biting behaviour, diapausing eggs and ecological plasticity (Hawley 1988). Its appearance is the one of a small black mosquito and is easily distinguishable for its white dorsal stripe and withe banded legs (Figure 1).

Figure 1. *Aedes albopictus* adult female captured on an adhesive sheet.

On the other hand, aquatic stages require some degree of expertise to be confirmed as *Aedes albopictus*. Eggs may be confused with ones of other aedine species and may be indistinguishable by simple observation under a binocular microscope (Capelli *et al.* 2011) thus requiring the eggs to be reared in the laboratory for identification or the use of biomolecular tools. Eggs are critical for *Aedes albopictus* success in spreading outside its place of origin as diapausing eggs allow overwintering in temperate region, survival to desiccation and passive transport (Reiter & Sprenger 1987; Reiter 1998). The physiological mechanism known as diapause inhibits the development of eggs when exposed to unfavourable environmental condition and is repeatedly observed in *Aedes albopictus* eggs during the cold season of the year (Hanson 1995). It is to be remarked however, that also adults can survive during winter under favourable conditions (Dutto & Mosca 2017).
Typical of a mosquito species, the *Aedes albopictus* life cycle could be summarized as follows:

- An egg is laid, typically in a small amount of corrugated water
- The egg hatches and develops in larva
- The larva grows through four developmental stages
- The IV instar larva develops into a pupa
- The pupa emerges from the aquatic stages as an adult.
- Female adults seek bloodmeals to develop the eggs

The length of developmental stages could vary depending on temperature as shown by previous studies (Alto & Juliano 2001b; Delatte *et al.* 2009). Temperature could have a lagged impact on *Aedes albopictus* population, with evidence that a mean temperature of 25°C during the warmest quarter provide optimum condition for population growth (Cunze *et al.* 2016). The activity of adults has been also linked to temperature, with a 13°C and a 9°C threshold for initiating and ending adult activity, respectively (Roiz *et al.* 2010). Nonetheless, mosquito survival and activity could be extended during cold month in urban environments whenever adult mosquitoes succeed in finding inside or near human habitation warmer resting and breeding locations. However, temperature of the coldest months has been suggested as a determining constrain in the occurrence of this species when below 0° C as it prevent the survival of diapausing eggs (Thomas *et al.* 2012). Temperature alone is not the only ecoclimatic variable affecting *Aedes albopictus*, also rainfall and the photoperiod have been associated with changes in population dynamic and activity (Waldock *et al.* 2013), but their role in shaping the mosquito population has not yet been entirely disentangled. Indeed, precipitations are necessary to replenish available breeding site (Alto & Juliano 2001a) or to create new ones thus allowing the population expansion (Roiz *et al.* 2015), but excessive rainfall could prevent adult mosquito flying activity or wash out eggs from small breeding sites.

Adult females could be spotted during the breeding seasons, typically from May to late October in temperate regions, flying near available host or resting in shaded vegetated area. The daytime adulthood activity is bimodal but field evidences suggest that under favourable condition females bite during all daylight hour if a bloodmeal is available. *Aedes albopictus* is not a great distance flyer. Studied on flight range and dispersal have been carried out by mark release recapture experiments using for ethical reasons blood-fed females (Honório *et al.* 2003; Marini *et al.* 2010). Their results show that the daily mean distance travelled is about 119 meter, with the furthest recapture happening at 290 meter on the boundaries of the study area after 17 days after release (Marini *et al.* 2010). *Aedes albopictus* is known to be an exophilic species, one that prefers biting outdoor, but there are increasing evidences that females in their hunt for a bloodmeal can show indoor biting behaviour. Females feed on a wide range of hosts, but are strongly attracted to human as proved by an experiment in Rome (Italy) that found the human blood index ranging from 79–96% in urban sites to 23–55% in rural sites (Valerio *et al.* 2010). Being particularly aggressive and being active during the day it has been reported as a source of not neglectable nuisance (Carrieri *et al.* 2008). On human hosts, the bites are usually found on ankles or on the lower part of the body. However, unprotected or exposed skin is a feasible spot for a bite on every part of the body. Obviously, this preference for human blood has serious consequences for the transmission of pathogens.

When not engaged in host seeking, females *Aedes albopictus* may rest and location of preference are shaded or cryptic spots generally with vegetation that could create optimal conditions of temperature and humidity. Similarly, preferred breeding sites are small or

Figure 2. Artificial container (bucket) used as breeding site by *Aedes albopictus* females, Procida island (Italy) September 2016. It is possible to observe both larvae in the water and adults resting on the leaves.

cryptic natural and artificial containers (Chandel *et al.* 2016) such as catch basins, man-made containers, buckets, basin of fountains, tires, vases/flowerpots and natural mosquito larval habitats. The positive relationship between vegetation and presence and abundance of *Aedes albopictus* population has been shown in urban settings (Landau & van Leeuwen 2012; Cianci *et al.* 2015). However, the case for urbanization increasing *Aedes albopictus* population is strong (Li *et al.* 2014) and the role of landscape variables should be further assessed. In the thesis, I investigated the competing role of vegetation and anthropic presence in the province of Rome by using an advanced statistical modelling approach, *ie* a negative binomial generalized additive mixed model fitted on field capture data. The results identify *Aedes albopictus* hot-spots characteristics that could help the planning of surveillance strategies and control interventions. Moreover, results show a particular bimodal pattern in the seasonal activity of the species that could have serious implication for pathogen transmission by extending the period when the introduction of an infected host could result in autochthonous transmission.

Aedes albopictus, exploiting passive transport, has become in less than 30 years a worldwide health threat by colonizing large temperate area in Europe and America (Kraemer *et al.* 2015). However, the introduction and establishment of invasive mosquito species alone does not pose a direct public health threat, it is necessary the introduction of infectious hosts from endemic area and the species being competent for the introduced pathogen. The introduction of infected hosts is relatively frequent nowadays that international travels are common and affordable. In fact, 169 viremic person-days in EU, of which 130 arriving in Italy, have been estimated among travellers from endemic countries between April and October (Seyler *et al.* 2009). In Europe public health authorities set up guidelines to tackle the event of autochthonous transmission of emerging vector borne diseases (Marrama Rakotoarivony & Schaffner 2012) subsequently the introduction of infected hosts. Suggested control interventions target mainly larval stages by using larvicides or removal of breeding sites, whereas control interventions targeting adults are recommended only as an emergency response during outbreaks or when nuisance became intolerable (Baldacchino *et al.* 2015). The competence of *Aedes albopictus* for arboviruses such as Dengue, Chikungunya and Zika has been studied and confirmed (Di Luca *et al.* 2001; Gratz 2004). Unfortunately, many local government and citizens perceived mosquito mainly as a nuisance problem (Dickinson & Paskewitz 2012), unconsciously neglecting the associated public health risk. The increasing number of local outbreaks happened in Europe during the last decade (Rezza *et al.* 2007; Gjenero-Margan *et al.* 2011; Tomasello & Schlagenhauf 2013), including the 2017 Chikungunya outbreak in Italy (Venturi *et al.* 2017), still ongoing up to the moment this thesis is being written, may change in future this perception. In the thesis is provided a quantitative characterisation of the outbreak of chikungunya currently (2017) ongoing in Lazio region, Italy, based on early epidemiological records published by the Italian National Institute of Health on October 10th and on a transmission model informed with previously collected data on mosquito abundance presented in the first chapter.

One of the key but unknown parameter needed to estimate the transmission risk is the vector to host ratio. The estimation of the vector population abundance and dynamics is crucial but not trivial. Active monitoring by traps that collects eggs (Johnson, Ritchie & Fonseca 2017), adults (Englbrecht *et al.* 2015) or passive monitoring (Kampen *et al.* 2015) have been employed in the ambitious goal of estimating vector abundance, nuisance and transmission risk. However, monitoring mosquito is challenging (Carrieri *et al.* 2017). The reliability as a tool for the straightforward estimation of adult abundance of the gold standard trap (ovitrap) used in surveillance program based on egg was yet to be proven. Following these premises in the thesis an evaluation of the relationship between trap captures and adult mosquito densities is carried out and the results used to inform risk threshold based on observed capture and give a preliminary evaluation of the transmission risk in Rome for Dengue, Chikungunya and Zika.

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out and the results used to inform risk threshold based on observed capture and give a preliminary evaluation of the transmission risk in Rome for Dengue, Chikungunya and Zika.
At present, mosquito surveillance and control programs are the main public health authorities' response to the presence of *Aedes albopictus* and the risk of mosquito borne diseases transmission, but communities play a vital role, including residential and private properties greatly improves the effectiveness of control measures (ECDC 2017). Many control strategies are available or in development and are manly based on source reduction, trapping, biological control (e.g. *Bacillus thuringiensis var. israelensis, Wolbachia*), chemical control (insecticide) and genetic engineering (sterile insect technique or genetically modified mosquitoes) (Baldacchino *et al.* 2015). Control interventions effectiveness ought to be evaluated by careful monitoring of mosquito population pre and post treatment. However, this is rarely the case especially when control intervention are paid or requested directly by citizens or to assess the control strategy as a whole rather than the single intervention. Moreover, when such assessment have been carried out for field insecticide treatments, results were not straightforward with treatment outcome being more effective in urban than in sub-urban sites (Fonseca *et al.* 2013). Given the lack of data on effectiveness of common use of insecticide field treatments, this thesis contributes in the field by providing an assessment of both a commonly applied control intervention in private properties as well as an evaluation of an area-wide control strategy carried out by public authorities.

Summary

During my PhD, I have exploited my background in statistical and mathematical modelling and the long standing entomological expertize of the Medical Entomology group of the DSPMI of Sapienza University to:

- *i)* contribute to clarify some relevant information for a better knowledge on the species distribution and temporal dynamics in Lazio region (where the 2017 chikungunya outbreak occurred) (chapter 1);
- *ii)* estimate CHIKV importation time, transmission dynamic, magnitude of the outbreak and associated health costs during the 2017 CHIKV outbreak (chapter 2);
- *iii)* assess whether the most widely used approach to monitor adult female adult densities (i.e. collections of eggs by ovitraps) allow precise estimations of mosquito-human contact (i.e. the most relevant parameter for epidemiological models) (chapter 3); and
- *iv)* assess the effectiveness of insecticide-based control interventions on the mosquito seasonal dynamics and abundance (chapters 4, and 5). Each chapter of the main body of the thesis corresponds to an article published on peer-reviewed journals. Thus, each chapter structure is the one of a scientific article, including an abstract, an introduction, a material and methods section, a result section and a conclusive discussion section. Any supporting information were included when present. References are listed at the end of the thesis.

Chapter 1

Spatial and temporal hot spots of *Aedes albopictus* abundance inside and outside a South European metropolitan area.

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Abstract

Aedes albopictus is a tropical invasive species which in the last decades spread worldwide, also colonizing temperate regions of Europe and US, where it has become a public health concern due to its ability to transmit exotic arboviruses, as well as severe nuisance problems due to its aggressive daytime outdoor biting behaviour. While several studies have been carried out in order to predict the potential limits of the species expansions based on eco-climatic parameters, few studies have so far focused on the specific effects of these variables in shaping its micro-geographic abundance and dynamics. The present study investigated eco-climatic factors affecting *Ae. albopictus* abundance and dynamics in metropolitan and sub-urban/rural sites in Rome (Italy), which was colonized in 1997 and is nowadays one of the most infested metropolitan areas in Southern Europe. To this aim, longitudinal adult monitoring was carried out along a 70 km-transect across and beyond the most urbanized and densely populated metropolitan area. Two fine scale spatiotemporal datasets (one with reference to a 20m circular buffer around sticky traps used to collect mosquitoes and the second to a 300m circular buffer within each sampling site) were exploited to analyze the effect of climatic and socio-environmental variables on *Ae. albopictus* abundance and dynamics along the transect. Results showed an association between highly anthropized habitats and high adult abundance both in metropolitan and sub-urban/rural areas, with "small green islands" corresponding to hot spots of abundance in the metropolitan areas only, and a bimodal seasonal dynamics with a second peak of abundance in autumn, due to heavy rains occurring in the preceding weeks in association with permissive temperatures. The results provide useful indications to prioritize public mosquito control measures in temperate urban areas where nuisance, human-mosquito contact and risk of local arbovirus transmission are likely higher, and highlight potential public health risks also after the summer months typically associated with high mosquito densities.

Introduction

The mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse) is classified among the 100 worst invasive species in the Global Invasive Species Database (http://www.issg.org/database/species) including all species of micro-organisms, fungi, plants and animals globally recognized as major threats to biodiversity and/or human activities. In the last 40 years, the species has been able to spread from its native range of distribution in rural tropical South-East Asia worldwide, largely through the transportation of its relatively cold-hardy and long-lived eggs via the international trade in used tires (Reiter & Sprenger 1987; Benedict *et al.* 2007) and to the capacity to colonize temperate regions by photoperiodic egg diapause (Hawley 1988; Benedict *et al.* 2007; Medlock *et al.* 2015). Another key element favouring *Ae. albopictus* expansion particularly to urban environments has been its ability to shift from natural larval habitats in forest edges (e.g. tree holes, bamboo stumps, and bromeliads) to anthropogenic containers (e.g. rain catch basins, tires, cemetery urns, vases, water storage containers) (Hawley 1988).

The first introductions of *Ae. albopictus* in Europe were documented in Albania in 1979 (Adhami & Reiter 1998) and 10 years later in Italy (Sabatini *et al.* 1990), where it has become a permanent pest in most regions (Medlock *et al.* 2012). In recent years the species gradually spread into other Mediterranean countries, including France, Spain, Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, and Greece (Medlock *et al.* 2015). Due to its aggressive daytime outdoor biting behaviour and to its ability to transmit a large variety of arboviruses (Medlock *et al.* 2015), the species represents an important nuisance as well as a major public health concern in all non-native countries where it has established (Gasperi *et al.* 2012). *Aedes albopictus* has been responsible for large chikungunya virus (CHIKV) epidemics in the Indian Ocean in 2005–2007 (Josseran *et al.* 2006; Renault *et al.* 2007) and of a CHIKV outbreak in northern Italy in 2007 (Rezza *et al.* 2007). Since then it has been associated with autochthonous transmission of CHIKV and dengue virus (DENV) in France (Ruche *et al.* 2010; Marchand *et al.* 2013; Delisle *et al.* 2015; 'L'épidémiologie 'Institut de veille sanitaire est. Chikungunya et dengue - Données de la surveillance renforcée en France métropolitaine en 2015 [Internet]' 2016) and of DENV in Croatia (Gjenero-Margan *et al.* 2011). Notably, these were the first autochthonous DENV cases reported in Europe since the outbreak in Greece in 1927–1928 caused by temporary establishment of a population of the tropical vector *Aedes aegypti* (Halstead & Papaevangelou 1980; Ruche *et al.* 2010). Models estimating climate change impact on spatio-temporal trends for risk exposure and season of transmission of CHIKV in Europe predict that Mediterranean regions will become increasingly climatically suitable for transmission, with highest risk of transmission by the end of the 21st century in France, Northern Italy and the Pannonian Basin (East-Central Europe) (Fischer *et al.* 2013). Moreover, a recent epidemic of Zika virus, with 440 000–1 300 000 estimated human cases in Brazil in 2015, is raising concerns about the risk of its introduction and local transmission by *Ae. albopictus* in Europe (Bogoch *et al.* 2016). Finally, its opportunistic biting behavior (Richards *et al.* 2006; Valerio *et al.* 2010) could involve *Ae. albopictus* in the transmission to humans of zoonotic pathogens such as West-Nile virus (Fortuna *et al.* 2015) and Dirofilaria canine nematodes (Cancrini *et al.* 2003, 2007).

Several authors used eco-climatic factors to predict the potential spatial distributions of *Ae. albopictus* and public health related threats. Most of these studies have focused on temperature to identify potential limits of the species range, indicating thresholds of minimum temperature in the coldest months and of heat accumulation (Nawrocki & Hawley 1987; Kobayashi, Nihei & Kurihara 2002; Neteler *et al.* 2011; Roiz *et al.* 2011) and producing maps to identify areas suitable for stable colonization (Bagny *et al.* 2009; Neteler *et al.* 2011; Fischer *et al.* 2013). Other studies exploited models to predict the species distribution using a broader range of climatic variables, including rainfall (Alto & Juliano 2001a; Benedict *et al.* 2007; Schaffner *et al.* 2009; Caminade *et al.* 2012; ECDC 2012; Fischer *et al.* 2014; Schaffner & Mathis 2014; Campbell *et al.* 2015). Recently, Kraemer et al. (Kraemer *et al.* 2015) developed an improved model combining climatic, environmental, land-cover and anthropogenic variables to predict the species probability of occurrence. Finally, Roche et al. (Roche *et al.* 2015) showed that human activities are particularly important for the species dispersion, while land use is a major factor for its establishment. Given the scale at which these studies were carried out, they are useful to predict *Ae. albopictus* future expansion and to improve surveillance programs by detecting the species introduction at its earliest stages when it is still possible to prevent its establishment (Marrama Rakotoarivony & Schaffner 2012).

However, in areas permanently colonized by *Ae. albopictus* it is crucial to identify potential spatial and temporal hot-spots of abundance which could be associated with higher nuisance biting and risk of disease transmission in order to prioritize mosquito control interventions. In fact, it has been demonstrated that treatment of hot-spot can be incorporated successfully into existing integrated mosquito management programs to increase their cost-effectiveness (Unlu *et al.* 2016). In general, availability of suitable breeding and resting sites along with the presence and abundance of competing species and of potential hosts (which in turn vary in relation to landscape composition, climatic conditions and host demography) are known to shape mosquito abundance at a local scale. In the absence of competition with *Aedes aegypti*, urbanization has been shown to favor high *Ae. albopictus* abundance (Li *et al.* 2014; Samson *et al.* 2015) and landscape and human activities have been found to be crucial to predict its actual local distribution and relative abundance (Vanwambeke, Bennett & Kapan 2011). To date, the specific effects of these variables in shaping *Ae. albopictus* micro-geographic abundance and dynamics at temperate latitude in Southern Europe are poorly understood. The few studies carried out so far showed a positive association between host-seeking female abundance and temperatures and a negative one with rainfall in north-east Italy (Roiz *et al.* 2010). Additionally, a positive association between number of eggs and vegetation around ovitraps was detected in a small highly urbanized site within Rome (Cianci *et al.* 2015).

The present study aims to investigate eco-climatic factors affecting *Ae. albopictus* abundance and dynamics in metropolitan versus sub-urban/rural sites in Rome (Italy), which was colonized by *Ae. albopictus* in 1997 and became one of the most infested metropolitan areas in Southern European temperate regions (Romi 2001; Severini *et al.* 2008).

Materials & Methods

Mosquito Sampling and Study Sites

Twenty-one study sites (hereafter referred as stations) were selected along a 70 km-transect across and beyond the most urbanized and densely populated metropolitan area of Rome (Italy), corresponding to the train route from the coast to Appennino mountains (Fig 1). All sites were below 300m asl, with the exception of station 20 (330m asl) and 21 (460m asl). Groups of four sticky traps (STs, (Facchinelli *et al.* 2007)) were located in a 300 m-radius area within each station (for a total of 84 STs), positioned on site and geo-referenced using GPS. The 300 m-radius was calculated from the centroid of the convex hull generated from groups of four neighbouring sampling points (i.e. STs) and corresponds to *Ae. albopictus* maximum dispersal range (i.e. 300 m; (Marini *et al.* 2010)). A 20 m circular buffer was calculated around each ST. This buffer at ST level corresponds to the largest one used in a similar study that showed an association between land cover variables and mosquito abundance (Cianci *et al.* 2015). Moreover, a 3 km-circular buffer was calculated from the centroid of the convex hull generated from groups of four neighbouring STs. Information obtained from the 3 km-circular buffer was used to assign each station to either metropolitan or suburban/rural area.

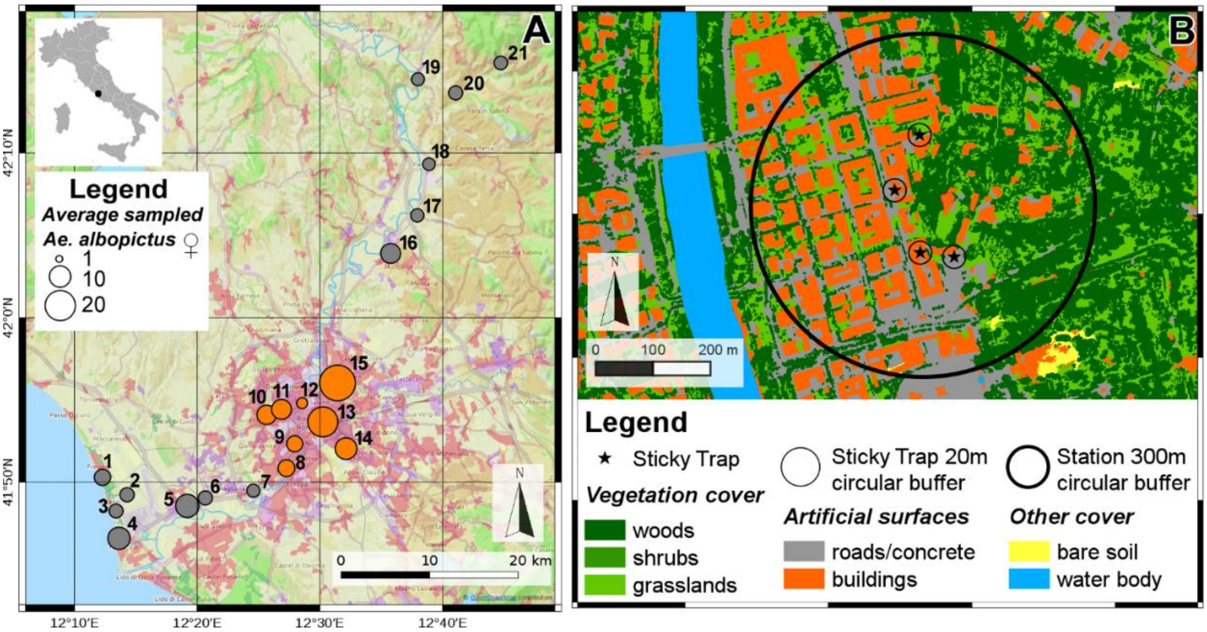


Fig 1: Mean abundance of *Aedes albopictus* collected along the 70 km-transect encompassing the metropolitan area of Rome. a) Map showing the weekly mean female abundance during the 18 sampling weeks in the 21 sampling stations (study sites); orange dot= "Metropolitan" site; grey dots= "Sub-Urban-Rural" site. b) Map showing the land cover variables in one of the 21 sampling sites, showing the 300 m-circular buffer calculated from the centroid of the convex hull generated from Sticky Traps (black star) and the 20 m-circular buffer around each Sticky Trap.

Sticky traps were monitored for 18 weeks from July 10th to November 8th 2012 on a weekly basis by substituting sticky panels with freshly glued ones and recording water leftovers. Trapped mosquitoes were morphologically identified and counted on site.

Dataset description

A fine scale spatio-temporal dataset was built to analyze the effect of a set of climatic and socio-environmental variables on *Ae. albopictus* abundances in the 21 stations.

Open source softwares were used for the construction of the spatial dataset: GRASS GIS (Neteler *et al.* 2012) for data processing and spatial analysis and QGIS (Quantum GIS Development Team 2012) for spatial analysis and layout generation. The spatial dataset included time-dependent climatic variables and time-independent socio-environmental variables, as follows:

Climatic variables:

i) Land Surface Temperature (LST) for each station was extracted from reconstructed temporal series of MODIS satellite data, collected by NASA (http://modis.gsfc.nasa.gov) and following methods described in Metz et al. (2014) (Metz, Rocchini & Neteler 2014). LST data represent the estimation of temperature detected at earth surface by the satellite sensor, with gap-filled pixels at 250m spatial resolution. Daily minimum, maximum and mean LST were extracted. These variables were used to compute the mean of daily LST minimum, maximum, mean, and range for each trap over each sampling week in the period June – November 2012. Mean LSTs were computed not only for the sampling week (Lag 0), but also considering different time-spans in the weeks before the sampling in order to take into account the role of climate variables during the larval development in affecting *Ae. albopictus* adult abundance and survival. A total of 4 temporal windows (Lag 1-4) were computed over the 1-4 weeks preceding the sampling weeks. Subsequently, the mean LST from Lag 1 to Lag 4 were also considered as climate variables.

ii) Growing Degree Days (GDD) were computed in order to take into account that heat accumulation influence *Ae. albopictus* life cycle and development. A value of 11° C was used as baseline temperature (Hawley 1988). Moreover, the weekly-accumulated GDD and a bounded estimate of accumulated GDD was calculated as in Roiz et al. (2015) (Roiz *et al.* 2015).

iii) Daily rainfall maps were generated by spatial interpolation of daily rainfall data acquired in 67 meteorological sampling stations, collected by the Hydrographic Service of Regione Lazio and disseminated through the hydrographic annals (http://www.idrografico.roma.it/annali). Daily rainfall maps were used to compute the total rainfall in each trap location for each week of the period June – November 2012. The same temporal windows as for LST (from Lag 0 to Lag 4) were considered.

Socio-environmental variables:

A land cover map was initially generated for the following 7 different classes retrieved from digital multispectral aerial imagery collected by optical sensor in the visible spectrum in 24 and 27 June 2008 at 0.5 m spatial resolution (Source: Italian National Geoportal, http://www.pcn.minambiente.it/GN/). Mapped land cover classes were ‘bare soil’, ‘roads/concrete’, ‘buildings’, ‘woods’, ‘shrubs’, ‘grasslands’, ‘water bodies’. Classification was performed using SMAP (Sequential Maximum A Posteriori) supervised classification

(Bouman & Shapiro 1994), in GRASS GIS 7, which segments multispectral images using a spectral class model known as a Gaussian mixture distribution and using spectral mean and covariance parameters. The SMAP segmentation algorithm improves accuracy and resolution of urban mapping by segmenting the image into regions rather than segmenting each pixel separately. Classification products were spatially filtered in order to partially remove classification errors and noise. Satellite data were confirmed by field workers during the initial positioning of STs. Collinear cover classes (representing proportions inside the buffer fixed space) were finally merged in two main land cover groups: 'artificial surfaces' (including 'roads/concrete' and 'buildings') and 'vegetation cover' (including 'woods', 'shrubs' and 'grasslands'). Only the latter one was used in the statistical analysis to avoid collinearity. Surface (in square meters) and cover percentage for the two main land cover classes were calculated for each station within: i) the 20 m-radius buffer around each ST, ii) the 300 m radius-buffer and iii) the 3 km radius-buffer. Population data was extrapolated from population 2011 census data (source http://gisportal.istat.it/). For the municipality districts encompassing the stations of the study area, recorded population has been divided by the area of the district in order to obtain the population density (inhabitants/km^2). The qualitative variable "environment" (Metropolitan or Sub-urban) was defined by evaluating the percentage of artificial surfaces within the 3 km-radius buffer and population density within the 300 m-radius buffer. Specifically, the environment around the centroid of the convex hull generated from groups of four neighbouring sampling points (i.e. ST) was defined "Metropolitan" when either the percentage of artificial surfaces exceeded 50% or the density of human population exceeded 6,000 human inhabitants per square kilometer, and "Sub-Urban/Rural" when neither these conditions were met.

Statistical analysis

The relationship between socio-environmental and climatic variables and *Ae. albopictus* abundance was investigated through generalized linear mixed models (GLMMs) and generalized additive mixed models (GAMMs). Response variable was the weekly number of *Ae. albopictus* adult females collected in each STs. Time-dependent (climatic) and time-independent (socio-environmental) variables were included in the model as predictors. In addition, the variable Day of Year (DoY) was also considered to investigate the temporal pattern of the population dynamic during the sampling period. Scatterplots, conditional boxplots, variance inflation factor (VIF) and concurvity (Buja, Hastie & Tibshirani 1989) were used to assess non-linear relationships and collinearity among variables.

Analysis of Aedes albopictus abundance during the entire sampling season.

A Negative Binomial GLMM was carried out to assess the effect of socio-environmental variables on *Ae. albopictus* adult abundance. The following explanatory variables were modelled linearly and centred to aid interpretation of model results (Schielzeth 2010): vegetation cover percentage computed at 20 meter-buffer around the STs, vegetation cover computed at 300 meter-buffer, environment, all the two-way interactions and the three-way interaction. Sampling stations were included as random effect to incorporate a dependency structure between observations taken by the four ST in the same station. However, the model residuals showed temporal patterns. Therefore, to account for the time varying abundance of *Ae. albopictus* dynamic time-dependent (climatic) variables were also considered. Preliminary analyzes were carried out to identify which climatic variables (i.e. temperature and rainfall) would be a feasible predictor of mosquito abundance and to identify its lagged effect (different temporal windows) on mosquito abundance. Although similar preliminary approaches are not advocated since they may result in *post hoc* hypothesis (Grueber *et al.* 2011), this step was necessary due to high collinearity both among climatic variables and within time windows of each variable (e.g. Temperature: LST Lag 0-4, max-min-mean LST, weekly-accumulated GDD, estimate of accumulated GDD). Specifically, univariate Negative Binomial GLMMs were carried out in the preliminary analyzes to investigate the relationship between mosquito abundance and each climatic variable in turn, computed for all temporal windows considered (see M&M: Dataset description, climatic variables). Climatic variables were standardized by subtracting their means and dividing by their standard deviations. All preliminary models presented bimodal temporal patterns in the residuals. Therefore, Negative Binomial GAMMs were carried out. The rainfall variables, the temperature variables and the variable Day of the Year (DoY) were included in turn as penalized thin plate regression spline smoother. Models were ranked using the Akaike Information Criterion (AIC) (Akaike 1974) separately for both climatic variables (temperature and rainfall) and DoY. Only the significant variable with its temporal window producing the lowest AIC was considered for inclusion in subsequent full model. Following the preliminary analysis, the variable DoY was included as a penalized thin plate regression spline smoother in the model assessing the effect of socio-environmental and climatic determinants on *Ae. albopictus* adult abundance (hereafter referred to as full model). Therefore, to account for the time varying abundance of *Ae. albopictus* the full model resulted a negative binomial GAMM instead of the initial GLMM.

Analysis of the two Aedes albopictus high-abundance phases.

Since *Ae. albopictus* adult seasonal dynamics showed a bimodal pattern, statistical models were carried out to investigate the major drivers of both high-abundance phases. Similar to the procedure followed by Roiz *et al.* (2015), one dataset associated to mosquito dynamics around the first peak (Phase-1) and another one associated with the second peak (Phase-2) were extracted. First, to focus on the high abundance phases only, only the observations collected in sampling dates with a positive upper confidence limit of the DoY smoother were considered. Afterwards, sampling dates prior the local minimum of the DoY smoother were assigned to Phase-1 dataset, while observations after the local minimum were used in Phase-2 dataset. Two separate analyzes were carried out to assess if socio-environmental and climatic variables may differently affect mosquito abundance in Phase-1 and 2. Following the same approach used for the entire season analysis, also here the full model for each Phase was carried out after the

preliminary analysis. Since no temporal pattern was found in the residuals of models, Negative Binomial GLMMs instead of GAMMs were carried out.
All analysis were carried out using R software version 3.1.3 (R Core Team 2017) and packages glmmADMB (Fournier *et al.* 2012), gamm4 (Wood & Scheipl 2014) and plyr (Wickham 2011).

Results

A total of 8,846 *Ae. albopictus* adult females and 1,932 males were collected by 84 STs in 21 sampling stations over the 18-week sampling period along a 70 km-transect across and beyond the highly urbanized area of Rome (Fig 1). All the following statistical analyzes were restricted to the female collections. The overall weekly mean of *Ae. albopictus* females catches was 7.6 (Standard Error, SE=0.5) and 5.6 (SE=0.2) in the Metropolitan and in Sub-Urban/Rural area, respectively (S1 Table). A great variability in mosquito abundance was observed among stations: an overall weekly mean of 19.0 (SE=2.2) and 1.8 (SE=0.3) mosquitoes/ST were collected in the most and least infested station, respectively. The mean percentages for Vegetation Cover around STs were 36.9% (ranging from 0% to 95.6%) and 46.5% (ranging from 28.5% to 68.5%) in the 20 m- and 300 m-radius buffer, respectively.

Predictors of *Aedes albopictus* abundance during the entire sampling season.

Results of GAMMs showed that *Ae. albopictus* population dynamics was better modelled (lowest AIC with significant coefficients) by the Day of the Year (DoY) smoother than by time-dependent climatic predictors (i.e. temperature and rainfall) computed in different temporal windows (Table S2). Therefore, DoY was taken as time-dependent variable in the full GAMM carried out to assess the effect of time-independent environmental predictors (i.e. Vegetation Cover) on *Ae. albopictus* abundance, and its role in Metropolitan vs Sub-Urban/Rural Environments. No multicollinearity among linear predictors was found (Variance Inflation Factor values < 2). On average, weekly *Ae. albopictus* abundance did not differ between Metropolitan and Sub-Urban/Rural environment (Table 1). However, a significant interaction term between Vegetation Cover and Environment was found in the 20 m-radius buffer, meaning that an increase of the proportion of Vegetation Cover was positively associated with *Ae. albopictus* abundance in the Metropolitan Environment, but not in the Sub-Urban/Rural one (Table 1, Fig 2). On the other hand, an increase of the proportion of Vegetation Cover in the 300 m-radius buffer was negatively associated with mosquito abundance in both Environments (Table 1).

Table 1: Results of GAMM of *Aedes albopictus* female abundance in Metropolitan vs. Sub-Urban/Rural Environments.

Variable	Coeff.	SE	z-value	Pr(>|z|)
Intercept	1.562	0.142	11.009	$2e^{-16}$ ***
Vegetation 20 m	2.059	0.271	7.597	$3.03e^{-14}$ ***
Vegetation 300 m	-3.147	1.201	-2.619	0.009 **
Environment (Sub-Urban/Rural)	-0.039	0.175	-0.222	0.824

Vegetation 20 m * Vegetation 300 m	1.200	2.214	0.542	0.588
Vegetation 20 m * Environment (Sub-Urban/Rural)	-2.470	0.342	-7.226	$4.96e^{-13}$ ***
Vegetation 300 m * Environment (Sub-Urban/Rural)	0.898	1.440	0.623	0.533
Vegetation 20 m * Vegetation 300 m * Environment (Sub-Urban/Rural)	5.015	2.734	1.834	0.067

Metropolitan Environment as reference level. Number of observation=1353, number of stations= 21. Standard deviation of random effects = 0.33. Value of dispersion parameter = 1.8. The model included a smoothing term with 8 estimated degrees of freedom (approximate p-values <0.0001). Significance code: *** <0.001, 0.001<**<0.01, 0.01<*<0.05.

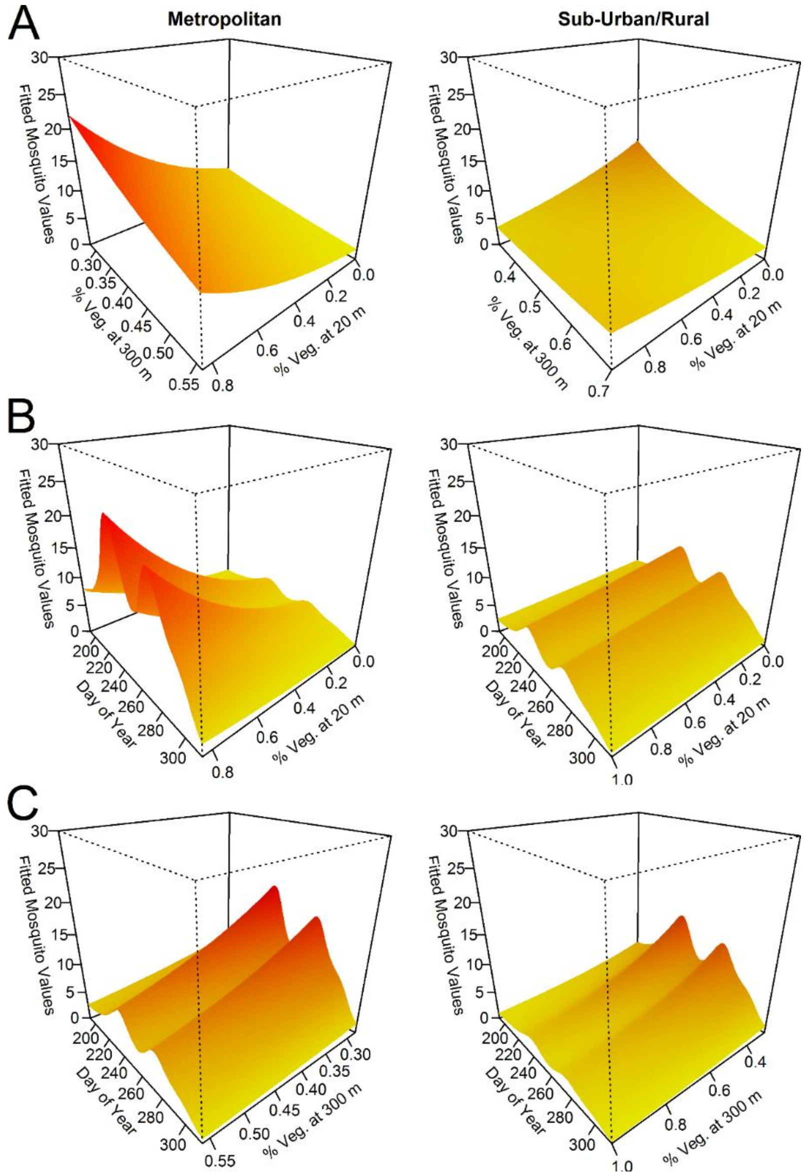


Fig 2: Fitted values (GAMM) of *Aedes albopictus* female abundance in Metropolitan and Sub-Urban/Rural Environments in Rome. Left column=Metropolitan Environment; right column=Sub-Urban/Rural Environment. Fitted mosquito values (Z-axis)= fitted values of females/station/week. A: interaction between Vegetation Covers

at 20 m and at 300 m buffers (scaled to the 0-1 interval) conditional to Days of the Year (DoY, considered at its mean values); B: interaction between Vegetation Cover at 20m and DoY conditional to vegetation cover at 300m (considered at mean values); C: interaction between Vegetation Cover at 300m and DoY conditional to Vegetation Cover at 20m (considered at mean values). Variables presented on the original scale (i.e. not centred).

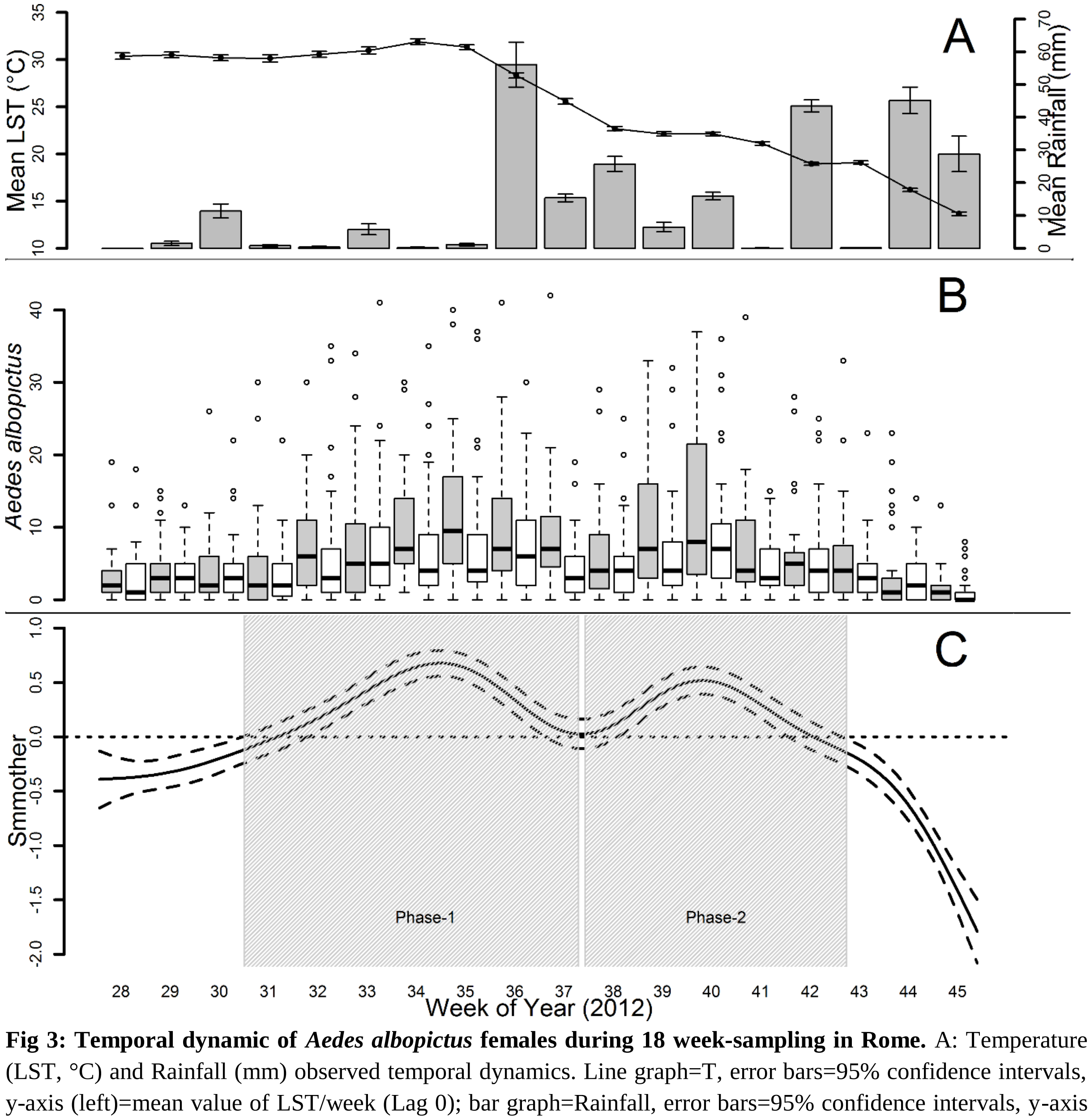


Fig 3: Temporal dynamic of *Aedes albopictus* females during 18 week-sampling in Rome. A: Temperature (LST, °C) and Rainfall (mm) observed temporal dynamics. Line graph=T, error bars=95% confidence intervals, y-axis (left)=mean value of LST/week (Lag 0); bar graph=Rainfall, error bars=95% confidence intervals, y-axis (right)= mean value of mm of rainfall/week (Lag 0). B: Observed mosquito temporal dynamics. Y-axis=boxplot of mosquito/week in Metropolitan (grey boxes) and Sub-Urban/Rural (white boxes) Environments. Boxes=first and third quartiles (the 25th and 75th percentiles). Line inside the box=median. The upper whisker extends from the boxes to the highest value that is within 1.5 * IQR (inter-quartile range: the distance between the first and third quartiles, so the height of the boxes). The lower whisker extends to the lowest value within 1.5 * IQR. Empty circles=outliers. C: Day of Year smoother (GAMM). Grey areas=phases exploited to investigate the climate drivers of the two peaks of mosquito abundance. X-axis=18 weeks of collections in 2012.

Figure 3A shows temperature and rainfall during the 18 week-sampling period. Figure 3B shows the observed *Ae. albopictus* temporal dynamics in Metropolitan and Sub-Urban/Rural

Environments. The shape of the estimated smoother (Fig 3C) shows bimodal mosquito population dynamics in both environments (no differences in the shape of the estimated smoothers in the two Environments was found), characterized by a first peak during the last weeks of August (weeks 34 and 35) and a second peak during the first week of October (week 40).

Predictors of *Aedes albopictus* during each of the two high-abundance phases.

The two phases (Phase-1 and -2) of highest mosquito abundance (in grey in Figure 3C) were analyzed separately in order to assess their respective climatic drivers. Results of univariate models - carried out in the preliminary analysis to assess which time-dependent variables would be the best predictor of mosquito abundance and to identify their lagged effect (different temporal windows) - showed that the accumulated LST at Lag 1 and the accumulated rainfall at Lag 3 were the most informative climatic predictors related to Phase-1 (S3 Table). However, when included in the full model with time independent variables, only LST at Lag 1 remained significant (Table 2). On the other hand, the accumulated rainfall at Lag 4 and the mean LST at the week of sampling (i.e. Lag 0) were the most informative climatic predictors in the preliminary analysis for Phase-2 (S3 Table). However, when they were included in the full model, only the accumulated rainfall at Lag 4 remained significant (Table 2). Socio-environmental time-independent variables in both models carried out for the two high abundance phases showed the same effects observed in the model carried out for the entire sampling season (Table 1). Specifically, a significant interaction term was detected between Vegetation Cover and Environment at 20 m-radius buffer, meaning that an increase of the proportion of Vegetation Cover was positively associated with *Ae. albopictus* abundance in the Metropolitan Environment, but not in the Sub-Urban/Rural one (Table 2). On the other hand, in both high-abundance phases an increase of the proportion of Vegetation Cover in the 300 m-radius buffer was negatively associated with mosquito abundance in both Environments (Table 2).

Table 2: **Results of GLMM of *Aedes albopictus* female abundance in Metropolitan vs. Sub-Urban/Rural Environments during the first and second phases of highest abundance**.

GLMM Phase-1			**GLMM Phase-2**		
Variable	**Coeff.**	**Pr(>|t|)**	**Variable**	**Coeff.**	**Pr(>|t|)**
Intercept	1.858	$2e^{-16}$ ***	Intercept	1.853	$2e^{-16}$ ***
Rainfall Lag 1	-0.0004	0.871	Rainfall Lag 4	0.007	$3.7e^{-7}$ ***
LST Lag 1	0.127	0.0008 **	LST Lag 0	0.030	0.355
Vegetation 20 m	1.906	$2.5e^{-6}$ ***	Vegetation 20 m	2.030	$3.1e^{-5}$ ***
Vegetation 300 m	-3.129	0.020 *	Vegetation 300 m	-3.673	0.021 *
Env. (Sub-urban/Rural)	0.045	0.822	Env. (Sub-urban/Rural)	-0.256	0.270

Veg. 20 m * Veg. 300 m	-2.027	0.536	Veg. 20 m * Veg. 300 m	1.650	0.661
Veg. 20 m * Env.	-2.186	$1.6e^{-5}$ ***	Veg. 20 m * Env.	-3.080	$4e^{-7}$ ***
Veg. 300 m * Env.	1.431	0.372	Veg. 300 m * Env.	2.912	0.125
Veg. 20m*Veg. 300m*Env.	5.960	0.130	Veg. 20m*Veg. 300m*Env.	6.841	0.139

Phase-1: number of observation=543, number of stations= 21, standard deviation of random effects = 0.35. The model included a smoothing term with 8 estimated degrees of freedom (approximate p-values <0.0001). Phase-2: number of observation=396, number of stations= 21, standard deviation of random effects = 0. 42. The model included a smoothing term with 8 estimated degrees of freedom (approximate p-values <0.0001). Significance code: *** <0.001, 0.001<**<0.01, 0.01<*<0.05.

Discussion

The results of the analysis of the spatial distribution and relative abundance of *Ae. albopictus* along the 70 km-long transect encompassing the metropolitan area of Rome highlighted a complex relationship between landscape composition and mosquito abundance.

When focussing the analysis to the scale of the estimated flight range of the species (Marini *et al.* 2010) - corresponding in the experimental set up to a 300 m-buffer within each sampling station - results showed that high adult abundance was on average associated with highly anthropized habitats (rather than with highly vegetated ones), in both the Metropolitan and the Sub-Urban/Rural areas. This is consistent with characteristics of highly anthropized habitats which favour the mosquito life-cycle, such as high human population density providing more opportunities for blood-feeding and larger numbers of artificial water containers (such as flowerpots, rain catch basins, abandoned tires, and disposable tins) suitable for container-breeding mosquitoes. Moreover, especially in temperate regions, the replacement of natural soil and vegetation with artificial surfaces is known to elevate temperatures and alter rainfall regimes with respect to surrounding regions (Oke 1982; Arnfield 2003; Han, Baik & Lee 2014), favouring mosquito development and gonotrophic cycle (Alto & Juliano 2001a). Indeed, *Ae. albopictus* has been shown to reach very high densities in highly anthropized areas, in particular in the absence of sympatric competing *Ae. aegypti* populations (Takken & Knols 2007; Li *et al.* 2014; Samson *et al.* 2015).

On the other hand, when the analysis was restricted to a 20 m-scale in order to focus on the landscape factors at sticky trap level (as in (Cianci *et al.* 2015)), a different pattern of mosquito abundance was observed depending on the location of the traps in Metropolitan or in Sub-Urban/Rural areas. In fact, while in the latter, the vegetation coverage at 20 m-scale did not affect *Ae. albopictus* abundance, in the Metropolitan area, sticky traps positioned within highly vegetated 20 m-buffers collected higher number of mosquitoes, especially when located in highly anthropized stations. Overall, results suggest that hotspots of *Ae. albopictus* abundance within the highly anthropized Metropolitan stations are associated with “small green islands”. Since the sticky traps used for mosquito sampling mostly collect ovipositing females as well as resting adults (Valerio *et al.* 2010), these “small green islands” may represent ideal sites in which the mosquito founds optimal conditions to lay eggs and rest. Moreover, it can be speculated that “small green islands” are associated with higher abundance of potential resting and larval sites characterized by more suitable temperatures for larval development (as shown in New Jersey, US (Bartlett-Healy *et al.* 2012)). Finally, “small green islands” in highly anthropized Metropolitan areas (such as children playgrounds, elderly people meeting places, small private gardens and condominium gardens) may be also attractive for host-seeking females, as they are largely exploited by people for outdoor activities especially during late summer afternoons when the species is most active. In fact, from an epidemiological perspective, it would be relevant in the future to extend the analysis in order to represent spatial heterogeneities not only in mosquito abundance but also in mosquito-to-host ratio (Vanwambeke, Bennett & Kapan 2011).

The results of the analysis of the *Ae. albopictus* seasonal dynamics showed a bimodal pattern (with a peak of abundance in August and one in October) in most sampling stations, revealing that the species may unpredictably reach very high abundance also after the summer season. The first peak in August was clearly temperature-driven, while rainfall accumulated in September (average value of 103 mm) following a month with very low rainfalls (average value in August=7.4 mm) seems to be the major driver of the second peak unexpectedly observed in mid-October. Afterwards, when temperatures and photoperiod became sub-optimal for the species life cycle, the mosquito abundance rapidly decreased despite frequent rainfall. The first peak in August followed by a decrease in mosquito abundance in the following weeks is consistent with data from the early stage of the species colonization of Rome (Di Luca *et al.* 2001; Toma *et al.* 2003), as well as from subsequent years when the species had already become an established urban pest (Facchinelli *et al.* 2007; Caputo *et al.* 2015). The second peak observed in the present work is less consistent with data reported in the past, although a second increase in the population abundance was frequently shown to occur in October, probably due to relatively high temperatures and rainfall accumulating after a few dry summer weeks, a common climatic pattern in Rome at least in the last 10 years (Figure S1). Moreover, subsequent data from human-landing catches carried out in Rome confirm a bimodal seasonal dynamics with very high host-seeking mosquito abundance in the final part of the reproductive season (Manica *et al.* 2017).

Some aspects of the experimental design deserve discussion. First, it may be argued that the seasonal-long trapping effort influenced mosquito abundance in the study sites. However, results from previous work clearly showed that only a small fraction of wild *Ae. albopictus* females is collected by STs even in the frame of a much more intense sampling scheme than the present one (Marini *et al.* 2010). Second, it is conceivable that competition of sticky traps with other potential oviposition sites likely non-homogeneously distributed in space and time may have created a bias in the comparison of mosquito abundance among sampling sites and among different phases of the season. Unfortunately, every collection approach may suffer of some kind of bias. Given appropriate resources, it would have been beneficial to monitor potential breeding sites during the field activities. Third, the results refer to ovipositing/resting females and may not directly reflect mosquito/human ratio, which is a more relevant epidemiological parameter to be assessed by the recording abundance of host-seeking females. Finally, inferences on the landscape determinants of the spatial and temporal distribution of *Ae. albopictus* abundance here presented are aggregated (i.e. vegetation cover includes trees, grass, bushes, etc.) to increase statistical power and do not take into account the land use associated with human activities.

Despite these study limitations, the results allow relevant speculations from a public health perspective. First, the analysis of the climatic determinants of *Ae. albopictus* seasonal dynamics highlights how the association of permissive photoperiod and temperatures associated with rainfall at the end of the summer period may result in a second phase of high *Ae. albopictus* abundance. This is likely to occur not only in the Rome area, but also in other Mediterranean regions colonized by the species and showing a similar climatic pattern. Roiz *et al*. 2015 showed that an extreme rainfall event increased and extended the species abundance in Montpellier (coastal France) leading to at least 11 cases of autochthonous CHIKV transmission. This led the authors to propose that mosquito control campaigns must be implemented after such heavy rainfall events. Our results extend this concept, as they suggest that also less extreme and repeated rainfall after a relatively long dry period (which characterized Rome in the past years and is predicted to became a typical scenario in Italy in future years due to climate changes (Zollo *et al.* 2015)), may cause the replenishment of peridomestic containers where desiccated eggs of *Ae. albopictus* are present, giving rise to increased mosquito abundance a few weeks later. This implies that in South European areas, characterized now or in the future by a similar climatic pattern, monitoring and control campaigns should be planned also after the end of the summer season to prevent a possible second peak of the mosquito population abundance and its associated health threats. Second, our spatial analysis emphasizes the need to prioritize public mosquito control activities in “small green islands” within highly anthropized metropolitan settings (such as children playgrounds or elderly people meeting places) where nuisance, human-mosquito contact and risk of local arbovirus transmission are likely to be higher (Seyler *et al.* 2009; Johansson *et al.* 2014). On the other hand, the study suggests that such a prioritization strategy might be ineffective outside the metropolitan areas, where no hot-spots of mosquito abundance have been identified.

Supporting Information

S1 Table. Weekly mean of *Aedes albopictus* adult females (±SE) in each of the 21 sampling station over 18-week sampling along a 70km-transect encompassing Rome metropolitan area. Each sampling station is characterized by population density (i.e. inhabitants/km^2) and vegetation cover (i.e. percentage of areas covered by "vegetation" vs "artificial surfaces") at a 300 m and 3 km radius areas. NA=not available.

	GPS						Weeks									
Station ID	**Latitude**	**Longitude**	**Ecology**	**Population Density**	**% Vegetation 3 km**	**% Vegetation 300 m**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	41.83824382	12.2046277	Sub-Urban/Rural	1706.325	31.19	41.97	0.25 + 0.25	3.25 + 1.18	3 + 1.53	2.5 + 1.5	6.25 + 2.95	10.5 + 3.88	8 + 3.72	8.75 + 3.12	8 + 2.38	7 + 4.02
2	41.82066696	12.23801976	Sub-Urban/Rural	11.368	34.25	45.99	2.75 + 1.89	3.25 + 0.25	8.5 + 2.25	6.25 + 1.65	13.75 + 7.7	10.75 + 4.8	5.25 + 1.65	9 + 3.24	6.5 + 2.9	1 + 0.41
3	41.8042066	12.2230366	Sub-Urban/Rural	2696.952	28.65	33.39	2 + 1.68	6.33 + 1.2	4 + 1.58	3.67 + 2.19	2.5 + 0.87	7 + 2.68	3.25 + 0.48	2.75 + 0.95	2.75 + 0.63	0.75 + 0.25
4	41.7763722	12.22691256	Sub-Urban/Rural	4959.511	29.4	38.94	8.33 + 5.04	5.75 + 1.75	5 + 1.47	4 + 1.63	6.75 + 3.09	9.25 + 1.49	15 + 3.54	16 + 3.89	13.75 + 3.52	9.5 + 2.25
5	41.80934886	12.31985942	Sub-Urban/Rural	20.459	51.83	37.56	7.75 + 1.89	9 + 1.68	4.5 + 1.44	4.75 + 2.32	14.75 + 6.76	20.5 + 7.9	35.5 + 8.72	17 + 7.84	12.25 + 2.29	NA
6	41.8174563	12.34435483	Sub-Urban/Rural	1820.085	61.54	44.58	3 + 0.58	1.75 + 0.48	3 + 0.71	4 + 2.27	8.75 + 3.12	6.75 + 1.8	7.5 + 1.26	6 + 4	5.75 + 1.25	11
7	41.82486978	12.40949916	Sub-Urban/Rural	21.425	65.58	56.86	2.67 + 2.19	2.5 + 2.18	4 + 3.67	4.25 + 2.1	6.5 + 3.1	1.75 + 0.48	5.75 + 2.46	2 + 0.91	1.75 + 1.75	NA
8	41.84758002	12.45450048	Metropolitan	9298.187	57.15	50.6	5 + 4.67	6.67 + 4.26	4 + 1.15	4.5 + 2.9	6.5 + 2.9	5.75 + 2.56	4.75 + 1.65	10.25 + 4.99	5.5 + 4.5	NA
9	41.87240106	12.465888	Metropolitan	18263.52	53.21	35.46	1.75 + 0.85	1.25 + 0.75	3.33 + 1.2	1 + 0.58	5.75 + 2.53	5 + 1	6.5 + 0.29	9.25 + 1.89	4.33 + 0.88	NA
10	41.90178235	12.42769521	Metropolitan	16844.138	60.12	28.73	3.33 + 0.33	6.75 + 2.56	5 + 1.96	5.75 + 2.1	5.75 + 2.46	4 + 1.91	7 + 1.87	12.75 + 3.64	7 + 2.97	6.75 + 1.49
11	41.90746141	12.44775396	Metropolitan	23260.09	54.6	30.42	1.25 + 0.63	2 + 0.71	3.5 + 0.96	3 + 1.08	7.33 + 1.2	1 + 0.41	11 + 3.34	10.25 + 3.09	9.25 + 2.14	6.25 + 2.02
12	41.91353299	12.47583383	Metropolitan	3117.546	47.45	55.91	2.25 + 1.31	2 + 1.41	0.75 + 0.75	1 + 0.58	2.67 + 1.76	3.25 + 2.29	3 + 1.15	3.5 + 1.26	1.75 + 1.44	2.75 + 1.8
13	41.89463452	12.50397023	Metropolitan	26655.809	39.48	28.52	4 + 3	2.25 + 0.85	3 + 2.04	10.5 + 5.33	16.25 + 6.06	11 + 3.14	29.75 + 13.55	40 + 15.58	13.5 + 5.2	19.25 + 7.91
14	41.86761525	12.53541125	Metropolitan	10817.479	62.13	46.98	5.25 + 2.87	3 + 1.15	0.75 + 0.48	1.75 + 1.11	3.75 + 0.85	5.25 + 2.66	10.5 + 3.01	7.75 + 2.78	6.75 + 2.69	9.5 + 3.84
15	41.93417412	12.52382362	Metropolitan	12483.623	59.11	51.03	NA	10.5 + 2.6	13 + 4.93	11.33 + 9.4	10 + 3.63	25.75 + 3.57	20 + 3.19	43.75 + 22.61	41.5 + 16.82	10.75 + 1.03
16	42.06513769	12.59593012	Sub-Urban/Rural	5786.351	61.81	36.38	NA	7	8.5 + 4.63	8.33 + 6.89	10.5 + 10.5	3 + 0.58	10 + 4.51	22 + 14	16.5 + 6.22	5 + 1.53
17	42.10367001	12.63226228	Sub-Urban/Rural	32.283	67.85	64.46	NA	3.75 + 1.25	3.5 + 0.96	3.25 + 1.49	5.75 + 1.11	5 + 1	4.75 + 1.18	6.5 + 2.47	3.5 + 1.55	2.67 + 0.33
18	42.15555727	12.6482546	Sub-Urban/Rural	2372.94	62.65	47.15	NA	3.5 + 0.96	4.67 + 2.6	3.33 + 1.45	4.25 + 1.97	5 + 2.68	5.5 + 1.55	5.5 + 2.22	3.25 + 0.25	3.67 + 0.33
19	42.24127405	12.63323956	Sub-Urban/Rural	833.476	72.73	67.75	NA	1 + 0.58	3.25 + 1.97	2.25 + 1.31	2 + 1	4.5 + 2.84	7 + 4.42	5.25 + 3.64	6 + 2.35	4.33 + 2.33
20	42.22748119	12.6844347	Sub-Urban/Rural	285.649	75.04	68.46	NA	NA	2.25 + 0.48	3 + 0.71	1.5 + 0.29	10.5 + 3.66	4.5 + 2.53	5.25 + 1.25	7.25 + 1.65	5.25 + 2.29
21	42.25766386	12.74593705	Sub-Urban/Rural	562.593	82.71	65.73	NA	0.33 + 0.33	1.33 + 0.33	1 + 0.41	0.25 + 0.25	2.75 + 0.85	5 + 1.08	9.25 + 4.27	8.25 + 1.11	5 + 1

11	12	13	14	15	16	17	18
3.5 + 1.76	6.5 + 2.06	13.5 + 6.38	6.25 + 1.8	10 + 4.71	2.5 + 0.96	3.5 + 2.25	0.75 + 0.48
0.5 + 0.29	2.75 + 1.49	1 + 0.71	2 + 0.41	3 + 1.53	1 + 0.71	0.25 + 0.25	0
3.25 + 1.6	5.25 + 2.59	15.25 + 4.66	3.5 + 1.66	3.75 + 1.03	6.25 + 1.31	2 + 0.71	1 + 0.41
11.5 + 4.73	16.75 + 10.53	24.75 + 9.2	11.25 + 2.5	17.75 + 3.68	7 + 1.08	8 + 1.15	3.25 + 1.11
9.25 + 3.71	12.75 + 7.16	12.5 + 3.28	5.33 + 1.33	8 + 2.86	11.5 + 4.33	8 + 2.04	4.67 + 1.2
4.67 + 0.33	9.25 + 5.12	4.33 + 1.76	3.25 + 1.6	1.25 + 0.63	2 + 0.58	2.25 + 0.48	0.75 + 0.48
4.67 + 4.18	11.75 + 6.02	6.67 + 3.18	1.75 + 0.85	2.5 + 1.32	1.5 + 1.19	1.25 + 0.95	1 + 0
3.67 + 1.67	4.5 + 2.5	4 + 1.87	3.67 + 0.67	3.75 + 0.85	0.75 + 0.48	0.25 + 0.25	0.5 + 0.29
0.75 + 0.75	3.5 + 1.19	11.33 + 5.36	8.25 + 3.22	3 + 1	2.5 + 1.19	0.5 + 0.29	0.5 + 0.29
5 + 1.87	7.5 + 3.3	10 + 5.9	3.75 + 1.25	2.75 + 1.25	4.5 + 1.66	3.25 + 2.29	0.5 + 0.29
6 + 2.42	13.25 + 6.76	15 + 7.52	6.25 + 2.14	5.75 + 0.75	4 + 1.08	1.25 + 0.63	0.75 + 0.48
0.5 + 0.29	3.5 + 1.71	3 + 2	1 + 0.41	0.5 + 0.29	1 + 0.58	0	0
12.75 + 5.57	27.5 + 17.56	7.75 + 2.66	19.33 + 10.53	8.25 + 2.66	11.5 + 3.66	2.25 + 0.75	2.5 + 1.04
7.25 + 3.01	13.5 + 4.79	16.75 + 6.1	11 + 3.39	6.75 + 2.95	12.5 + 7.01	3.5 + 3.18	2 + 1.08
13.75 + 4.87	17.75 + 4.23	33.5 + 6.01	8.5 + 4.5	22.75 + 4.61	11 + 3.08	17.25 + 2.39	4.5 + 2.84
6 + 1.35	6.33 + 2.19	12.33 + 1.67	4 + 1	8.5 + 3.38	3.33 + 0.88	7 + 1.22	3.67 + 2.19
5.75 + 1.75	5.5 + 0.96	3.5 + 1.44	3 + 0.41	3 + 1.08	1.75 + 0.75	3.75 + 2.25	0.25 + 0.25
1.67 + 0.67	1.75 + 0.48	4.5 + 1.19	3 + 3	4 + 1.47	2.25 + 0.48	2 + 1.35	0
3 + 1	5 + 0.58	3.75 + 1.49	5 + 2.65	4 + 1.22	3.25 + 0.95	1 + 0.58	0
5 + 1.35	6.25 + 1.75	5.75 + 0.85	5.33 + 0.67	2.25 + 0.63	3.25 + 1.31	1.25 + 0.63	0
3.67 + 0.88	4.75 + 0.48	6.25 + 0.85	8 + 1.78	4.75 + 0.25	4.33 + 0.33	1.25 + 0.48	0

S2 Table. Result of Generalized Additive Mixed Models (GAMMs) of time-dependent climatic predictors during the whole sampling season. Rainfall variables were modelled in turn either non-linearly or linearly with the inclusion of a Day of Year (DoY) smoother in each model. Temperature (T) variables were modelled in turn non-linearly (GDD=Growing Degree Days; LST=Land Surface Temperature). For T variables, the DoY smoother was not included due to high collinearity (i.e. concurvity) between T and DoY. s() denotes the smoother term.

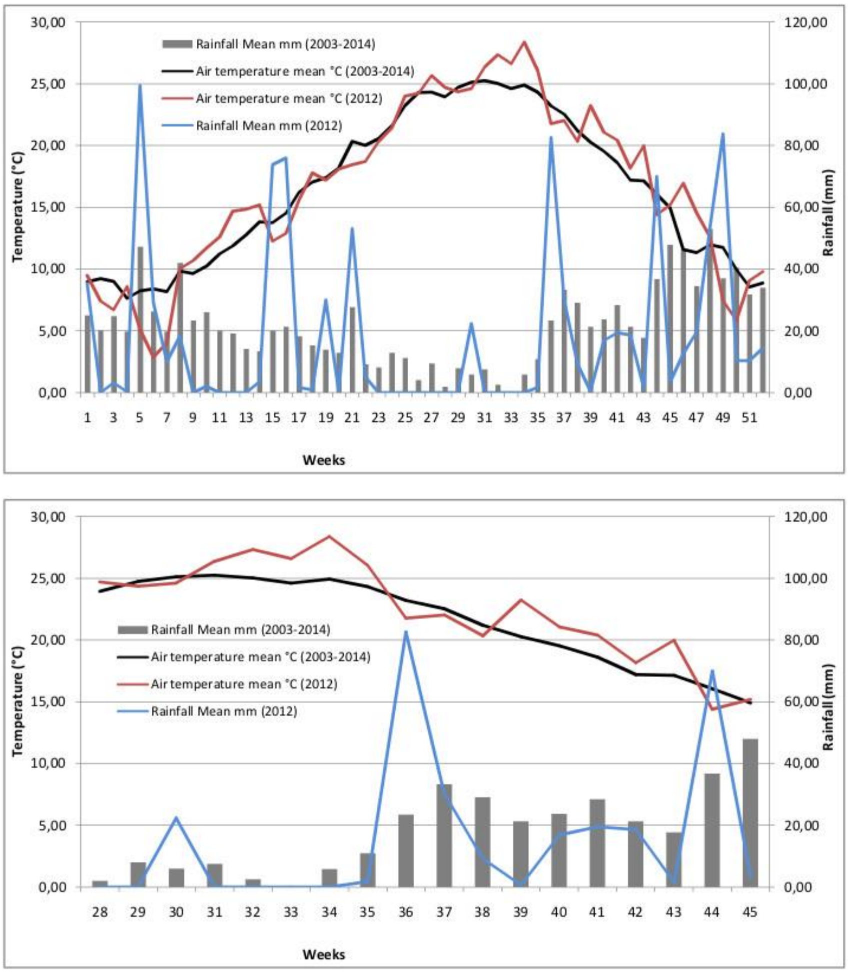
Model	**Rainfall Variables**	**AIC**	**Delta AIC**	**Intercept**	**Coeff**	**Statistic coeff**	**Statistic smoother**
GAMM-5	s(Rainfall Lag 4)	6717.86	0.00	1.70	-	-	<0.0001
GAMM-3	s(Rainfall Lag 2)	6733.64	15.78	1.71	-	-	<0.0001
GAMM-1	s(Rainfall Lag 0)	6738.16	20.29	1.72	-	-	<0.0001
GAMM-2	s(Rainfall Lag 1)	6746.40	28.54	1.72	-	-	<0.0001
GAMM-4	s(Rainfall Lag 3)	6755.81	37.95	1.71	-	-	<0.0001
GAMM-6	Rainfall Lag 0 + s(DoY)	6501.57	0.00	1.62	-0.06	0.06	<0.0001
GAMM-10	Rainfall Lag 4 + s(DoY)	6502.98	1.42	1.62	0.08	0.13	<0.0001
GAMM-8	Rainfall Lag 2 + s(DoY)	6504.80	3.23	1.62	-0.03	0.50	<0.0001
GAMM-7	Rainfall Lag 1 + s(DoY)	6505.21	3.64	1.62	0.00	0.92	<0.0001
GAMM-9	Rainfall Lag 3 + s(DoY)	6505.21	3.64	1.62	0.00	0.94	<0.0001
	Temperature Variables						
GAMM-15	s(LST Lag 4)	6543.76	0.00	1.63	-	-	<0.0001
GAMM-14	s(LST Lag 3)	6543.79	0.03	1.63	-	-	<0.0001
GAMM-11	s(LST Lag 0)	6546.24	2.48	1.63	-	-	<0.0001
GAMM-13	s(LST Lag 2)	6553.69	9.93	1.64	-	-	<0.0001
GAMM-19	s(GDD)	6556.81	13.05	1.64	-	-	<0.0001

GAMM-16	s(LST Min)	6557.84	14.08	1.64	-	-	<0.0001
GAMM-12	s(LST Lag 1)	6563.77	20.01	1.64	-	-	<0.0001
GAMM-17	s(LST Max)	6564.95	21.19	1.64	-	-	<0.0001
GAMM-20	s(Accumulated GDD)	6689.23	145.46	1.69	-	-	<0.0001
GAMM-21	s(Bounded GDD)	6690.06	146.30	1.69	-	-	<0.0001
GAMM-18	s(Temperature Range)	6755.43	211.66	1.72	-	-	<0.0001
	Day of Year Variable						
GAMM-22	s(DoY)	6510.73	0	1.62	-	-	<0.0001

S3 Table. Result of Generalized Linear Mixed Models (GLMMs) of time-dependent climatic predictors during the two high *Aedes albopictus* abundance phases. (GDD=Growing Degree Days; LST=Land Surface Temperature).

		Phase-1		
Model	**Rainfall Variable**	**AIC**	**Delta AIC**	**Statistic coeff**
GLMM-2	Rainfall Lag 1	3277.56	0.00	0.0103
GLMM-3	Rainfall Lag 2	3280.32	2.76	0.0573
GLMM-4	Rainfall Lag 3	3281.64	4.08	0.1347
GLMM-1	Rainfall Lag 0	3282.80	5.24	0.3117
GLMM-5	Rainfall Lag 4	3283.14	5.58	0.4058
	Temperature Variable			
GLMM-7	LST Lag 1	3264.76	0.00	<0.0001
GLMM-15	Accumulated GDD	3267.50	2.74	<0.0001
GLMM-11	LST Min	3267.52	2.76	<0.0001
GLMM-16	Bounded GDD	3267.98	3.22	0.0001
GLMM-8	LST Lag 2	3269.76	5.00	0.0002
GLMM-9	LST Lag 3	3271.74	6.98	0.0007
GLMM-10	LST Lag 4	3273.94	9.18	0.0023
GLMM-14	GDD	3275.82	11.06	0.0042
GLMM-6	LST Lag 0	3277.76	13.00	0.0128
GLMM-12	LST Max	3280.32	15.56	0.0591
GLMM-13	Temperature Range	3282.88	18.12	0.3390
		Phase-2		
Model	**Rainfall Variable**	**AIC**	**Delta AIC**	**Statistic coeff**
GLMM-5	Rainfall Lag 4	2295.44	0	<0.0001
GLMM-1	Rainfall Lag 0	2309.06	13.62	0.0011
GLMM-4	Rainfall Lag 3	2316.4	20.96	0.0797
GLMM-3	Rainfall Lag 2	2317.12	21.68	0.1209

GLMM-2	Rainfall Lag 1	2317.74	22.3	0.1869
	Temperature Variable			
GLMM-6	LST Lag 0	2310.74	0	0.0027
GLMM-12	LST Max	2313.54	2.8	0.0140
GLMM-7	LST Lag 1	2314.66	3.92	0.0263
GLMM-14	GDD	2314.72	3.98	0.0277
GLMM-8	LST Lag 2	2317.2	6.46	0.1288
GLMM-13	Temperature Range	2317.22	6.48	0.1333
GLMM-11	LST Min	2318.24	7.5	0.2654
GLMM-9	LST Lag 3	2319.08	8.34	0.5260
GLMM-10	LST Lag 4	2319.4	8.66	0.7785
GLMM-16	Bounded GDD	2319.46	8.72	0.8876
GLMM-15	Accumulated GDD	2319.46	8.72	0.8638



S1 Fig. Climatic pattern in Rome (2003-2014). Data collected by the Hydrographic Service of Regione Lazio and disseminated through the hydrographic annals (http://www.idrografico.roma.it/annali). Meteorological sampling stations of Roma Sud. Upper panel: whole year data, Lower panel: highlight week 28-45 from whole year data.

Chapter 2

Transmission dynamics of the ongoing chikungunya outbreak in Central Italy: from coastal areas to the metropolitan city of Rome, summer 2017

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Abstract

A large chikungunya outbreak is ongoing in Italy, with a main cluster in the Anzio coastal municipality. With preliminary epidemiological data, and a transmission model using mosquito abundance and biting rates, we estimated the basic reproduction number R_0 at 2.07 (95% credible interval: 1.47–2.59) and the first case importation between 21 May and 18 June 2017. Outbreak risk was higher in coastal/rural sites than urban ones. Novel transmission foci could occur up to mid-November.

Background

On 7 September 2017, Italian public health authorities reported three autochthonous cases of chikungunya in Anzio, a coastal city 50 km south of Rome, located in the Lazio region (Venturi *et al.* 2017). However, the symptom onset for the first cases was retrospectively considered to have occurred between 26 and 27 June. The outbreak continued spreading in the Lazio region with the number of notified cases reaching 297 (of which 170 were confirmed) on 13 October. Although most cases were reported from Anzio, a distinct cluster of transmission was also detected in the metropolitan area of Rome(Ministero della Salute 2015a). The index case has not been identified, but the mosquito vector implicated in the chikungunya virus (CHIKV) transmission was confirmed to be *Aedes albopictus*, as was the case in a previous Italian CHIKV outbreak, which occurred in the region of Emilia Romagna in 2007 (Venturi *et al.* 2017). In the same period than the Lazio outbreak in 2017, a further outbreak was detected in Guardavalle Marina, a small coastal town in the Calabria region, 600 km south of Anzio, with 54 additional notified cases (nine confirmed). It is still unknown whether the Guardavalle outbreak is epidemiologically linked to the epidemic occurring in Lazio. Here, we provide a quantitative characterisation of the ongoing outbreak, using available epidemiological data (Ministero della Salute 2015a) and a transmission dynamics model (Poletti *et al.* 2011; Guzzetta *et al.* 2016a, 2017) informed with data on mosquito abundance (Manica *et al.* 2016) and biting rate on humans (Manica *et al.* 2017) from previous collections in 18 sites within Lazio region.

Reproduction numbers from epidemiological data

The instantaneous reproduction number R_t (Ajelli *et al.* 2016) was estimated from the time series of notified cases in Anzio, Rome and Guardavalle Marina under the assumption of gamma distributed generation time (shape = 4.67; scale = 3; mean = 4 days) (Salje *et al.* 2016) (Figure 1). By averaging R_t over the first 3 weeks of August (initial period of exponential growth), we estimated the basic reproduction number R_0 for Anzio at 2.07 (95% credible interval (CI): 1.47–2.59), a value slightly lower than that estimated for the 2007 outbreak in Emilia Romagna (i.e. R0 = 3.3; 95% CI: 1.8–6.0) (Poletti *et al.* 2011). The decrease in R_t corresponded with the first date of reactive vector control interventions, namely 7 September (ECDC). The robustness of this estimate was confirmed by computing the basic reproduction number from the exponential growth rate (Wallinga & Lipsitch 2007) yielding a very similar result (R_0 = 1.88; 95% CI: 1.55–2.27). The hypothesis of sub-exponential growth in Anzio was subsequently ruled out (Chowell *et al.* 2016). For Rome and Guardavalle Marina, the number of cases was too small to compute a reliable estimate of R_0; however, peak values of R_t for these two outbreaks were smaller compared with the Anzio outbreak (Figure 1).

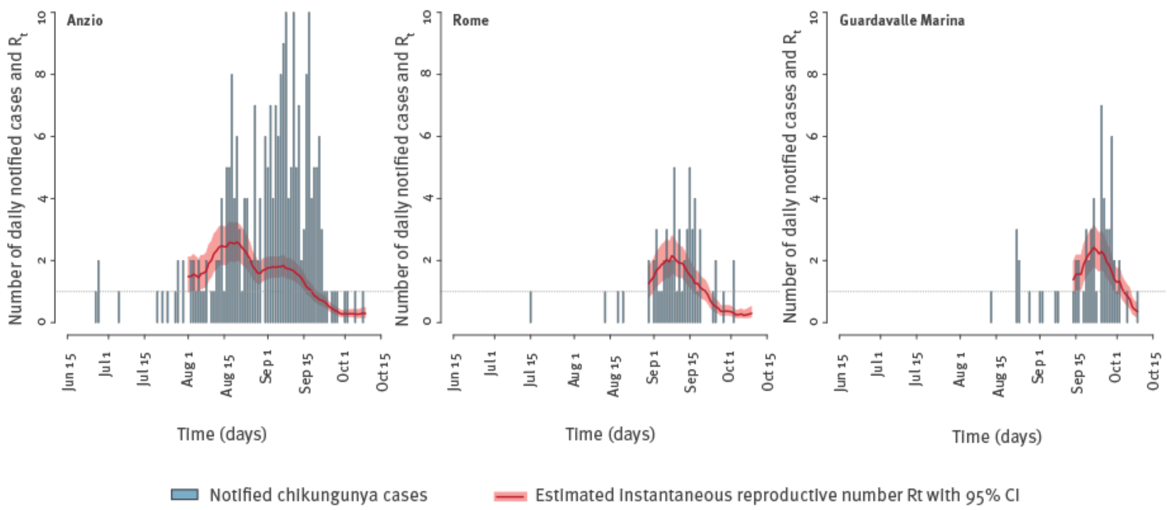

Figure 1. Time series of notified chikungunya cases with estimates of the instantaneous reproductive number R_t over time, averaged over a moving window of 14 days, Anzio, Rome and Guardavalle Marina, Italy, 2017

R_t was estimated by Markov chain Monte Carlo applied to the Poisson likelihood associated to the renewal equation $C(t) = Pois(R_t \sum_{s=1}^{t} T_g(s)C(t-s))$ [8], where C(t) is the number of new cases at time t and Tg is the generation time distribution [9].

Mosquito abundance

We calibrated a mosquito population model (Guzzetta *et al.* 2016a) to *Ae. albopictus* capture data obtained at several time points throughout the period July to November 2012 from 18 sites along a 70 km-transect from the Lazio coast (four sites) to rural inland areas (5 sites), and encompassing the metropolitan area of Rome (nine sites) (Manica *et al.* 2016) (Figure 2). Coastal sites have a human density (5–50 inhabitants/ha) close to that of Anzio (roughly 30 inhabitants/ha, increasing during summer months due to touristic influx) and similar eco-climatic conditions, and were therefore considered representative for the analysis of the main outbreak; urban sites (with human density up to 267 inhabitants/ha) were considered representative for the Rome outbreak. The model takes as input daily temperature records obtained from the closest weather station to each sampling site (‘Regione Lazio, Ufficio Idrografico e Mareografico’).

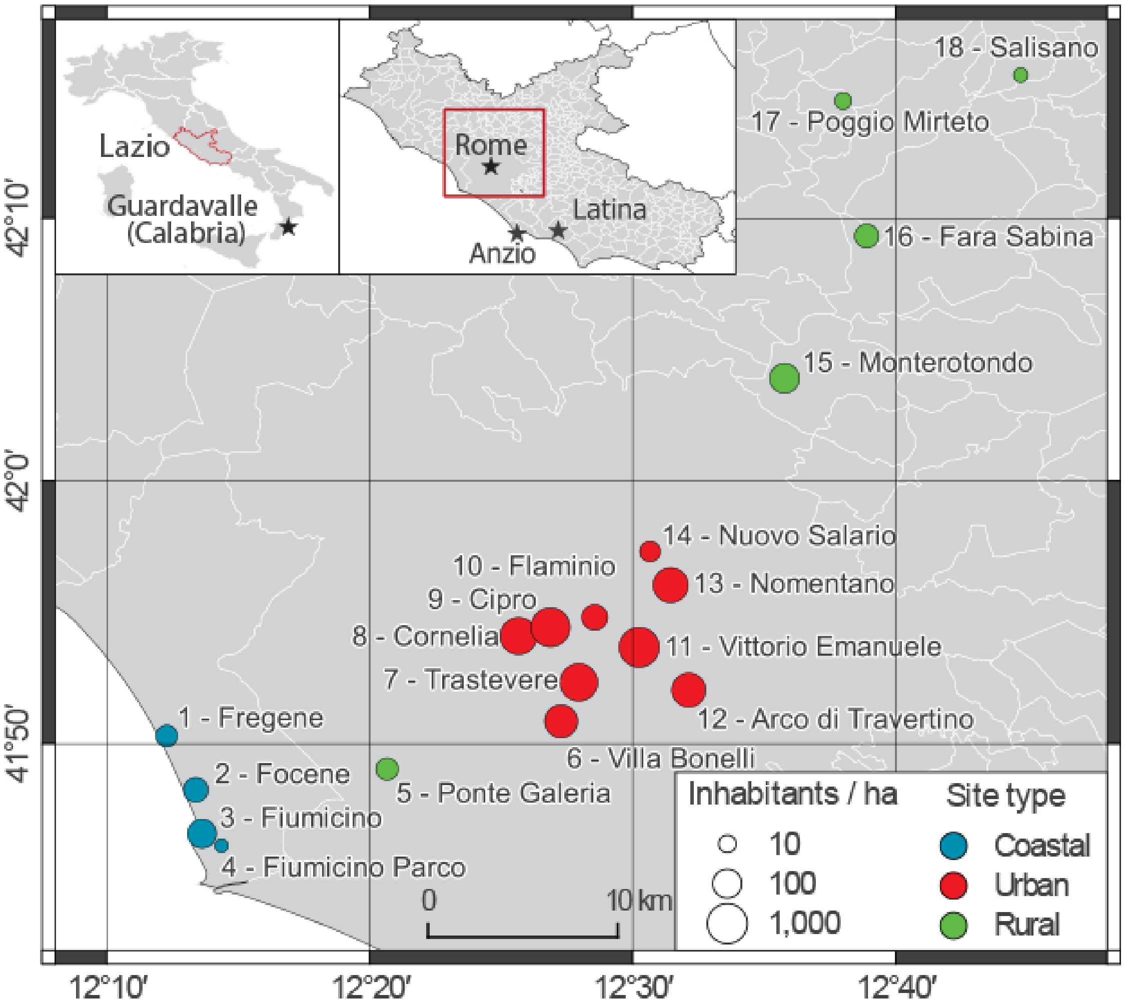

Figure 2. Location within the Lazio region of sites from which mosquito sampling in 2012 provided data for estimation of mosquito abundance in 2017, Italy (n = 18 sites). Stars represent locations with ongoing outbreaks in 2017 in Italy.

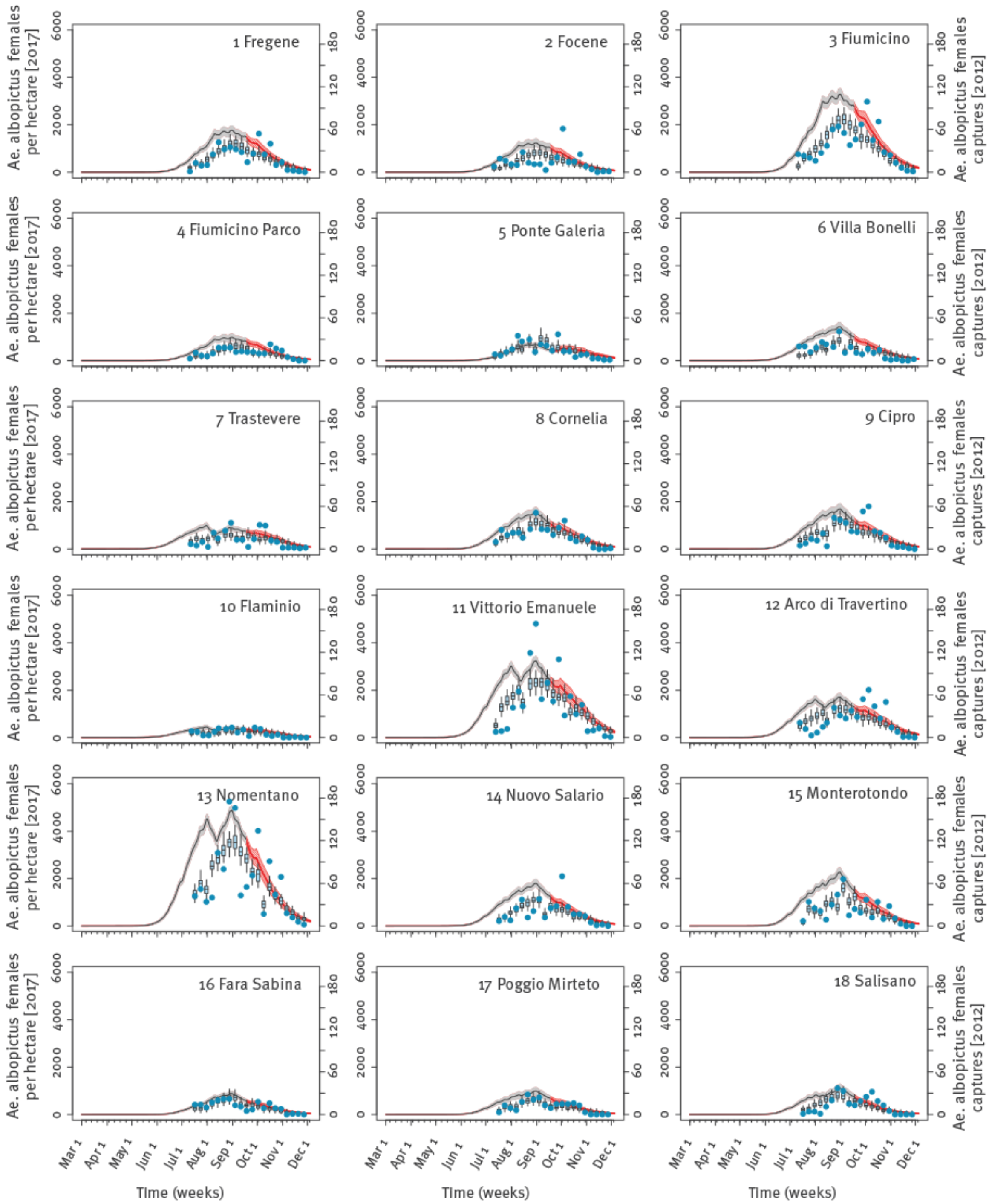

Figure 3. Number of *Aedes albopictus* adult females per hectare over time, as estimated in the absence of interventions for 2017 in the 18 mosquito sampling sites, Lazio region, Italy. For each study site, the abundance of *Aedes albopictus* adult females per hectare in 2017 is presented over the March to December period (line: mean number; shaded area: 95% credible interval); the grey colour is used to depict estimates based on recorded temperatures ('Regione Lazio, Ufficio Idrografico e Mareografico'), while red is used for estimates from predicted temperatures based on previously observed trends (scale on the left).

The calibrated model was re-run with 2017 temperatures to estimate the mosquito abundance during the ongoing outbreak (Figure 3). Human landing capture experiments performed in 2014 within a highly *Ae. albopictus* infested area in Rome re used to estimate the mosquito biting rate (Guzzetta *et al.* 2016b). Remarkably, the biting rate was found to be nearly constant over the season and its value (range: 0.08–0.1, as shown in the Table) complies with the 0.09 (95%CI: 0.05–0.16) estimate from the 2007 CHIKV outbreak (Poletti *et al.* 2011; Guzzetta *et al.* 2016b).

In addition, for each site, the observed (blue dots) and estimated (boxplots) total number of capture female adults during 2012, are shown from March to December (scale on the right). Boxplots represent 2.5%, 25%, 75%, and 97.5% quantile and mean of model estimates.

Table. Epidemiological parameters used in the estimation of transmission in an outbreak of chikungunya in Central Italy, 2017

Parameter	**Unit**	**Distribution**	**Min and max[a] parameter value**	**Reference**
Date of imported infection	Date	Uniform	1 May; 15 Nov	NA
Mosquito biting rate	Bites/ mosquito /day	Uniform	0.08; 0.10	Own estimate from [1]
Probability of vector-to-human transmission per bite	%	Uniform	14; 84	[2]
Probability of human-to-vector transmission per bite	%	Uniform	75; 90	[3]
Extrinsic incubation period	Days	Uniform	2; 3	[4]
Intrinsic incubation period	Days	Uniform	1; 12	[5]
Human infectious period	Days	Uniform	2; 7	[5]
Probability of developing symptoms	%	Uniform	65; 93	[6]
Probability of being detected	%	Uniform	44; 80	[6]
Delay between symptom onset and detection	Days	Gamma	Scale: 8.53; shape: 1.725	Own estimate from [7]

Max: maximum; min: minimum; NA: not applicable.

[a] Unless otherwise specified.

Transmission dynamics

The probability of a CHIKV outbreak, the number of symptomatic and asymptomatic cases and the daily number of notified cases at different sites were computed using a previously published stochastic transmission model (Guzzetta *et al.* 2017) (Figure 4) simulated over an area of radius 300 m (i.e. ca 28 ha), according to mosquito abundance data(Manica *et al.* 2016), epidemiological data (ECDC) and mosquitoes flight range (Marini *et al.* 2010). Potential delays between symptom onset and notification were also accounted for (Table). A set of 10,000 model simulations was run for each site by sampling epidemiological parameters from known distributions and considering a single imported case at different times within the 1 May–15 November time window (Table). In order to predict the time of virus introduction, the symptom onset for the first notified case was considered to have occurred between 23 and 29 June in coastal sites (first recorded symptoms in Anzio: 26 June) and between 12 and 18 July in urban sites (first recorded symptoms in Rome: 15 July). The likely time of virus introduction was identified by selecting simulations with compliant symptom onsets.

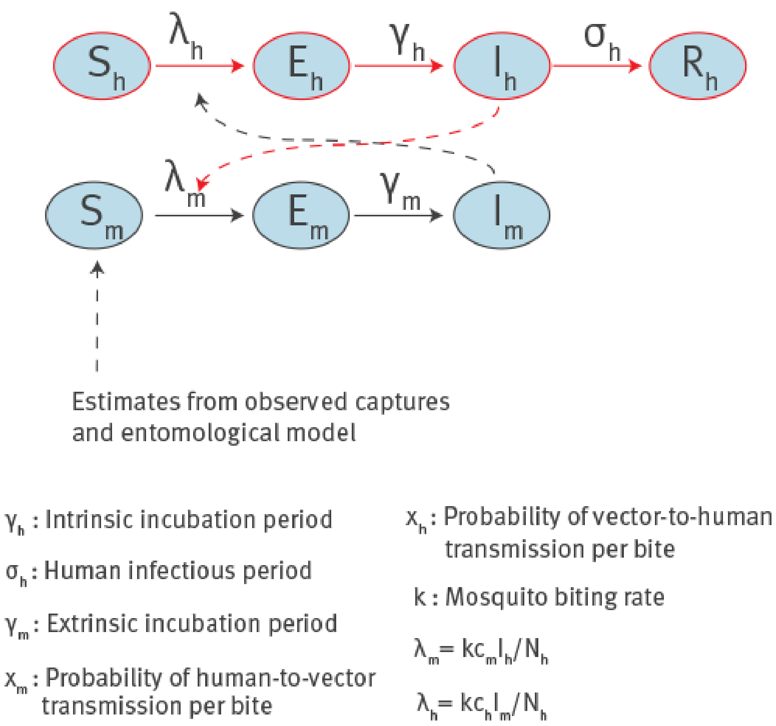

Figure 4. **Schematic representation of the model used to estimate chikungunya transmission, Lazio region, Italy, 2017**. E: exposed; I: infectious; □: force of infection, i.e. the probability per unit of time for a susceptible to become infected; N: total population; R: recovered; S: susceptibles. Subscripts h and m refer to humans and mosquitoes respectively. Human cases are notified with probability pspn, which represent the probability of developing clinical symptoms and the probability of being detected respectively, with a delay d between symptom onset and detection. Parameters values are reported in the Table.

According to model estimates, the first CHIKV case is likely to have been imported in the first week of June in Anzio (range: 21 May–18 June, sites 1–4 in Figure 5) and in early July in Rome (range: 28 May–16 July, sites 7–14 in Figure 5). In early June the probability of occurrence of an outbreak is estimated to be higher in coastal sites (11–44%) compared with urban sites (3–34%) (Figure 6). However, in the latter sites, the probability of outbreak increases to 22–82% at the predicted time of arrival of the infection in Rome. The risk of large outbreaks is estimated to be higher in coastal and rural sites than in urban sites (Figure 6), despite the high *Ae. albopictus* abundance in some urban areas (Figure 2). This is explained by the higher human density in urban sites, which reduces the mosquito/human ratio and thus the risk of infection. Specifically, at the predicted time of the first case in Anzio, the number of mosquitoes per person ranged between 1.9 and 7.3 in coastal sites and between 0.4 and 2.6 in urban areas. The probability of observing additional transmission foci in unaffected areas is estimated to remain significant up to mid-November. This analysis was not performed for Guardavalle Marina due to the lack of entomological data.

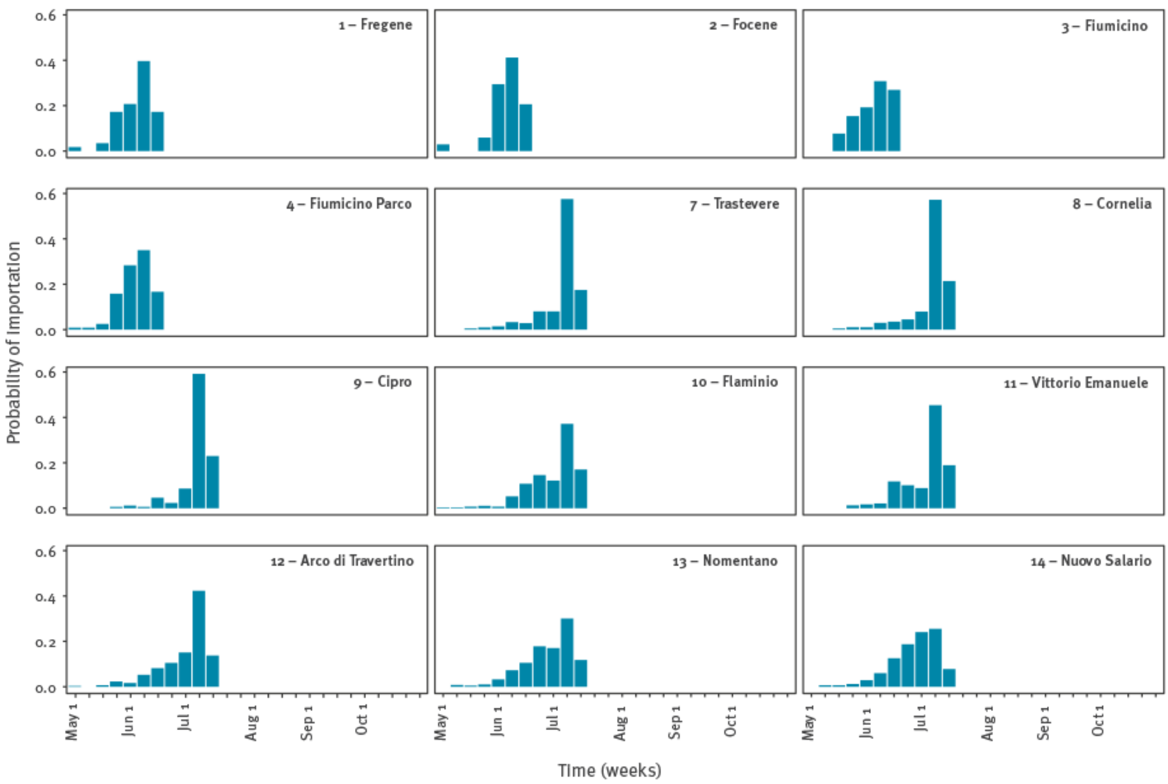

FIGURE 5. Distributions of the probable time of first chikungunya virus introduction in coastal sites (sites from 1 to 4), which were considered as representative of Anzio, and in urban sites considered as representative of Rome (sites from 7 to 14), Italy 2017

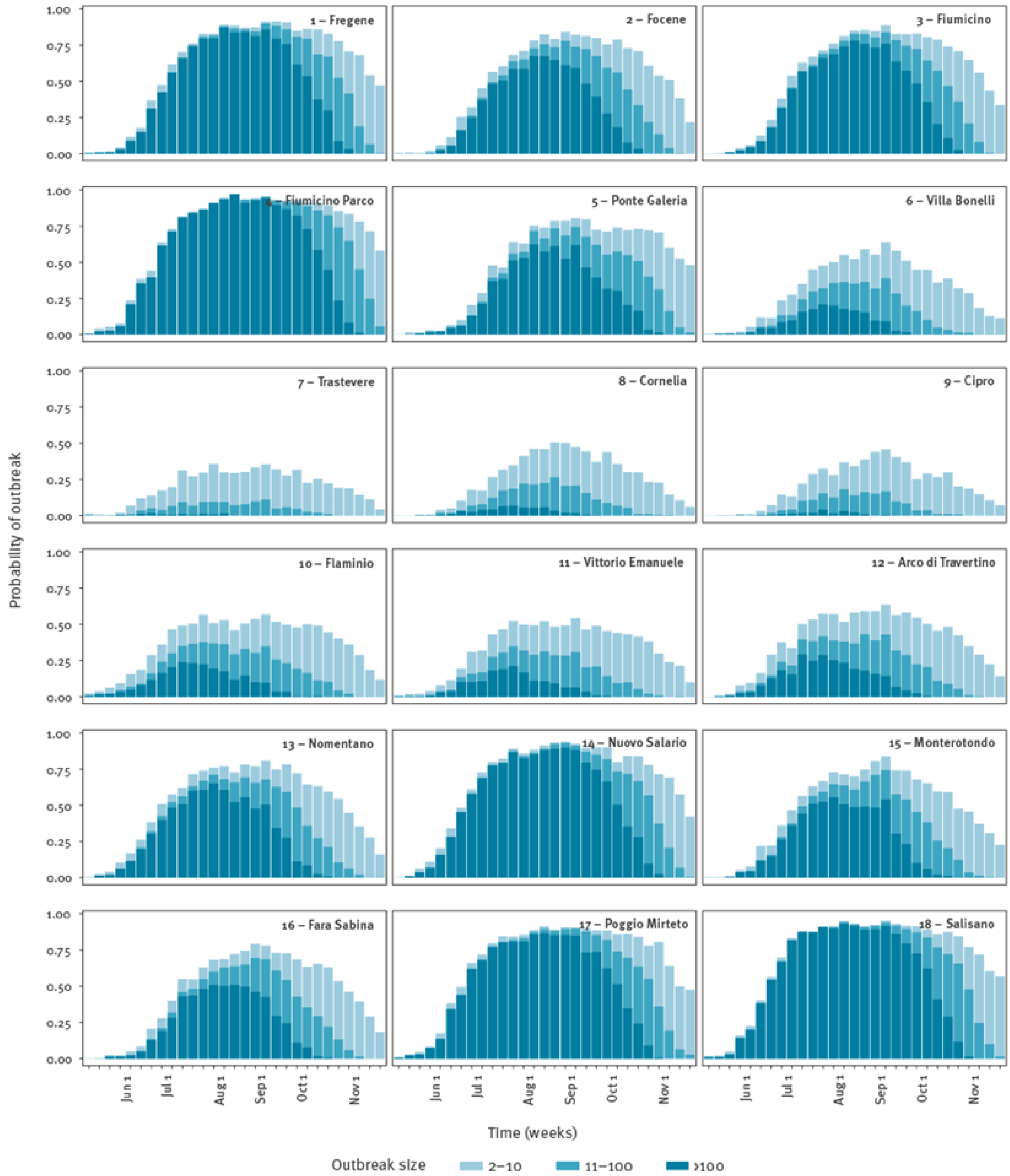

FIGURE 6. Model estimates of the probability of autochthonous transmission of chikungunya virus in 18 mosquito sampling sites in Lazio region, disaggregated by potential outbreak size, in case of a single imported case at different weeks of the year from 1 May to 15 November, Italy 2017

Estimates do not account for the different probabilities of importation (which depend on the absolute number of infected travellers) in urban, rural and coastal sites.

Estimates of health and economic burden

Based on observed cases that occurred before the restriction of blood donations in Lazio on 12 September , the estimated time of virus introduction, the notification rates (Table), the durations of infection (Table) and the available estimates on the daily blood donation rates (Grazzini), we estimated the probability that one blood sample might have been collected from an infected individual to be ca 0.73% (95% CI: 0.28–1.34%) in Anzio and 0.15% (95% CI: 0.05–0.29%) in Rome. Based on average costs and Disability Adjusted Life Years (DALY) lost per observed symptomatic CHIKV case (Guzzetta *et al.* 2017), the economic burden as at 13 October is estimated at 322,000 EUR (95% CI: 222,000–477,000) with a loss of 341 DALYs (95% CI: 235–505). These estimates exclude costs related to the management of blood supplies after restrictions.

Discussion

Our modelling estimates are subjected to uncertainties related to the actual mosquito abundance in Anzio and to the provisional nature of epidemiological data available up to now, including possible changes in the detection rates after the outbreak identification. Furthermore, the model is not suitable to evaluate the potential geographical spread of the epidemic, as it provides estimates only at the scale of 30 ha-patches, with the assumption of homogenous mixing within the patch. Critically, the high spatial heterogeneity in mosquito abundance, especially in urban areas, suggests the need to rely on information about mosquito populations at the local scale in order to assess the impact of current and future outbreaks. As shown by past surveillance records (Ministero della Salute 2015b, 2017), the number of imported chikungunya cases in Lazio range from zero to seven per year, therefore suggesting that multiple importations from abroad in the city of Anzio during the summer of 2017 were unlikely; however, multiple introductions in Rome (e.g. infected tourists coming back from Anzio) are possible. This is a further possible limitation to the interpretation of results related to Rome.

Despite these limitations, the model provides relevant estimates to characterise the ongoing CHIKV outbreak in Central Italy. First, the R_0 in Anzio is shown to be lower, but comparable to R_0 associated with the 2007 CHIKV outbreak in Emilia Romagna and other outbreaks worldwide (Poletti *et al.* 2011). Second, perhaps counter-intuitively, the highest transmission potential is predicted in coastal and rural areas (due to the higher mosquito to human ratio compared with densely populated metropolitan areas), consistently with the higher incidence of CHIKV observed in Anzio compared with Rome (ECDC). Third, the model estimates the health and economic burden related to the outbreak, which are instrumental to evaluate cost–benefits of preventive interventions aimed to reduce mosquito vector densities. In fact, availability of information on insecticide treatments carried out after CHIKV notifications would also allow predicting their effect on mosquito population dynamics. Finally, the model predicts a risk of autochthonous transmission in Lazio region up to mid-November, as a consequence of the expected persistence of favourable climatic conditions in the area (Regione Lazio). Although the number of cases is declining, with only 23 cases notified in October 2017, the foci of CHIKV transmission identified in the city of Latina (22 km east of Anzio; Figure 2) (Regione Lazio) and in Guardavalle Marina (Figure 2) highlight the need to continue monitoring the outbreaks.

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Chapter 3

From eggs to bites: do ovitrap data provide reliable estimates of *Aedes albopictus* biting females

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Abstract

Background. *Aedes albopictus* is an aggressive invasive mosquito species that represents a serious health concern not only in tropical areas, but also in temperate regions due to its role as vector of arboviruses. Estimates of mosquito biting rates are essential to account for vector-human contact in models aimed to predict the risk of arbovirus autochthonous transmission and outbreaks, as well as nuisance thresholds useful for correct planning of mosquito control interventions. Methods targeting daytime and outdoor biting *Ae. albopictus* females (e.g. Human Landing Collection, HLC) are expensive and difficult to implement in large scale schemes. Instead, egg-collections by ovitraps are the most widely used routine approach for large-scale monitoring of the species. The aim of this work was to assess whether ovitrap data can be exploited to estimate numbers of adult biting *Ae. albopictus* females and whether the resulting relationship could be used to build risk models helpful for decision-makers in charge of planning of mosquito-control activities in infested areas.

Method. Ovitrap collections and HLCs were carried out in hot-spots of *Ae. albopictus* abundance in Rome (Italy) along a whole reproductive season. The relationship between the two sets of data was assessed by generalized least square analysis, taking into account meteorological parameters.

Result. The mean number of mosquito females/person collected by HLC in 15' (i.e. females/HLC) and the mean number of eggs/day were 18.9±0.7 and 39.0±2.0, respectively. The regression models found a significant positive relationship between the two sets of data and estimated an increase of one biting female/person every 5 additional eggs found in ovitraps. Both observed and fitted values indicated presence of adults in the absence of eggs in ovitraps. Notably, wide confidence intervals of estimates of biting females based on eggs were observed. The patterns of exotic arbovirus outbreak probability obtained by introducing these estimates in risk models were similar to those based on females/HLC ($R0>1$ in 86% and 40% of sampling dates for Chikungunya and Zika, respectively; $R0<1$ along the entire season for Dengue). Moreover, the model predicted that in this case-study scenario an $R0>1$ for Chikungunya is to be expected also when few/no eggs/day are collected by ovitraps.

Discussion. This work provides the first evidence of the possibility to predict mean number of adult biting *Ae. albopictus* females based on mean number of eggs and to compute threshold of eggs/ovitrap associated to epidemiological risk of arbovirus transmission in the study area. Overall, however, the large confidence intervals in the model predictions represent a caveat regarding the reliability of monitoring schemes based exclusively on ovitrap collections to estimate numbers of biting females and plan control interventions.

Introduction

Aedes albopictus (Skuse) (Diptera: Culicidae) is an aggressive daytime biting invasive mosquito species (Hawley 1988) which represents a serious health concern not only in tropical areas, but also in temperate regions of Europe, US and China where it is now well established (Medlock *et al.* 2015). In fact, the species is a competent vector for many arboviruses (Gratz 2004), such as the most recent pandemic Zika virus (Di Luca *et al.* 2016), and has been responsible for large Chikungunya virus epidemics in Indian Ocean islands and in India (Higgs

2006; Enserink 2006; Roth *et al.* 2014). In Europe, it was responsible for the first outbreak of an exotic arbovirus (i.e. >200 confirmed Chikungunya cases in Ravenna Province, north-east Italy in 2007) and of the transmission of autochthonous cases of Dengue and Chikungunya in France and Croatia in more recent years (Angelini *et al.* 2007; Rezza *et al.* 2007; Gjenero-Margan *et al.* 2011; Grandadam *et al.* 2011; Delisle *et al.* 2015; Succo *et al.* 2016).
Estimates of mosquito biting rates are essential to account for vector-human contact in models aiming at predicting the risk of autochthonous transmission and outbreaks of mosquito-borne diseases, as well as mosquito nuisance. These estimates can be obtained by collecting mosquitoes on human volunteers (i.e. human landing collection, HLC), a very labour-intensive process, unethical in areas of proven disease transmission (Silver 2008). Other methods targeting biting females of daytime outdoor biting species (e.g. BG-sentinel traps for *Ae. albopictus*) are expensive and difficult to implement in large scale schemes. Thus, models aimed to predict the risk of autochthonous transmission and outbreaks of arbovirus by *Ae. albopictus* are constrained by the difficulty to obtain fine-scale entomological data.
On the other hand, the most widely available entomological data for *Ae. albopictus* come from egg-collection by ovitraps, a routine large-scale monitoring approach. This has been largely exploited by public administrations to survey the species abundance, due to its limited implementing costs (Marrama Rakotoarivony & Schaffner 2012). The use of egg abundance in risk models can be convenient, provided this can be proved to be a good predictor of biting adults. However, the relationship between mosquito eggs and biting females is not straightforward (Qiu *et al.* 2007; Marrama Rakotoarivony & Schaffner 2012) and may be differently affected by climatic (e.g. temperature, rainfall, wind; (Hawley 1988; Waldock *et al.* 2013; Vallorani *et al.* 2015), ecological (e.g. number of alternative oviposition sites (Davis *et al.* 2015)) and demographic (e.g. human and alternative hosts densities) factors.
As of today, no studies have attempted to quantitatively predict numbers of adult biting Aedes females from ovitrap data, although a study from Indonesia showed a positive correlation between eggs in ovitrap and number of host-seeking *Aedes aegypti* females in BG-sentinel traps (Tantowijoyo *et al.* 2016). The aims of the present study were to i) investigate the relationship between the mean number of human-biting *Ae. albopictus* females and number of eggs in ovitraps along the mosquito reproductive season and ii) assess the accuracy of this relationship. An accurate prediction of numbers of adult biting females from ovitrap data would in fact provide decision-makers in charge of planning of mosquito-control activities with a straightforward measure of high mosquito densities, associated to higher nuisance, as well as higher risk of arbovirus outbreaks. In order to achieve these goals, we carried out parallel ovitrap and human landing collections in two hot-spots of high *Ae. albopictus* abundance in Rome (Italy) and assessed the relationship between the two sets of data by regression analysis.

Materials & Methods

Study Sites

Human Landing Collections (HLC) and ovitrap collections were carried out from July 21th to October 31th 2014 in two *Ae. albopictus* heavily infested study sites (~1-hectar each) inside the metropolitan area of Rome (Italy), at about 400 m distance from each other: the botanical garden inside the campus of La Sapienza University of Rome (Site A, 41°54'12.6"N and 12°30'59.7"E;

see (Cianci *et al.* 2015) and the enclosed garden of the Institute of Anatomy (Site B, 41°54'23.32"N and 12°30'57.35"E; see (Caputo *et al.* 2012).

Mosquito collections

Human Landing Collections were performed 3 days per week (i.e. on Monday, Wednesday and Friday) by two qualified operators in two outdoor spots located at a distance of approximately 100 m within each study site. The operators gave their consent to carry out HLC after being informed of potential risks. At planned day, collections started 1 hour before sunset and finished within 30 minutes. Each HLC (i.e. a single collection made by a single operator in one spot) lasted for 15 minutes; after rotating between spots within the site, operators moved to the second site. In the following day of collection, the first site sampled was the second one sampled in previous collection day. In case of rain immediately before or during HLC time, collections were postponed to the next scheduled day. During each HLC, the operator seated exposing a ~4200 cm^2 naked area in one foreleg. Biting female mosquitoes were killed with a racket zapper as soon as they landed on the skin. Killed mosquitoes were identified and counted directly in the field.
Egg collections were carried out by ovitraps filled with 300 ml water and internally lined with a germination paper on which mosquito females lay their eggs (Velo *et al.* 2016). Ten ovitraps were positioned in site A and 5 in site B (this difference in number of ovitraps is due to lack of open space derived by the presence of a large building in site B). In the same day of HLC, operators collected germination papers in sealed plastic bags, emptied ovitraps, and replenished them with tap water. Egg counting was carried out under a stereomicroscope in the laboratory. Each month, approximately 1/10 of collected eggs were hatched and reared to the adult stage in order to confirm exclusive presence of *Ae. albopictus*.

In view of the following considerations we assume that removing *Ae. albopictus* adult females and their eggs from the field doesn't significantly affect the mosquito population size and temporal dynamics: i) collections were carried out in typical hot-spots of high *Ae. albopictus* density (Manica *et al.* 2016) in heavily infested areas (Marini *et al.* 2010; Caputo *et al.* 2015; Cianci *et al.* 2015); ii) the arrival in an infested area a human host can attract all the females present within a radius of only 4-7 m in 15' HLC (Mogi & Yamamura 1981); iii) the time required by HLC represents only a small fraction of the overall female daily biting activity (Hawley, 1988); iv) the number of ovitraps employed is to be considered negligible compared to number of potential natural breeding sites in the study sites (e.g. catch basins, vases, pots, flowerpot saucers).

Meteorological Data

Meteorological data (i.e. hourly records of temperature at 2 m from ground, wind speed and precipitation) were obtained by the opendata archive of the "Ministero delle Politiche Agricole, Alimentari e Forestali" (weather station Roma Collegio Romano https://www.politicheagricole.it/flex/cm/ pages/ServeBLOB.php/L/IT/ IDPagina/7012, accessed 2 June 2015). Meteorological data were aggregated to obtain the following variables of interest:

- daily average wind speed, average temperature and total mm of rainfall;

- a binary rainfall index indicating the occurrence of rainfall during the day;
- average temperature and accumulated mm of rainfall recorded over one, two, three weeks prior to collection day.

Statistical analysis

All analyses were carried out using the software R version 3.3.1 (R Core Team 2017) and the packages nlme (Pinheiro J, Bates D, DebRoy S 2014), MuMIn (Barton 2016), AICcmodavg (Mazerolle 2015) and ggplot2 (Wickham 2009).
A Pearson correlation between the mean number of female/site/day (i.e. the mean number of biting *Ae. albopictus* females collected by the two operators in the two spots within a site in a single day) and the mean number of eggs/site/day at lag 0 (i.e. the mean number of eggs from each ovitrap within each site divided by the number of days the ovitrap was active) was computed.,

Basic estimate of biting females based on mean number of egg/day in ovitrap (Model-I).

This relationship was tested by means of regression analysis also accounting for meteorological variables that could affect HLC sampling. Response variable was the mean number of female/site/day (i.e. the mean number of biting *Ae. albopictus* females collected by the two operators in the two spots within a site in a single day). Explanatory variables were site, mean number of eggs/site/day at lag 0 (i.e. the mean number of eggs from each ovitrap within each site divided by the number of days the ovitrap was active), mean number of eggs/site/day at lag 1 (i.e. the mean number of eggs/site/day in the seven days preceding HLC sampling), the mean number of eggs/site/day at lag 2 (i.e. the mean number of eggs/site/day from 7 to 14 days preceding HLC sampling). The choice of lag 0, 1 and 2 was based on: i) the mean time from egg oviposition to first blood-meal, which during the summer months in temperate areas is <14 days (authors' personal observation), and ii) the fact that routine ovitrap surveillance in large-scale monitoring schemes is usually carried on a weekly base, at least in Italy (ISS 2016).
In addition, some explanatory variables were included, i.e. meteorological variables recorded on the day of HLC sampling such as the precipitation occurrence (yes or no) and the average daily values for wind speed, temperature and temperature quadratic term. Temperature and wind data were centred (subtracted its mean) to help interpretation of results (Schielzeth 2010). Due to irregularly observed data and the longitudinal structure of the data, a continuous auto-regressive correlation structure of order 1 was considered in the model. The resulting model was fitted using the generalized least squared method by maximizing the restricted log-likelihood (REML). Model assumptions were verified by checking the model normalized residuals for any pattern or dependency. This model, hereafter-defined "full model", including all the ecologically relevant parameters available, was used to generate a set of all plausible sub-models. The model considering the temperature quadratic term included also the linear one. A multi-model selection approach (Burnham & Anderson 2002) was then employed to compare all models in the set. Models were ranked by AICc (Burnham & Anderson 2002) using maximum likelihood estimation (ML) (Faraway 2006). Results of the ranking process were used to calculate weights and the relative importance for each variable by summing the Akaike weights for each model that contains the parameter of interest. The model having the lowest

AIC was then selected and refitted using REML Model, performance was assessed using in-sample errors by computing the root mean squared error (RMSE), which represents the sample standard deviation of the differences between predicted values and observed values and could be interpreted as an estimation of the standard deviation of the unexplained variance. Pearson correlation between observed and fitted mean values was also computed.

Improved estimate of biting females based on mean number of egg/day in ovitrap (Model-II).

Following the same approach, we built a new regression model aiming at improving the basic prediction of biting females obtained from Model I where only egg counts and short-term meteorological variables were considered. Specifically, we added average values for meteorological variables (temperature and precipitation) computed for a longer period preceding HLC sampling (till three weeks before) in order to take into account the effect of climatic variables not only on HLC sampling, due to mosquito activity, but also on mosquito population dynamics. Explanatory variables were the same used in Model-I: site, mean number of eggs/site/day (only at lag 0), the precipitation occurrence (yes or no) and the average values for wind speed and temperature quadratic term recorded on the day of collection. In addition, in this case, the average daily temperature and accumulated precipitation, with their quadratic terms, recorded over the previous one, two, three weeks were also included as explanatory variables. Again, temperature, wind and rainfall variables were centred and a continuous auto-regressive correlation structure of order 1 was considered. A set of plausible sub-models was then generated. The model set was tailored in order to retain models considering at most three meteorological variables (one for temperature, one for rainfall and one for wind) in order to avoid collinearity among meteorological explanatory variables. Models considering the quadratic terms included also the corresponding linear one. All models in the set were then compared and ranked by AICc (Burnham & Anderson 2002) using ML estimation (Faraway 2006). The model having the lowest AIC was then selected and refitted using REML. RMSE and Pearson correlation between observed and fitted mean values were computed. Collinearity was investigated using the function corvif (Zuur *et al.* 2009). During the model validation process, a simulation study was carried out to assess how the relationship between the mean number of egg/day in ovitraps and biting females from HLC, obtained from the best Model II, is influenced by the number of ovitraps considered. To test this, Model-II was re-fitted on simulated subsets of the original dataset; precisely, subsets were simulated by fixing at each step the number of ovitraps included in the analysis (from 1 to 15 traps, that is the actual number used in the best Model II) and then resampling with replacement (1000 times each step) the number of ovitraps to be considered. Model-II was re-fitted on every subset in order to obtain mean values and 95% confidence intervals for the parameters of interest (i.e. the estimated value of the mean number/eggs/day parameter, its significance, the RMSE and the Pearson correlation) for each fixed number of ovitraps.

Basic reproduction number and outbreak probability of exotic arbovirus

The basic reproduction number (R_0) for mosquito-borne arboviruses such as Chikungunya, Dengue and Zika virus can be calculated from densities of human and mosquito populations and several epidemiological parameters according to the following formula $R_0 = R_0^{HV} R_0^{VH}$

(Smith *et al.* 2012). Symbols, interpretations, values and literature references for each parameter are reported in the Table 1. Specifically, $R_0^{HV} = \frac{k\chi_V}{\gamma}\frac{V}{H}\frac{\omega_V}{\omega_V + m}$ could be interpreted as the product of the number of infectious mosquitoes generated from an infectious human while $R_0^{VH} = \frac{k\chi_H}{m}$ as the number of infectious humans generated by the infectious mosquitoes surviving the extrinsic incubation period. When $R_0 < 1$ (epidemic threshold), the probability of observing sustained arbovirus transmission after importation of a case is negligible. When $R_0 > 1$, the outbreak probability is given by the following formula: $p = 1 - \frac{R_0^{VH} + 1}{R_0^{VH}(R_0^{HV} + 1)}$.

Table 1 Epidemiological parameters. Symbols, values and references for the parameters use

		CHIKV		DENV		ZIKAV	
Parameter	**Description**	**Value (range)**	**Reference**	**Value (range)**	**Reference**	**Value**	**Reference**
k	Human biting rate (the number of bites to humans per mosquito per day)	0.09 (0.05 – 0.16)	(Poletti *et al.* 2011)	0.09 (0.05 – 0.16)	(Poletti *et al.* 2011)	0.09 (0.05 – 0.16)	(Poletti *et al.* 2011)
m	Mortality rate (1/g = average mosquito life-span in days)	Function (Temperature)	(Poletti *et al.* 2011)	Function (Temperature)	(Poletti *et al.* 2011)	Function (Temperature)	(Poletti *et al.* 2011)
χ_H	Susceptibility to infection of humans, transmission efficiency from an infected mosquito to human	65% (50% – 80%)	(Dumont, Chiroleu & Domerg 2008)	31% (10%-50%)	(Manore *et al.* 2014)	50% (1% - 100%)	(Wong *et al.* 2013; Chouin-Carneiro *et al.* 2016)
χ_V	Susceptibility to infection of mosquito, transmission efficiency from an infected human to mosquito	85% (70% – 100%)	(Talbalaghi *et al.* 2010; Vega-Rua *et al.* 2013)	31% (10%-50%)	(Manore *et al.* 2014)	50% (0.8% – 100%)	(Wong *et al.* 2013; Chouin-Carneiro *et al.* 2016)
$1/\omega_V$	Length of extrinsic incubation period	2.5 (2 – 3) days	(Dumont, Chiroleu & Domerg 2008; Dubrulle *et al.* 2009)	10 (7-14) days	(Manore *et al.* 2014)	10.5 (7 – 14) days	(Guzzetta *et al.* 2016b)
$1/\gamma$	Infectious period in human hosts	4.5 (2 – 7) days	(Parola *et al.* 2006; Dumont, Chiroleu & Domerg 2008)	6 (3-7) days	(Manore *et al.* 2014)	5.8 (4 – 7) days	(Guzzetta *et al.* 2016b)

X	Correction factor	0.101	(Carrieri *et al.* 2012)	0.101	(Carrieri *et al.* 2012)	0.101	(Carrieri *et al.* 2012)
k V/H	Ratio of mosquito per human	Time dependent	Observed by human landing collection	Time dependent	Observed by human landing collection	Time dependent	Observed by human landing collection

HLC-observed data and HLC-predicted values obtained from Model-2, multiplied by a correction factor x as in (Carrieri *et al.* 2012), were used to estimate the number of bites on human per mosquito (kV/H).

Results

Ovitrap and HLC collections.

A total of 5,678 biting *Ae. albopictus* adult females and 25,120 *Ae. albopictus* eggs were collected. The mean number of females/person collected by HLC in 15' (hereafter females/HLC) was 20.8 (±0.9 SE) and 17.1 (±0.9 SE) in Site-A and in Site-B, respectively. The maximum number of females/HLC was 47 in Site-A and 45 in site-B. The mean number of eggs/day was 35.6 (±3.4 SE) and 40.7 (±2.4 SE) in Site-A and Site-B, respectively. The maximum number of eggs collected in one ovitrap in a single sampling was 288 in Site-A and 300 in Site-B. No eggs were found in 109 out of 644 ovitrap collections (16.9%). A bimodal temporal pattern of egg and adult abundance, consistent with the pattern observed in previous years (Manica et al. 2016), was observed in both study sites (Fig. 1). A significant Pearson correlation was found between the mean number of female/site/day and the mean number of eggs/site/day at lag 0 ($r = 0.47$, df=71, pvalues = <0.0001).

Basic estimate of biting females based on mean number of egg/day in ovitrap.

Results of regression analysis carried out to estimate biting females based on mean number of egg/day accounting for meteorological variables that could affect HLC sampling - show that the model with lowest AIC had as explanatory variables the mean number of eggs/site/day at lag 0 and average daily wind measured at day of sampling (Model-I; Table 2; Table S1). The estimated parameter for the continuous AR1 correlation is 0.85.

Table 2. Coefficient and statistics of the parameters for Model-I.
Coefficient and statistics of the parameters for the best (lowest AIC) generalized least square model with continuous AR1 correlation structure analysing the relationship between the mean numbers of biting *Ae. albopictus* females/site/day and themean number eggs/site/day

Coeff.	Value	SE	T-value	p-value
Intercept	14.719	2.493	5.904	<0.0001
Mean number of eggs/site/day	0.233	0.071	3.280	0.0016
Wind	-1.855	1.221	-1.519	0.1334

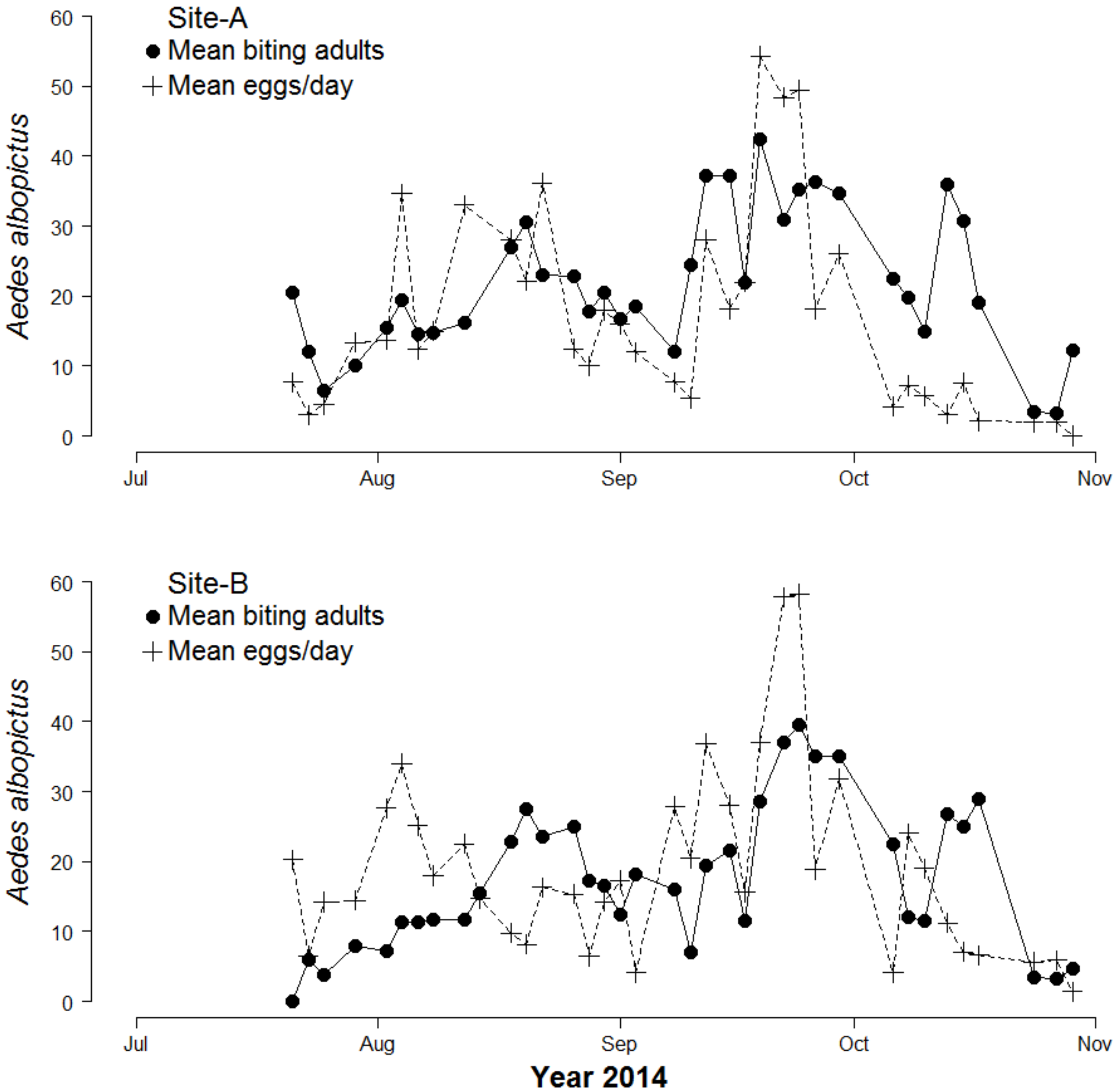

Figure 1. Seasonal patterns of *Aedes albopictus* eggs and adults. Seasonal patterns of eggs and adults per site per day in botanical garden (A) and the enclosed garden of the Institute of Anatomy (B) in Sapienza University, Rome, Italy.

The model-averaged importance of terms computed after the multi-model selection process (192 models) are mean number/eggs/day lag 0 (0.81) and temperature (0.52). Other explanatory variables with values <0.50 are mean number/eggs/day lag 1 (0.50), wind (0.44), rain occurrence (0.37), site (0.37), mean number/eggs/day lag 2 (0.35), and temperature^2 (0.28). A positive relationship between the mean numbers of females/HLC and the mean numbers eggs/site/day is observed (Fig. 2A). The estimated coefficient for the mean number of eggs/site/day is 0.233 However, Model-I does not satisfactory explain the variability of the collected number of adult females (Pearson correlation=0.53; RSME=8.9; Fig. 2B) and only partially describes the observed temporal pattern of biting females (Fig. 2C and 2D).

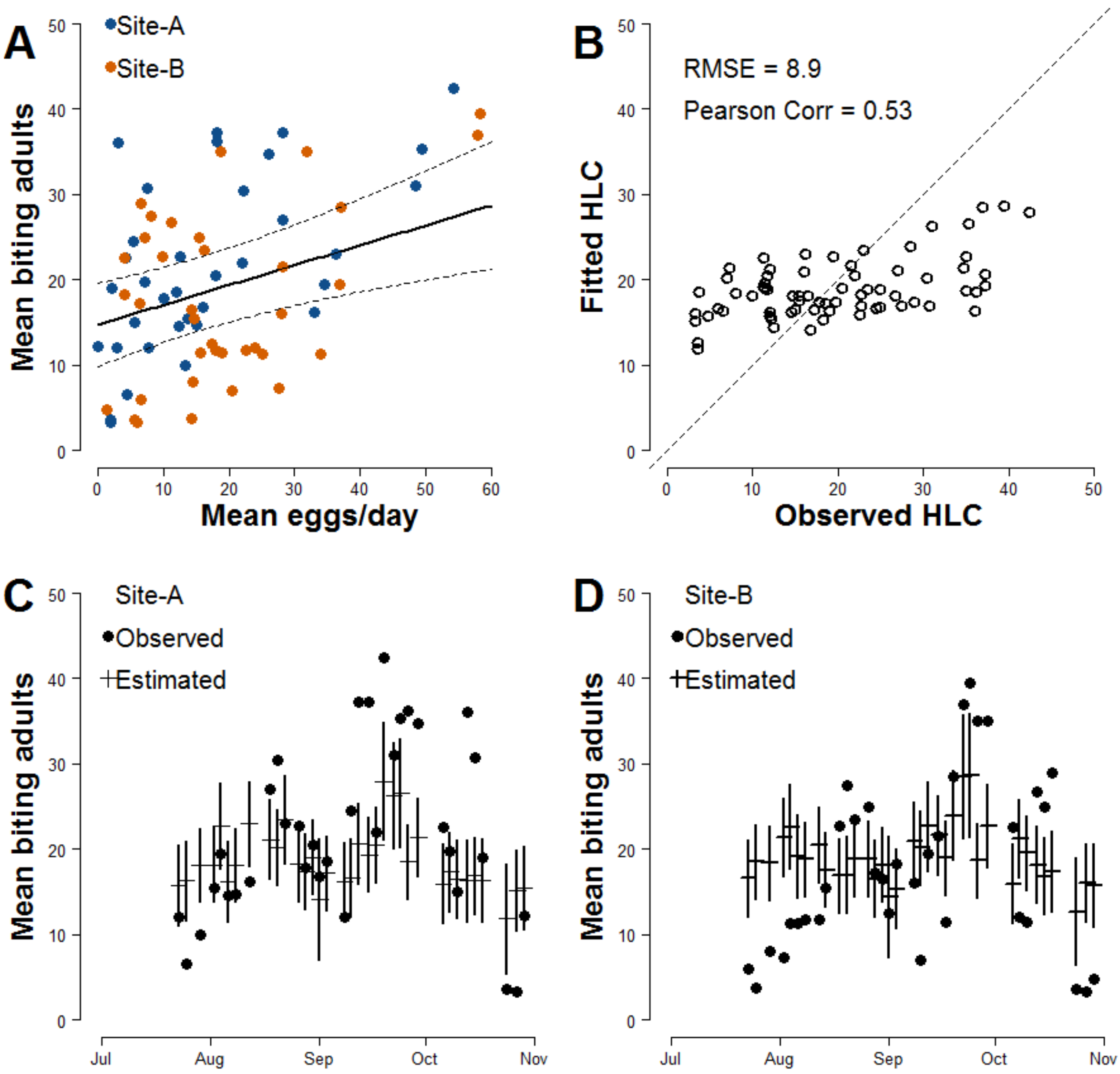

Figure 2. Basic relationship between ovitrap collections and HLC (Model-I). A) x-axis=mean number of eggs/site/day; y-axis=mean number of *Ae. albopictus* biting females. Solid line = fitted values, dashed lines = 95% confidence intervals for the regression line, dark dots = observed data. B) Observed *vs* Fitted HLC values. C and D) Observed and fitted values of the mean number of biting females collected during HLC along the season. x-axis = date of collection; y-axis the mean number of biting females; horizontal mark = fitted values, vertical solid lines = 95% confidence intervals; dark dots = observed data.

Improved estimate of biting females based on mean number of egg/day in ovitrap.

In order to improve the accuracy of estimates, meteorological variables that may affect the mosquito population dynamics were added to Model-I. After model ranking (Table S2), the explanatory variables of the model with lowest AIC (Model-II) are the mean number of eggs/site/day, the wind, the mean temperature in the day when HLCs were carried out and its quadratic term, the mean rainfall during two weeks before HLC and the two Sites (Table 3). The parameter estimate for the continuous AR1 correlation is 0.70. As for Model-I, a positive relationship between the mean numbers of females/HLC and the mean numbers eggs/site/day is observed; the estimated coefficient for the mean number of eggs/site/day is 0.245 (Fig. 3A). Compared to Model-I, Model-II better explains the variability of the collected number of adult females (Pearson correlation=0.76; RSME=6.9; Fig. 3B) and better predicts their temporal pattern (Fig. 3C and 3D). Results of the simulation study indicated that 10 traps were sufficient to give 80% power in detecting the mean number/eggs/day effect and that a further increase of the number of ovitraps would have a low probability to improve the results (Fig. S2).

Table 3. Coefficient and statistics of the parameters for Model-II.
Coefficient and statistics of the parameters for the best (lowest AIC) generalized least square model with continuous AR1 correlation structure analysing the relationship between the mean numbers of biting *Ae. albopictus* females/site/day and the mean number eggs/site/day accounting for the lagged effects of meteorological variables

Coeff.	**Value**	**SE**	**T-value**	**p-value**
Intercept	20.109	2.438	8.247	<0.0001
Mean number of eggs/site/day	0.245	0.073	3.337	0.0014
Temp	-0.891	0.471	-1.891	0.0630
$Temp^2$	-0.289	0.086	-3.348	0.0013
Rain 2 week lag	-0.141	0.081	-1.739	0.0867
Wind	-2.943	1.321	-2.228	0.0293
Site-B	-4.648	2.620	-1.774	0.0807

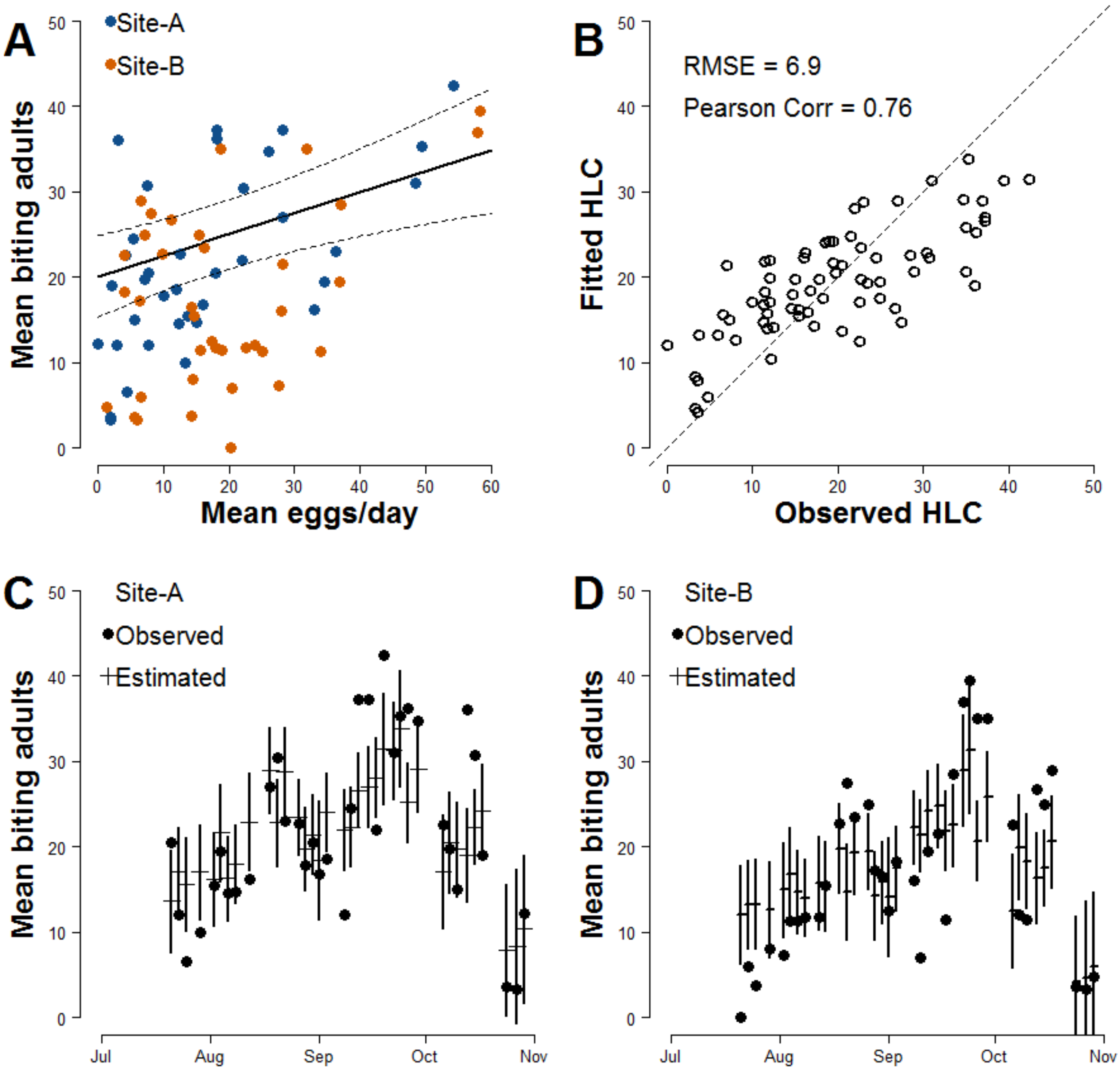

Figure 3. Improved relationship between ovitrap collections and HLC (Model-II). A) x-axis=mean number of eggs/site/day; y-axis=mean number of *Ae. albopictus* biting females. Solid line = fitted values calculated at mean values of other predictors; dashed lines = 95% confidence intervals for the regression line, dark dots = observed data. B) Observed *vs* Fitted HLC values. C and D) Observed and fitted values of the mean number of biting females collected during HLC along the season. x-axis = date of collection; y-axis the mean number of biting females; horizontal mark = fitted values, vertical solid lines = 95% confidence intervals; dark dots = observed data.

Estimates of risk of exotic arbovirus autochthonous transmission.

Estimates of R_0 for CHIKV in the study area range from 1 to 2.4 when calculated both on the basis of observed and fitted biting females, with the exception of few dates at the beginning and at the end of the sampling period (Fig. S1). On the contrary $R_0<1$ is always obtained for DENV and ZIKAV, with the exception of few sampling dates between late August and October, when

R_0 for ZIKAV ranges between 1 and 1.5 (Fig. S1). Figure 4 shows the relationship between the mean number of eggs/site/day and the values of R_0 for CHIKV computed using average HLC values (solid lines) with their confidence intervals (grey area) predicted by Model II during *Ae. albopictus* reproductive season (from June to September). Despite the large confidence intervals in the estimation of R_0 values for CHIKV based on fitted biting females, results indicate that R_0 is >1 when at least 28, 20, 20, 3, 12 and 79 eggs/day are collected between June and November, respectively. Below these numbers of eggs/day, R_0=1 is included within the confidence intervals and does not allow to predict the onset of the outbreak with 95% of confidence. Similar patterns of the risk of outbreak for arboviruses in the study area are obtained either based on HLC data or on estimates of biting females from Model-II (Fig. 5). Risk of CHIKV outbreak ranges from 40 to 80% from the second half of August to the end of the October, with only few exceptions (Fig. 5A e 5B). Risk of ZIKAV ranges between 0 and 20% up to second half of September when it raises up to 40% and decreases afterwards (Fig. 5C e 5D). No risk of outbreak (p=0) is predicted for DENV (not shown).

Discussion

Ovitrap data are considered appropriate to assess presence/absence of *Ae. albopictus* in a given site but not adult abundance, due to the several biases potentially affecting the outcome of ovitrap collections and their relationship with the adult mosquito population (Qiu *et al.* 2007; Straetemans 2008; Marrama Rakotoarivony & Schaffner 2012). However, due to feasibility and economic reasons, the number of eggs in ovitraps represents the most commonly available data provided by large-scale routine monitoring activities carried out by public administrations in infested areas, at least in Europe (Severini *et al.* 2008; Carrieri *et al.* 2011; Collantes *et al.* 2015; Flacio *et al.* 2015). Thus, number of eggs in ovitraps is often taken as the only indicator of high nuisance or of higher risk of disease transmission and used for planning mosquito control interventions. Establishing a threshold in the number of eggs/ovitrap over which nuisance could affect the quality of life (Halasa *et al.* 2014) and represent a risk of arbovirus transmission could serve as a very useful tool for decision-makers in charge of planning mosquito-control activities in infested areas.

This work provides the first evidence of a significant positive relationship between ovitrap data and data from HLC, i.e. the gold standard for assessing biting rate of human-biting mosquito (Silver, 2008) and estimating nuisance and risk of arbovirus transmission.
Results also highlight the possibility to predict mean number of adult biting females based on mean number of eggs. Counterintuitively, the mean number of eggs at Lag 0 provided a better fit than the lagged effects. Indeed, eggs have a double significance: they may reflect either eggs from which the collected adults were originated (Lag 1 and 2) or eggs laid by collected adults (Lag 0). The reason why the latter provided the best fit may be that blood-feeding follows oviposition in a short time. This would imply that the number of biting females is correlated with those of ovipositing females in few previous days. On the other hand, larval development is more affected by climatic conditions over a long time and the relationship with production of adults eventually seeking for host is likely to change along the season, weakening the significance of Lag 1 and 2.

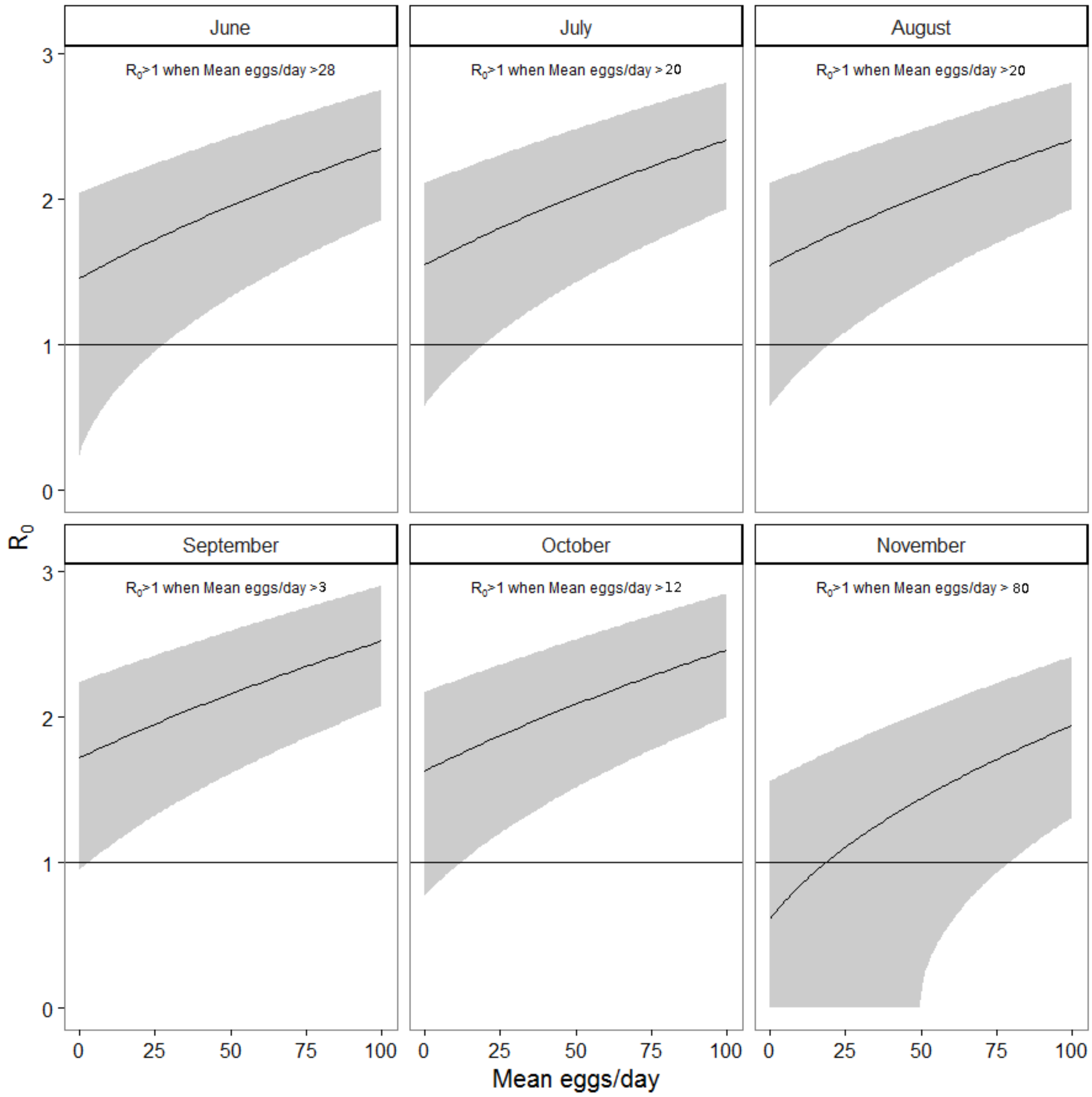

Figure 4. Relationship between mean number eggs/day/ovitrap and R0 estimates along *Aedes albopictus* reproductive season in a highly infested area in Rome. Solid black line = mean R_0 value computed using average HLC values predicted by Model II for the given value of mean eggs/day. Grey area = confidence intervals. Meteorological variables were considered at their monthly mean values.

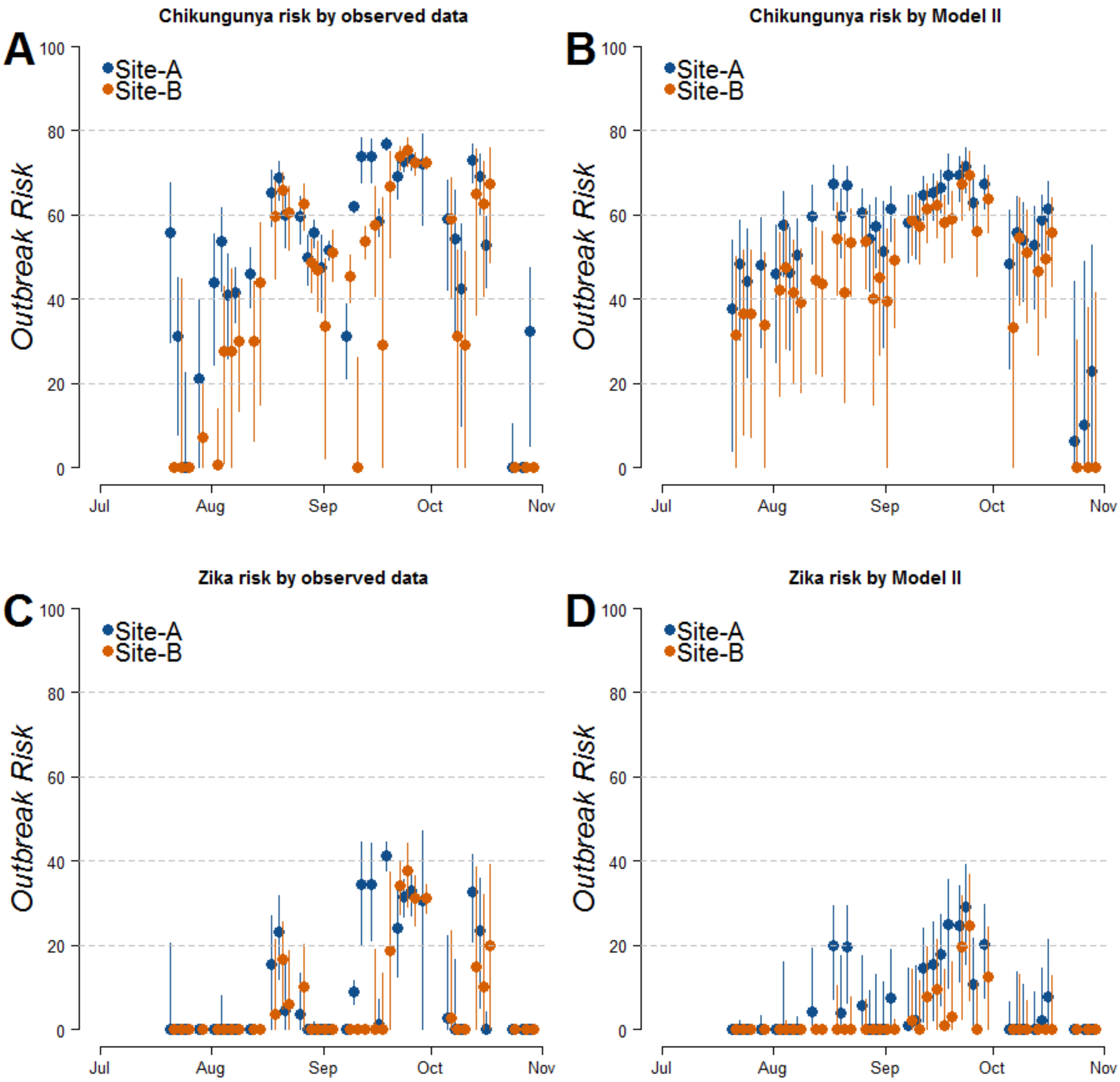

Figure 5. Estimated risk of exotic arbovirus outbreaks from an infected case in a highly infested area in Rome. Estimated risk of exotic arbovirus outbreaks based either on observed HLC data (A,B), or on the mean number eggs/site/day and its estimated relationship with biting *Ae. albopictus* females by Model-II (C,D). x-axis: months, y-axis: outbreak probability. Dots=mean values; solid lines=confidence intervals; blue=Site-1; red=site-2.

In addition to this, it is likely that the same climatic conditions affect in the same way oviposition and host-seeking behaviours of the population at a given time strengthening the effect of Lag 0. In order to improve the prediction, several variables are considered: daily temperature, daily wind speed and the lagged effect of rainfall, reflecting the negative effect of not-optimal temperatures, of strong winds and of precipitation on adult mosquito flight and survival (Hawley 1988; Waldock *et al.* 2013). However, despite this significant relationship, the accuracy of the prediction is relatively low, as indicated by wide confidence intervals on the predicted values (e.g. for a prediction of 20 females, the observed value is predicted to be between 6 and 34 in 95% of the cases). This low accuracy was expected due to the several local eco-climatic factors potentially affecting mosquito biting and oviposition activities, as well as

to possible migration from neighbouring areas and the experimental scheme adopted. In particular, it should be noted that in the present work, a 15'-long HLC on unprotected volunteers in the daily peak of *Ae. albopictus* activity (Hawley 1988; Delatte *et al.* 2010; Carrieri *et al.* 2012) was taken as a proxy of the number of biting female/person/day. Moreover, the competition of other human hosts present during the HLC and of natural oviposition sites alternative to ovitraps were not taken into account.
Model prediction accuracy is also affected by sampling effort; on one hand, increasing the number of traps would decrease uncertainty of model prediction, on the other hand, at small scale as in our experimental design, an intensive sampling effort could affect mosquito population dynamic. Here we detect that our choice of using 15 traps well compensate both aspects, in fact power analysis (Fig. S2) indicates that 15 traps are sufficient to have a good statistical power (higher than 80%) but are negligible compared to the number of natural breeding sites in the study sites (botanical and enclosed gardens).

In the study area, the models predicted an increase of one biting female/person every 5 additional eggs found in ovitraps, possibly reflecting that each female had a high number of oviposition sites alternative to ovitraps where to lay its eggs, consistent with the species skip-oviposition behaviour (Hawley 1988; Davis *et al.* 2015; Davis, Kline & Kaufman 2016). The models estimated the presence of adult biting females also at zero mean number of eggs/day, as also observed during the experiment. This is counterintuitive, as each adult female releases tens of eggs each gonothrophic cycle, and questions the widely accepted concept that ovitraps are a very sensible tool to detect the presence of adult females.

From the epidemiological perspective, the observed number of biting female/person was in the range of those estimated in Emilia Romagna during the 2007 CHIKV-outbreak (Poletti *et al.* 2011) and of those observed in other north-east Italy sites (Marini *et al.* 2015), where similar models predicted a non-negligible risk of exotic arbovirus outbreaks (Guzzetta *et al.* 2016b,a). Risk models predicted that the extremely high biting rates observed in the study area were associated to an R0>1 along most of the season for CHIKV and in only a few weeks during the peak of mosquito abundance for ZIKAV. It is interesting to note that risk models also showed that risk of CHIKV and ZIKAV outbreak was higher not only at the peak of the summer season (i.e. August), but also in October, reflecting the bimodal population dynamics already reported for the species in Rome (Manica *et al.* 2016). Notably, these patterns are not to be extended to the whole metropolitan area of Rome, as both study sites are hot-spots of *Ae. albopictus* abundance, due to the presence of small green islands within a highly urbanized environment (Manica *et al.* 2016).

When estimates of adult biting *Ae. albopictus* females based on ovitrap data were exploited in risk models, the patterns of exotic arbovirus outbreak probability were similar to those obtained based on collected adults. The model allowed to predict the dynamics of the risk of arbovirus outbreak in the study area based on the number of eggs in ovitraps and to obtain threshold values of mean number of eggs/day above which interventions to prevent the transmission need to be implemented. For example in the case of CHIKV, which had the highest outbreak probability, mean numbers of eggs/ovitrap/day ranging from 3 to 20 were associated to actual risk of transmission from June to October. This range is frequently observed in Rome (Di Luca *et al.* 2001; Toma *et al.* 2003), suggesting that the city has high risk of CHIKV outbreak in the presence of infected human hosts. However, it remains to be established whether the relationship between eggs and biting adults is maintained also in areas less suitable for high mosquito densities than the study sites.

The models here applied to estimate adult biting *Ae. albopictus* females based on ovitrap data could be further improved by introducing other variables (e.g. number of oviposition sites alternative to ovitraps) or by a more intense sampling effort with ovitraps, thus resulting in more accurate epidemiological estimates. However, the results here obtained represent a caveat regarding the significance of relying on large scale ovitrap monitoring schemes for estimating numbers of biting females and planning control interventions aiming at preventing risk of arbovirus transmission (or of high nuisance). In order to fill the gap between entomological studies, operational field surveillance and planning of mosquito control activities, efforts should be concentrated on the development and validation of new strategies to predict risk of arbovirus outbreaks and possibly provide straightforward warning thresholds.

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Chapter 4

Not in my backyard: effectiveness of outdoor residual spraying from hand-held sprayers against the mosquito *Aedes albopictus* in Rome, Italy.

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Abstract

BACKGROUND: WHO guidelines state that adulticide interventions are recommended only in case of disease outbreak. However, peridomestic sprayings are carried out routinely to reduce *Aedes albopictus* (Skuse) nuisance, at least in Italy. Failing in keeping low adult abundance overtime triggers the need for further applications. The aim of the study was to investigate the effectiveness of a common control strategy routinely performed by citizens in highly infested urban sites inside the metropolitan city of Rome using a freely purchasable pyrethroid and a hand-held sprayer. Moreover, the effectiveness evaluated in three field experiments was compared to the one achieved by blending the pyrethroid with a new carbon-based liquid additive.

RESULTS: 86% post-treatment reduction in *Ae. albopictus* abundance was observed in gravid and host-seeking females, while the population recovery time was 10 days. Blending the insecticide with the additive lengthened mosquito recovery time over 14 days.

CONCLUSION: Peridomestic sprayings largely reduce mosquito population immediately after treatment but fail to keep low mosquito abundance on a longer period, partially explaining the uncontrolled repetitions of treatments. An optimal control application could benefit from research in the field of additive to improve the mosquito abatement and the overtime performances of pyrethroids.

Introduction

Mosquitoes are nuisance pests and potential human disease vectors. Nuisance-vector control aims at improving the life quality of people by reducing the nuisance level and preventing or controlling mosquito-borne diseases (EMCA & WHO Europe 2013). In the multidisciplinary practice of mosquito control, numerous methods have been developed (Rose 2001; Becker *et al.* 2010; Baldacchino *et al.* 2015) to achieve these objectives. Among others, surveillance and conventional control methods targeting larvae population integrated by social awareness and public education are essential (Gubler & Clark 1996; Chaki *et al.* 2012; Healy *et al.* 2014; Naranjo *et al.* 2014). Meanwhile, alternative control methods are being developed (Gravitz 2012; McGraw & O'Neill 2013). In vector control, the major concern is to maintain mosquito population under a threshold at which disease transmission is unlikely (Gratz 2004; Medlock *et al.* 2012). When larval control does not suffice, control measures targeting adult population are needed in order to reduce the risk for transmission of arboviral diseases (WHO 2009a; Bellini, Zeller & Van Bortel 2014). Nuisance control faces similar concerns in maintaining mosquito population under a tolerance threshold. However, the level of infestation above which control measures (and the type of) are justifiable has not yet been established (Becker *et al.*

2010; Baldacchino *et al.* 2015) despite considerable efforts to assess the public tolerance of mosquito biting (Carrieri *et al.* 2008).

In a study carried out in New Jersey (US), citizens reported being affected by *Ae. albopictus* in their social life and outdoor activities (Halasa *et al.* 2014). Another study in Wisconsin (US) showed that they were more willing to pay intervention to reduce mosquito nuisance rather than to limit the risk of disease transmission (Dickinson & Paskewitz 2012). Invasive mosquitoes as *Ae. albopictus* are of major concern and nuisance in people's daily life not only for the increased risk of the arrival and the spread of arbovirus as Dengue and Chikunguya (Lambrechts, Scott & Gubler 2010; Abramides *et al.* 2013) but also for the numerous bites (Carrieri *et al.* 2008; Weaver 2014) due to their daytime feeding behaviour (Medlock *et al.* 2012). Therefore, the burden of high adult mosquito density on human host is not neglectable even in areas of low or near zero epidemic risk.

Chemical spray treatments provide a rapid method to reduce adult population (WHO 2003, 2006). In its broadest sense, the term "spray treatments" refers to various forms of intervention carried out with hand-operated sprayers or from trucks or aircrafts (WHO 2003). Previous studies have investigated thoroughly how droplet size, type of spraying device used, and environmental and meteorological condition determine treatment effectiveness (Mount, Biery & Haile 1996; Hoffmann *et al.* 2007, 2008, 2009, 2010; Lothrop *et al.* 2007; WHO 2009b; Bonds 2012). The substance sprayed could be either a liquid insecticide which is dispensed in aerosol droplets and kills mosquitoes on contact (WHO 2003) or an insecticide that kills mosquitoes by secondary contact on sprayed surfaces (WHO 2006). In commercial formulations, the activity of insecticide such as pyrethroids is usually enhanced by the addition of a synergist (Metcalf 1967) such as piperonyl butoxide (Bingham *et al.* 2011), which inhibits metabolic degradation of the active ingredient. Hand-operated compression sprayers are typically designed to apply insecticide mixed with water on surfaces or breeding sites (EMCA & WHO Europe 2013). Strict safety and effectiveness requirements need to be satisfied before the usage approval of a specific insecticide formulation (WHO 2009b, 2012). The use of adulticide is recommended only in case of outbreak (EMCA & WHO Europe 2013) but in Italy it is also accepted when dealing with a severe mosquito infestation (Carrieri *et al.* 2008; Becker *et al.* 2010; Baldacchino *et al.* 2015). It is of Public Health interest to constrain the health concerns and the costs that spray applications imply. Hence, WHO guidelines advice not to misuse them, also because of the risk of inducing resistance in mosquitoes and the possibility of causing damage to human and non-target species (WHO 2009b, 2011).

In Italy, the first reports of *Ae. albopictus* establishment date back to 1990 (Sabatini *et al.* 1990). During the early stages of *Ae. albopictus* invasion, adult treatments were usually carried out in heavily infested areas using deltamethrin and permethrin. In 1997, the estimated amount of insecticides used and the total operational cost was about 300 kg and US $700,000, respectively (Romi, Di Luca & Majori 1999). Nowadays, peridomestic spray applications are routinely performed as a nuisance-reduction practice (Carrieri *et al.* 2008). As a matter of fact, municipalities have issued guidelines for public notice (Sindaco di Roma 2014) and pest control companies advertise both area-wide and peridomestic spray applications. The latter are sometimes carried out by private citizens themselves using hand-operated compressor sprayers. By public notice, in Rome spray applications have to be realised only when strictly necessary in specific highly infested areas (Sindaco di Roma 2014) to constrain the excessive use of insecticide made by citizens in order to reduce mosquito nuisance.

Mosquito population recovery after treatment is a major drawback especially outdoors, where spray applications are routinely carried out against *Ae. albopictus*. Evidence suggests that outdoor space sprayings, defined as "the application of small droplets of insecticide into the air in an attempt to kill adult mosquitoes in and around homes", are not a recommended control measure unless their application is part of a wider vector control strategy (Esu *et al.* 2010). Due to the population recovery and the short residual effectiveness of the insecticide, adult reduction is only transient. Therefore, successive treatments are suggested in vector control programmes (WHO 2003). On the other hand, in nuisance control failing in reducing the burden of high adult abundance triggers the need for further applications by inhabitants. The improvement of insecticide performance in adverse conditions could lead to an increase in treatment effectiveness and a reduction in follow-up treatments.

Effectiveness of peridomestic residual spraying using hand-held sprayers against *Ae. albopictus* was evaluated during field experiments in a highly infested urban area in Rome (Italy). Two different insecticide treatments were carried out using i) a registered pyrethroid based product available in stores (Microsene) composed by tetramethrin, known as a rapid knockdown agent, and permethrin with a residual activity permethrin) and ii) a mixture of Microsene with a new additive (i.e. Carbonxide) which has been previously tested to improve trap performances (Koehler, Ragasa & Pereira 2013), in order to determine and compare post-treatment mosquito reduction, mosquito recovery time and daily mosquito recovery rate.

Materials and Methods

Study Area

The study was conducted in a highly urbanized area (Ponte Mammolo: Lat. 41°54'59.6"N, Long. 12°33'59.9"E) in Rome, Italy. Eight sites (classified from A to H) were selected. Sites were considered independent without migration of *Ae. albopictus* among sites. The minimum distance among sites (about 300 m) was greater than the maximum daily distance travelled by *Ae. albopictus* (maxODT: about 290 m) as reported in a comparable location in Rome (Marini *et al.* 2010). The selected sites shared five common features: i) the presence of a hedge (maximum height 3 m, observed species: Pittosporum tobira, Rhyncospermum jasminoides, Hedera helix), ii) the presence of a residential building (minimum height 7 m), iii) half pavement and half grass terrain, iv) an approximate area of 100 m^2, v) previous reports by the inhabitants of high mosquito nuisance. At the beginning and during the experiments all the recognized breeding sites that could be exploited for oviposition by *Ae. albopictus* gravid females were removed from each site. Owners were informed about the risk related to the use of insecticide and additives and gave their consent. All applications were carried out following the manufacturer's instructions and in compliance with the relevant regulations.

Products and Materials

Microsene® (Italian Ministry of Health Reg. No. 18735) (I.N.D.I.A. INDUSTRIE CHIMICHE 2010) was purchased from I.N.D.I.A. (I.N.D.I.A. INDUSTRIE CHIMICHE S.p.A, Padova, Italy) and Carbonxide® (Patent number: 7098249) (Ture 2006) was purchased from Società Isolpaint International srl, Taranto, Italy. Microsene is a water microemulsion concentrated solvent-free and pyretroid-based insecticide that is currently used to control adults of mosquitoes. 100 g of Microsene contain: Permethrin (95% min.) g 15, Tetramethrin (94% min.) g 2.5, Piperonyl Butoxide (94% min.) g 5, and co-formulating agents and water up to 100 g. 41 Carbonxide is a carbon-based liquid additive which ensures high mechanical performances, thermal shock resistance and low thermal conductivity to products. Carbonxide is a highly stabilized colloidal system comprising: 30 to 45 volume percent of a liquid phase comprising C15–C20 saturated hydrocarbons, C18–C25 unsaturated hydrocarbons and paraffinic mineral oil and 55 to 70 volume percent of a solid phase comprising a carbon fraction, a thickener, calcium carbonate and alumina. The producer reports that Carbonxide improves the physical, mechanical and chemical properties of materials, including thermal isolation, UV-rays resistance, corrosion resistance and mechanical resistance, elasticity, scratch resistance, fixing to the supporting base, and the reduction of thermal expansion (Ture 2006). Carbonxide has been previously employed in a dual-action lethal trap for which a patent has been requested (WO Patent App. PCT/US2013/037422) (Koehler, Ragasa & Pereira 2013). Carbonxide was added to the coating to enhance stability of the insecticide active ingredients and to slowly release insecticide during field deployment. Each experiment was carried out using for each site: either i) one litre of mixture of Microsene blended with water at concentration of 0.8% per litre (as suggested by the producer for mosquito control outdoors (I.N.D.I.A. INDUSTRIE CHIMICHE 2010)), or ii) one litre of mixture of Microsene at concentration of 0.8% per litre plus Carbonxide (1+1 by volume) blended with water. The same technician performed all the treatments using a hand-pumped insecticide sprayer (Hudson® hand compression sprayer with a flat nozzle (SS 8002), recommended for the application of public health insecticides (Thornhill 1991)). Adulticide was applied to both sides of the hedge and inside the perimeter of the study area, always maintaining the same distance between the sprayer and the surfaces.

Experimental Design

Three experiments, from now on referred as i) Pre- and Post-Treatment Monitoring, ii) Short-Term Post-Treatment Monitoring of Multiple Treatments and iii) Long-Term Post-Treatment Monitoring of Single Treatment, were carried out from July to October 2014 (Table 1). The abundance of adult females of *Ae. albopictus* has been monitored using two different adult traps: Sticky Traps (Facchinelli *et al.* 2007) designed for gravid females. BG Sentinel™ trap (BG trap, Biogents AG, Regensburg, Germany) designed for host-seeking females. BG traps were used in combination with BG-Lure and carbon dioxide. Fermenting yeast was used as source of carbon dioxide (Saitoh *et al.* 2004; Harwood *et al.* 2014) and renewed every 3 days. Each trap remained active for 24 hours. Collected mosquitoes were brought to the laboratory and identified by a qualified technician using a stereomicroscope and a taxonomic key (Severini *et al.* 2009).

Table 1: Experimental Design

	Pre- and Post-Treatment Monitoring		*Short-Term Post-Treatment Monitoring of Multiple Treatments*			*Long-Term Post-Treatment Monitoring of Single Treatment*		
Product	c+m[a]	m[b]	c+m	m	u[c]	c+m	m	u
Site ID	A	B	C	D	E	F	G	H
BG[d]	0	0	1	1	1	0	0	0
ST[e]	5	5	3	4	3	5	5	5
Number of treatment per site	1		1+1 after 10 days			1		
Monitoring Days Pre-Treatment	14		-			-		
Monitoring Days Post-Treatment	14		28			40		

[a] c+m = Carbonxide&Microsene, [b] m = Microsene, [c] u = untreated, [d] BG = BG Sentinel™ trap, [e] ST= Sticky Traps

Pre- and Post-Treatment Monitoring

The experiment was carried out from the 3rd to the 31st of October 2014 in site A and B. An adulticide sprayed treatment (in the evening of the 12th of October) was applied in: i) site A using Carbonxide & Microsene (c+m), ii) site B using Microsene (m). Five STs were placed in each site and collected four times per week. In each site a total of 19 collection days per trap were realised, 5 collections before the treatment and 14 collections after it. Monitoring was carried out the same days for both sites.

Short-Term Post-Treatment Monitoring of Multiple Treatments

The experiment was carried out from the 1st to the 20th of July 2014 in site C, D and E. Two consecutive adulticide sprayed treatment (in the evening of the 30th of June and in the evening of the 9th of July) were applied in: i) site C using Carbonxide & Microsene (c+m), ii) site D using Microsene (m). Site E was left untreated (u). The daily monitoring started the day after the first treatment and lasted for ten days after the second treatment. A BG trap and four STs were located in site D, while a BG trap and three STs were located in site C and E. A total of 20 collection days per trap were realised, 9 collections after the first treatment and 11 collections after the second one. Monitoring was carried out the same days for all sites.

Long-Term Post-Treatment Monitoring of Single Treatment

The experiment was carried out from the 8th of September to the 17th of October 2014 in site F, G and H. An adulticide sprayed treatment (in the evening of the 7th of September) was applied in: i) site F using Carbonxide & Microsene (c+m), ii) site G using Microsene (m). Site H was left untreated (u). Five STs were located in each site. The daily monitoring started the day after the treatment and a total of 40 collection days per trap were realised. Monitoring was carried out the same days for all sites.

Statistical analysis

All statistical analyses were carried out using R version 3.2.0 (R Core Team 2017) and R2jags statistical software packages (Su & Yajima 2012). Prior to them, graphical exploratory techniques were used to check for outliers and collinearity, following the protocol outlined in (Zuur, Ieno & Elphick 2010). A Bayesian approach was used to infer the relationship between the number of *Ae. albopictus* adult females collected daily and the independent variables considered in the models (see Sections 2.4.1, 2.4.2 and 2.4.3). The response is a count data and preliminary analyses showed overdispersion, possibly caused by extreme variation. Hence, a negative binomial distribution with logarithmic link was applied. Diffuse normal priors were used for regression parameters (Zuur, Saveliev & Ieno 2009), while half-Cauchy priors were used for standard deviation parameters (Gelman 2006). Models featured a burn-in of 75,000 iterations, a thinning rate of 10, three chains and a total number of 95,000 iterations, resulting in 6,000 iterations per parameter for the posterior distributions. Finally, a model validation was carried out to assess mixing of chains and integrity of models' statistical assumptions (Zuur, Saveliev & Ieno 2009).

Pre- and Post-Treatment Monitoring

A Generalized Linear Mixed Model (GLMM) was used to investigate whether the number of *Ae. albopictus* adult females collected daily differs between pre- and post-treatment and depending on sites. The period (categorical variable: pre- or post-treatment) and the site (categorical variable: site A with c+m, site B with m) were included as independent variables.

Their interaction was taken into consideration to investigate if a different reduction in the amount of daily collected females after the treatment occurred between sites. In order to deal with the dependency structure in sampling, the collection day and the trap identification code were both modelled as crossed random factors. The random structure was selected a priori (Bolker *et al.* 2009). The percentage reduction in mosquito population was computed as the subtraction between the mean abundance pre-treatment and the mean abundance post-treatment divided by the mean abundance pre-treatment.

Short-Term Post-Treatment Monitoring of Multiple Treatments

A Generalized Linear Mixed Model (GLMM) was used to investigate the relationship between Ae. albopictus adult females collected daily and four independent variables: the trap method (categorical variable: ST or BG trap), the insecticide treatment (categorical variable: first, second treatment), the site (categorical variable: site C with c+m, site D with m, site E untreated), and the days following the treatment (quantitative variable: Day) including their interaction with sites. The aim was to evaluate the recovery rate post sequential treatments. Trap identification code was modelled as a random effect to impose a correlation structure between observations from the same trap. The random structure was selected a priori (Bolker *et al.* 2009).

Long-Term Post-Treatment Monitoring of Single Treatment

A Generalized Additive Mixed Model (GAMM) was used to investigate the relationship between *Ae. albopictus* adult females collected daily and two independent variables: the site (categorical variable: site F with c+m, site G with m, site H untreated), and the days following the treatment (quantitative variable: Day) including the interaction between them. The aim was to model as a function of time the post-treatment recovery rate to assess the presence of any plateau effect. The model contains: i) a parametric term for site H (untreated) and ii) two time smoothers, one for site F (c+m) and one for site G (m), respectively. Time was standardized to improve mixing of chains (Zuur, Saveliev & Ieno 2009). O'Sullivan splines with four internal knots for standardized time were used as time smoothers (Wand & Ormerod 2008). In the GAMM, the Bayesian approach provides flexibility to define smoothers bases and gives the possibility to easily compare smoothers by subtracting one from the other and compute credible intervals. 51 Therefore, to establish whether the time effect was the same in site F and site G, the difference between the 6,000 smoothers (see Statistical Analysis) in site F (c+m) and the 6,000 smoothers in site G (m) was computed, obtaining a posterior mean and a 95% credible interval. Trap identification code was modelled as a random effect to impose a correlation structure between observations from the same trap. The random structure was selected a priori (Bolker *et al.* 2009).

Results

Pre- and Post-Treatment Monitoring

Overall, 865 adult females of *Ae. albopictus* were collected in the two treated sites (a total of 10 traps for 19 collection days). The overall mean of *Ae. albopictus* per 24h/trap in STs was: i) in site A (c+m) 5.84 (± 0.96 Standard Error, n=25) pre-treatment and 1.31 (± 0.22 SE, n=70) post-treatment, ii) in site B (m) 8.56 (± 1.40 SE, n=25) pre-treatment and 5.90 (± 0.79 SE, n=70) post-treatment (Figure S1). The result of GLMM analysis showed that there was a comparable pre-treatment abundance of *Ae. albopictus* in the two sites whereas a significant post-treatment decrease was detected in site A (c+m) (Table 2, Figure 1).

Table 2: Result Pre/Post Treatment of GLMM in site A (Carbonxide & Microsene) vs B (Microsene). Posterior mean, standard error and 95% credible interval for independent variables. If, for a given parameter, 0 is in the 95% CI then it is not significantly different from 0. Pre-treatment *Aedes albopictus* adult females collected daily on Sticky Traps in site A (c+m) as reference level (intercept). Posterior mean for the standard deviation of random effect trap identification code = 0.353 and collection day = 0.892.

	Mean	SE	2.50%	97.50%
Intercept	1.719	0.485	0.792	2.663
Post-Treatment	-1.710	0.533	-2.733	-0.629
Site B (m)	0.290	0.319	-0.334	0.924
Site B * Post-Treatment	1.095	0.285	0.539	1.658

The statistical significance of the interaction term (Site B * Post-Treatment) and its positive sign suggested that the difference in *Ae. albopictus* abundance between pre- and post-treatment was greater in site A (c+m) than in site B (m) (Table 2, Figure 2).

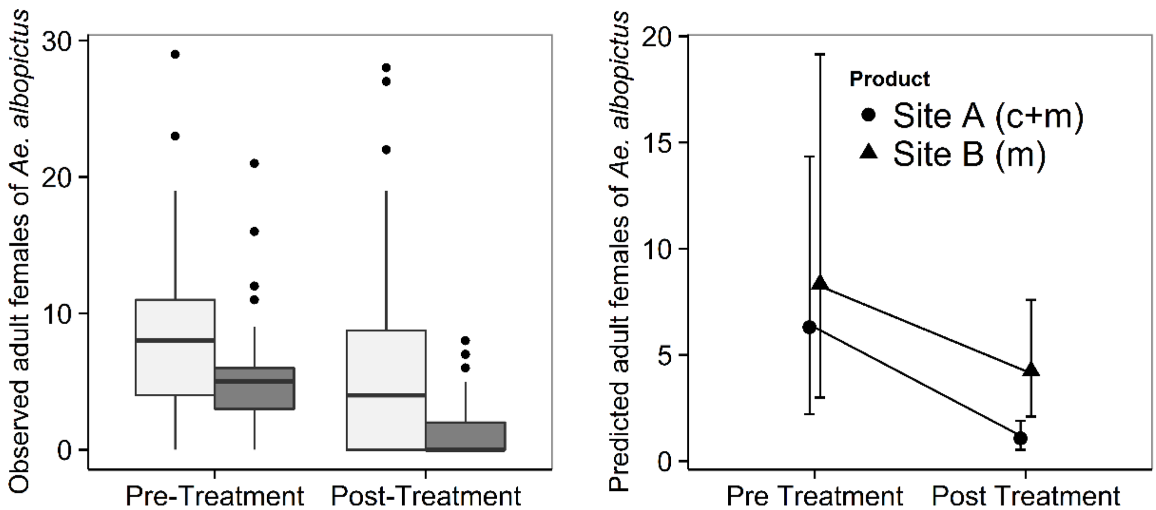

Figure 1: *Left:* Boxplot of observed *Ae. albopictus* adult females collected by sticky traps pre- and post-treatment in the two sites (site A = Carbonxide & Microsene light grey, Site B = Microsene dark grey). The boxes identify

the first and third quartiles (the 25th and 75th percentiles). The upper whisker extends from the boxes to the highest value that is within 1.5 * IQR (inter-quartile range: the distance between the first and third quartiles, so the height of the boxes). The lower whisker extends to the lowest value within 1.5 * IQR. Points beyond the end of the whiskers are outliers. *Right: Ae. albopictus* predicted values of negative binomial GLMM, the circle and the triangle are the posterior mean in the site A (c+m) and in the site B (m), respectively. The band represent 95% credible interval

More specifically, restricting the database to the sole site B (m), it emerged that the mean abundance post-treatment was not statistically different from pre-treatment (mean estimate: -0.611, SE: 0.524, credible interval: -1.673, 0.421). However, in site B if we take into consideration only the first 10 days post-treatment a statistical difference between pre- and post-treatment can be noticed (mean estimate: -1.368, SE: 0.527, credible interval: -2.459, -0.352).

Short-Term Post-Treatment Monitoring of Multiple Treatments

Overall, 1,332 adult females of *Ae. albopictus* were collected in 20 days (147 in site C (c+m), 447 in site D (m) and 738 in site E (untreated)). On average, the number of *Ae. albopictus* collected with STs was 66% less than with BG-trap. The mean of *Ae. albopictus* per 24h/trap in STs and BG are shown in Table S1. The result of GLMM analysis indicated that all independent variables considered were significant predictors of the number of *Ae. albopictus* collected daily (Table 3). A statistically significant difference in *Ae. albopictus* abundance was found between treated sites (C, D) and untreated site (E), while no difference was detected between the two treated sites (C vs D). The number of *Ae. albopictus* adult females collected daily was positively associated with the independent variable Day (see Section 2.4.2) in both treated sites (Table 3, Figure 2a). The interaction effect showed a statistically significant slower increase in site C (c+m) compared to site D (m) (Figure 2a). As for the untreated site, no temporal pattern on adult female abundance was observed during the experiments (Table 3, Figure 2b).

Table 3: Result of GLMM for Short-Term Post-Treatment Monitoring of Multiple Treatments in site C (c+m), D (m) and E (u). Posterior mean standard error and 95% credible interval for independent variables. If, for a given parameter, 0 is in the 95% CI then it is not significantly different from 0. *Aedes albopictus* adult females collected daily on BG Trap in site C (Carbonxide & Microsene) after the first treatment as reference level. Day: days following the treatment. Posterior mean for the standard deviation of random effect = 0.245

	Mean	**SE**	**2.50%**	**97.50%**
Intercept	0.363	0.364	-0.368	1.068
Day	0.174	0.043	0.093	0.257
Site E (u)	2.788	0.399	1.993	3.564
Site D (m)	-0.630	0.448	-1.532	0.229
II Treatment	-0.454	0.111	-0.664	-0.230
Sticky Trap	-0.956	0.217	-1.371	-0.508
Day* Site E (u)	-0.206	0.051	-0.304	-0.108
Day* Site D (m)	0.218	0.056	0.107	0.329

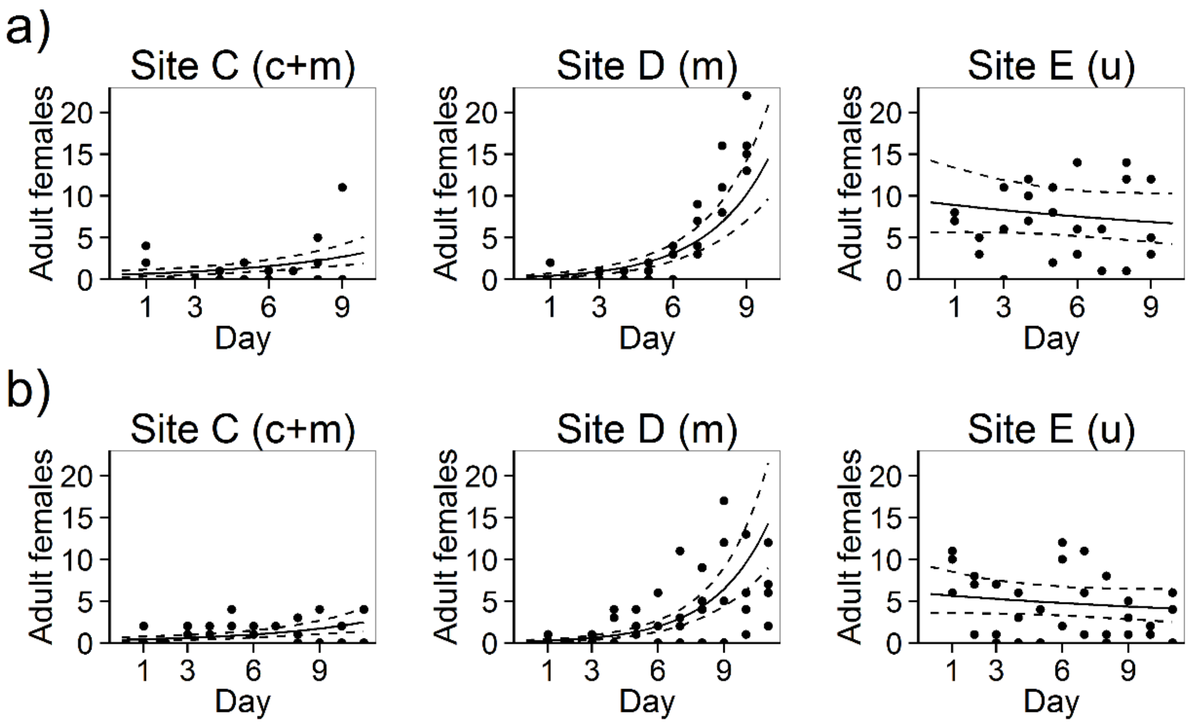

Figure 2: Result of Short-Term Post-Treatment Monitoring of Multiple Treatments GLMM: posterior mean (solid line) and 2.5% and 97.5% quartiles (dotted lines). *Panel a):* First treatment. *Panel b):* Second treatment. On the x-axis the days following the treatment, on the y-axis the predicted number of *Ae. albopictus* adult females. Dots are the observed number of *Ae. albopictus* adult females collected daily versus days following the treatment for Sticky Trap in the three sites.

Long-Term Post-Treatment Monitoring of Single Treatment

Overall, 2,552 adult females of *Ae. albopictus* were collected during the 40 days post-treatment period in the three sites (171 in site F (c+m), 813 in site G (m) and 1,568 in site H (untreated)). The mean of *Ae. albopictus* per 24h/trap in STs was 1 (± 0.11 SE) in site F (c+m), 4.78 (± 0.42 SE) in site G (m), and 9.28 (± 0.68 SE) in site H (untreated) (Figure 3). The mean posterior distribution and their 95% credible interval (Table 4) indicated a statistically lower abundance in the treated sites compared to the untreated site.

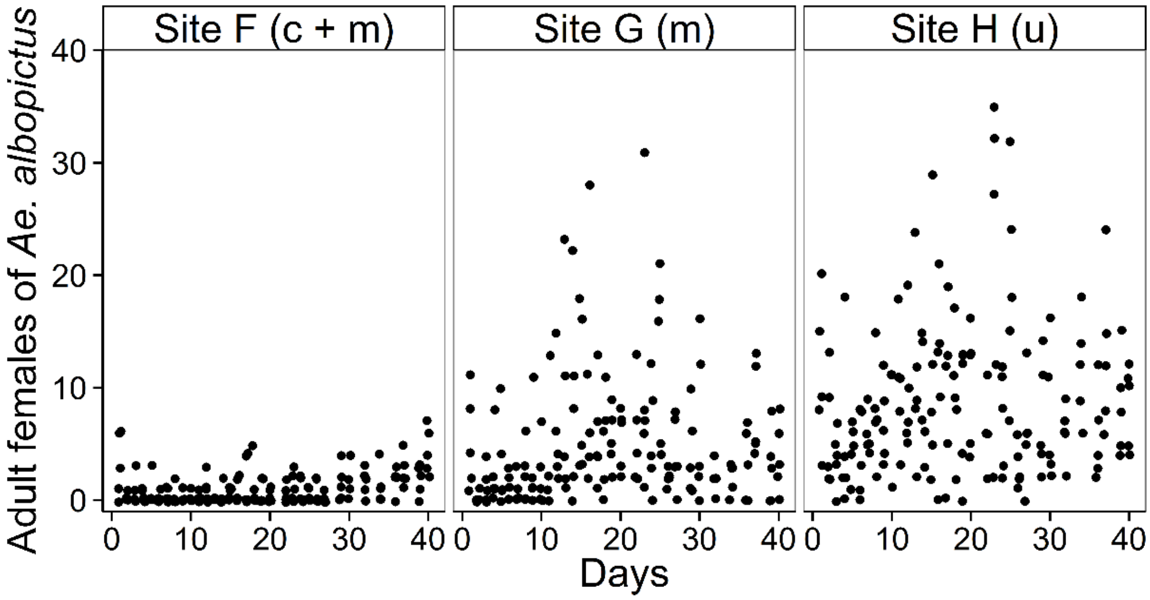

Figure 3: Number of *Ae. albopictus* adult females collected daily versus days following the treatment. On the x-axis days following the treatment, on the y-axis observed number of *Ae. albopictus* adult females collected daily in each of the five Sticky Traps. Observations were jittered on the x- and y-axis and two observations in site C (x-value=10, y-value=52 and 71) were not displayed to allow for a better graphical representation.

Table 4: Result Long-Term Post-Treatment Monitoring GAMM. Posterior mean, standard error and 95% credible interval for independent variables. If, for a given parameter, 0 is in the 95% CI then it is not significantly different from 0. Site F (Carbonxide & Microsene) as reference level. Posterior mean for the standard deviation of random effect = 0.472

	Mean	**SE**	**2.50%**	**97.50%**
Intercept	-0.105	0.248	-0.613	0.379
Site G (m)	1.312	0.338	0.646	1.976
Site H (u)	2.282	0.348	1.601	2.997
Difference Between Site G and Site H	-0.970	0.343	-1.683	-0.321

Moreover, site F (c+m) showed a lower post-treatment abundance compared to site G (m). Figure 4 shows two non-linear smoothers for treated sites (Figure 4A) and one smoother obtained by their subtraction (Figure 4B). In the site E (untreated), no effect over time was detected (results not shown). This is consistent with the results obtained in the previous experiment. The smoother for the time effect in site F (c+m) indicated that after the treatment there was a decreasing trend in mosquito abundance in the first 10 days followed by an increasing trend from the 10th day until the 40th (Figure 4A). On the other hand, the smoother for the time effect in site G (m) showed that after the treatment there was an initial decreasing trend for a shorter period (less than 10 days), followed by an increase until the 20th day, and a further decrease (Figure 4A). The difference between the two smoothers of treated sites (F-G, see Figure 4B) highlighted a different post-treatment pattern between 10 and 25 days post-treatment where this difference was always negative and was due to a slower increase in the number of *Ae. albopictus* collected daily in site F compared to site G. After 30 days from the treatment, the difference between the two smoothers became positive since the time effect in site G was not significant anymore while a positive effect was still detected in site F.

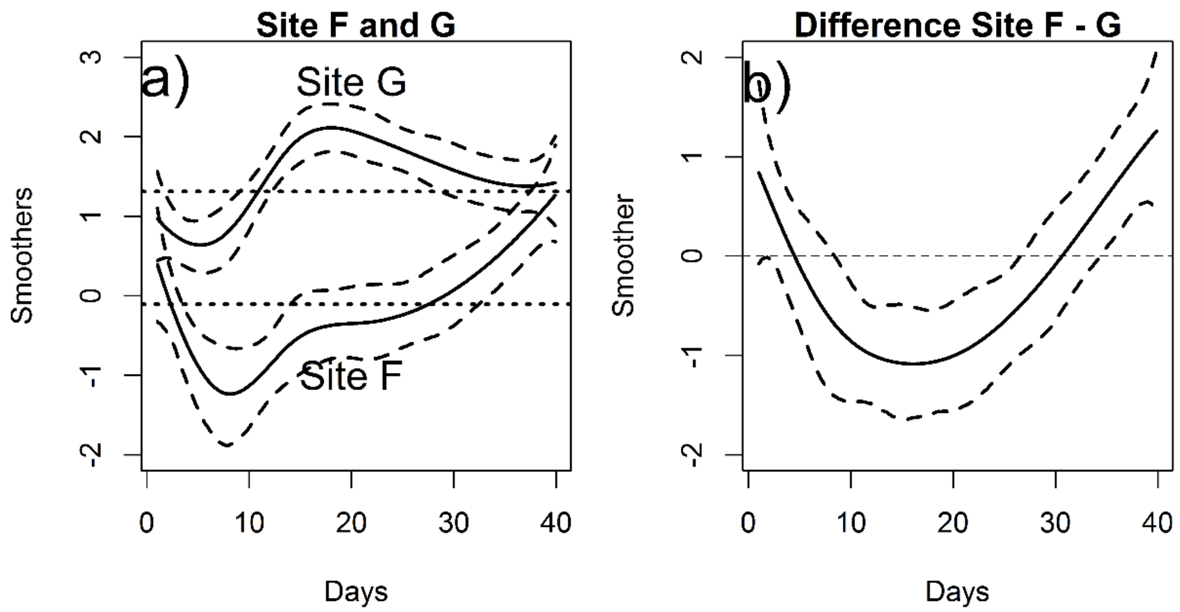

Figure 4: *Panel a)*: Estimated smoothed curve of the time effect on the number of *Ae. albopictus* adult females collected daily in site F (treated with Carbonxide & Microsene) and in site G (treated with Microsene). The solid black line pictures the posterior mean. Dashed black lines represent 95% confidence interval. The horizontal dotted line represent the estimated posterior mean for the smoothers intercept *Panel b):* Estimated smoother for the difference between the 6,000 time smoothers for site F and G. The solid line pictures the posterior mean and the dashed lines are the 95% credible interval. When the horizontal dotted line at 0 is in the 95% credible interval, then there is no significant difference between the time smoothers for site F and G.

Considering both smoothers with the intercept (dashed point in Fig4B) it is noticeable that the abundances in the two sites at the end and at the beginning of the experiment (after 35 days) were not statistically different.

Discussion

Results of Microsene sprayings in the three experiments provided evidence of: i) effectiveness of outdoor hand spraying insecticide (i.e. Microsene) in significantly reducing *Ae. albopictus* female abundance immediately after treatment (86% the day after) but not when comparing the whole pre- and post-treatment period (31% in 14 days), ii) rapid re-introduction of *Ae. albopictus* adult population even after a second treatment, with a recovery rate of 48% daily increase in mean abundance, iii) a recovery time of 10 days, after which the adult abundance was comparable to the pre-treatment mean abundance. Blending the adulticide (Microsene) with the new carbon-based additive (Carbonxide) showed an enhanced effectiveness over time: i) 88% reduction the day after and a greater mosquito reduction with 77% reduction when comparing the whole pre- and post-treatment period, ii) a lower recovery rate of 19% daily increase, and iii) recovery time longer than 14 days.

A great effectiveness of adulticides treatments was observed immediately after treatments (i.e. Abbott formula) as observed by an almost complete reduction of flying *Ae. albopictus* adults. This is in agreement with the results observed for pyrethroid usage to control *Ae. albopictus* in Italy as well as in other nations, also with different spraying methods (Trout *et al.* 2007; Suman *et al.* 2012; Marini *et al.* 2015). Spray treatments were carried out in order to reproduce the way they are routinely realised in private backyards and gardens in Italy. In these sites, mosquito control concerns mainly nuisance reduction than disease transmission (Carrieri *et al.* 2008). Nuisance is tightly connected to mosquito abundance and spray application may provide an immediate and economical relief from it. The high performance of the knock down pyrethroid present in insecticide formulations (e.g Tetramethrin in the formulation used in this work) clearly deals with the need of private citizens to immediately suppress local infestation of biting *Ae. albopictus*. However, in field settings, post-treatment mosquito either are survivors of the insecticide intervention, or freshly emerged ones, or mosquito entering the area from non-treated surroundings. The sprayed insecticide formulation contained also a residual-action pyrethroid (Permethrin in the formulation used in this work). The latter should guarantee a longer insecticidal effect by acting as a barrier to mosquito reintroduction in the sprayed area (Trout *et al.* 2007; Amoo *et al.* 2008). Therefore, several days of post-treatment monitoring could provide important additional information to assess the effectiveness of an adulticide intervention. If the objective of an adulticide intervention is nuisance reduction, the loss of effectiveness could be described and estimated by: i) daily increase in post-treatment mosquito and ii) the reestablishment of pre-treatment mosquito abundance (i.e. recovery time) and/or the failure of achieving a certain reduction threshold for a specific number of days. The identification of these events could provide information on the mosquito reintroduction and on the lifespan of an intervention.

The percentage of daily increase in post-treatment mosquito was hereby-defined recovery rate and assessed in the second experiment. The recovery rate detected in the site treated with Microsene resulted in a 48% daily increase in the mean number of *Ae. albopictus* (obtained by exponentiation of the sum of parameters Day and Day*Site D (m) in Table 3). A limitation of this study is that it did not explicitly considered meteorological conditions in the statistical analysis. Hence, in further experiment would be interesting to evaluate the interaction between residual effectiveness and a wide range of different meteorological conditions. Assuming for the sake of argument that no other environmental factor (e.g. high rainfall) influence the recovery rate, this result estimates in about 12 days the time needed for a mean post-treatment population of 0.1 mosquito to reach a mean population level of 10. The reduction in mosquito population had a longer duration in the site treated with Carbonxide & Microsene compared to the one treated only with Microsene. In fact, the recovery rate detected in the site treated with Carbonxide & Microsene resulted in a 19% daily increase in the mean number of *Ae. albopictus*. This means that about 27 days would be needed for a mean post-treatment population of 0.1

mosquito to reach a mean population level of 10. We argue that the effect detected on adult abundance was the result of an improvement in impingement capabilities and stability of the insecticide active ingredients on sprayed surfaces (the hedge) due to the addition of Carbonxide. So, an estimation of the number of daily collected mosquitoes after the treatment could represent either a good measure to evaluate performances of insecticide additive but also an important parameter to plan and minimize the number of sequential adulticide treatments. In conclusion, the computation of recovery time and rate in specific settings for a particular product could be extremely helpful for both citizens and vector control services in order to: i) obtain an estimate of the number of post-treatment days in which mosquito abundance would remain under a given threshold indicating a not tolerable nuisance or risk factor for vector disease transmission, ii) rationalise the application of adulticide sprayings by a quantifiable parameter and not only as a reply to a request for service from citizens, or by pre-planned seasonal interventions.

The adulticide tested provided an immediate relief from high mosquito abundance. As observed in previous studies which focused on the evaluation of adulticide performances (Alimi *et al.* 2013; Fonseca *et al.* 2013), the reduction was not permanent, resulting in mosquito population recovery to pre-treatment abundance after 10 days. This could lead private citizens to make frequent applications of freely purchasable adulticides, thus increasing the development of mosquito resistance (Nauen 2007; Marcombe *et al.* 2014). The combination of Carbonxide & Microsene lengthened mosquito recovery time to pre-treatment abundance (over 14 days), resulting in a 40% increase compared to the pyrethoid alone, and it could be proven effective in other settings (e.g. indoor residual spraying). Further studies will need to investigate the trade-offs between multiple sequential treatments that grant an abundance reduction for short periods or less treatments that achieve a mosquito reduction for longer periods. Moreover, given the relative small size (100 m^2) and characterization of the study areas and the removal of all breeding sites, we are confident that most of the mosquitoes trapped after the treatment came from outside the sprayed area. These results confirm that effectiveness of small-scale spray applications is only transient inside highly infested area. Applications of these insecticide products ought to be carried out parsimoniously and with great care, taking into account the effects they produce and the risks they pose to the environment and the human health. Therefore, it is necessary to highlight the importance of creating social awareness and educating people on chemical use. It would also be useful to coordinate interventions in larger areas as well as to complement spray applications with other control methods (Baldacchino *et al.* 2015).

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Chapter 5

Assessment of the effectiveness of a seasonal-long insecticide-based control strategy against *Aedes albopictus* nuisance in a urban area.

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Abstract

Seasonal-long larvicide treatments and/or outdoor space-spray applications of insecticides are frequently applied to reduce *Aedes albopictus* nuisance in urban areas of temperate regions, where the species has become a permanent pest affecting people's health and quality of life. However, assessments of the effectiveness of sequential interventions is a difficult task, as it requires to take into account the cumulative and combined effect of multiple treatments, as well as the mosquito seasonal dynamics (rather than mosquito abundance before and after single treatments). We here present the results of the effectiveness assessment of a seasonal-long calendar-based control intervention integrating larvicide treatments of street catch basins and night-time adulticide ground spraying in the main University hospital in Rome (Italy). Cage-experiments and an intensive monitoring of wild mosquito abundance in treated and untreated sites were carried out along an entire season. Sticky traps were used to monitor adult abundance and site-specific eco-climatic variations (by recording water left over), in order to disentangle the effect of insecticide treatments from eco-climatic drivers on mosquito seasonal dynamics. Despite the apparent limited impact of single adulticide sprayings assessed based on mortality in caged and wild mosquitoes, the results of the temporal analysis showed that mosquito seasonal patterns were initially comparable in the two sites, diverged in the absence of diverging eco-climatic conditions and remained stable afterwards. This allowed to attribute the lack of the expected Ae. albopictus population expansion in the treated site to the combined effect of multiple adulticide sprayings and larvicide treatments carried out during the whole season. The approach proposed was proved to be successful to assess effects of seasonal-long control treatments on adult mosquito population dynamics and could represent a valuable instrument to separate the relative impact of larvicides and adulticides, to evaluate their actual cost-benefits and to possibly minimize space-spraying applications to reduce mosquito nuisance.

Introduction

In the case of major malaria and Dengue vector species, which are the most frequent targets of insecticide-based interventions, the most important parameter to define the effectiveness of a treatment is its impact on disease transmission and morbidity/mortality. In the absence of disease transmission, standardized methodological and statistical approaches and guidelines to assess the effectiveness of insecticides against mosquitoes mostly focus on the assessment of the effectiveness of single treatments (WHO 2009b). This is carried out by measuring either mortality in caged mosquitoes spread in the target area, or percentages of reduction in wild mosquito abundance between pre- and post-treatment in treated vs untreated sampling areas (e.g. by Abbot and Henderson's formula). The former approach provides information on the extent to which variation in the observed level of efficacy is due either to the chemical itself, or to technical aspects of the treatment (e.g. droplet size) or to meteorological conditions (e.g. wind). The latter approach, on the other hand, allows to assess the effectiveness of the formulated space spray product in operational settings against field populations of a target species.

Assessments of the effectiveness of sequential insecticide-based interventions is a much more difficult task, as it requires to take into account the cumulative and combined effect of multiple treatments, as well as the mosquito seasonal dynamics, rather than mosquito abundance only. Moreover, in order to compare mosquito populations over time it is recommended that similar paired sites (treated and untreated) are selected according to mosquito population parameters (e.g. density, population dynamics, isolation), as well as ecological (e.g. landscape, availability of breeding sites, presence of competing species), climatic and socio-economic factors (Unlu *et al.* 2011; Iyaloo *et al.* 2014). Ideally, in order to provide significant preliminary data, the two sites should be selected and monitored along the mosquito reproductive season before the treatments are carried out or, in case of feasibility constraints, at least a few weeks before the treatments. This exercise is very laborious and costly, and even if results show similar vector densities and dynamics during the preliminary monitoring, eco-climatic changes arising in one of the two sites may interfere with the subsequent assessment of the effectiveness of a seasonal long control intervention.

Seasonal-long outdoor space-spray applications of insecticides, either alone or in the frame of integrated mosquito control activities, are frequently applied to reduce Aedes albopictus nuisance in urban areas from temperate regions. In fact, this originally Asiatic tropical species has become a permanent pest and is affecting people's health and quality of life (Carrieri *et al.* 2008) in US and Europe since its introduction in the '80 (Hawley *et al.* 1987; Hawley 1988) and '90 (Sabatini *et al.* 1990; Adhami & Reiter 1998), respectively. Due to above mentioned constraints, so far only few field assessments of seasonal-long area-wide strategies exploited to reduce *Ae. albopictus* densities (and nuisance) have been carried out (Richards *et al.* 2008; Abramides *et al.* 2011). Source reduction campaigns have been shown to achieve temporary suppression of immature *Ae. albopictus* in Spain and in North Carolina (Richards *et al.* 2008), while it was not enough to maintain adult counts below a nuisance threshold in New Jersey (Fonseca *et al.* 2013). Fonseca et al. (2013) showed that integrated area-wide control strategies (i.e. active source reduction, larviciding, adulticiding and public) resulted in a substantial reduction in *Ae. albopictus* populations in urban sites in New Jersey, but only modest reductions in suburban sites.

In Italy - where *Ae. albopictus* represents a major pest in urban and periurban areas and has already been responsible of a chikungunya virus outbreak (Romi *et al.* 2009) - seasonal-long outdoor interventions are frequently carried out during the species reproductive season to control its nuisance either in large public urban areas or in private residential areas. These interventions include multiple sequential larvicide treatments of street catch basins, which are considered the major not-removable urban larval sites in Italy (Carrieri, M., Bacchi, M., Bellini, R., Maini 2003; Caputo *et al.* 2015), and/or outdoor cold fog adulticide applications using vehicle-mounted sprayers. Data by Caputo et al. (2015) suggest that the major phase of *Ae.*

albopictus population expansion in Rome can be prevented by seasonal-long larvicide treatments of street catch basins in association with adulticide sprayings carried out during sunset.

We here present the results of the assessment of the effectiveness of a seasonal-long calendar-based control intervention integrating larvicide treatments of street catch basins and night-time adulticide ground spraying to reduce *Ae. albopictus* density in the ground of the main University hospital in Rome. In order to do this, we carried out cage-experiments and a fine-scale monitoring of wild mosquito abundance in the study site, as well as in a control site, along an entire season. At the same time, an ad hoc developed easy-to-use approach was implemented to measure micro eco-climatic changes in the two sites. Results were exploited to assess the effectiveness of single adulticide treatments on mosquito abundance before and after single sprayings, as well as the overall effectiveness of the integrated intervention on the mosquito population dynamics.

Materials and Methods

Study sites

Experiments were carried out in two sites in central Rome at a 1.4 km distance from each other (Figure 1), where presence of *Aedes albopictus* was previously detected (BC, personal observation). The first was a ~ 40 h-area of the Sapienza University hospital "Policlinico Umberto I" (41°54'21" N 12°30'41" E), characterized by 14 m high XIX centuries buildings and large boulevards lined by Platanus trees and pedestrian walkways occasionally lined with bushes. The second site was ~2.5 h-area of the Department of Philosophy of Sapienza University (41°55'07" N 12°31'01" E) including a central 14 m high XIX centuries building and a neighbouring area characterized by tall trees, bushes, pedestrian walkways. While insecticide treatments were planned in the "Policlinico Umberto I" (hereafter treated site) during summer 2013 (see below), no treatments were planned in Department of Philosophy (hereafter untreated site).

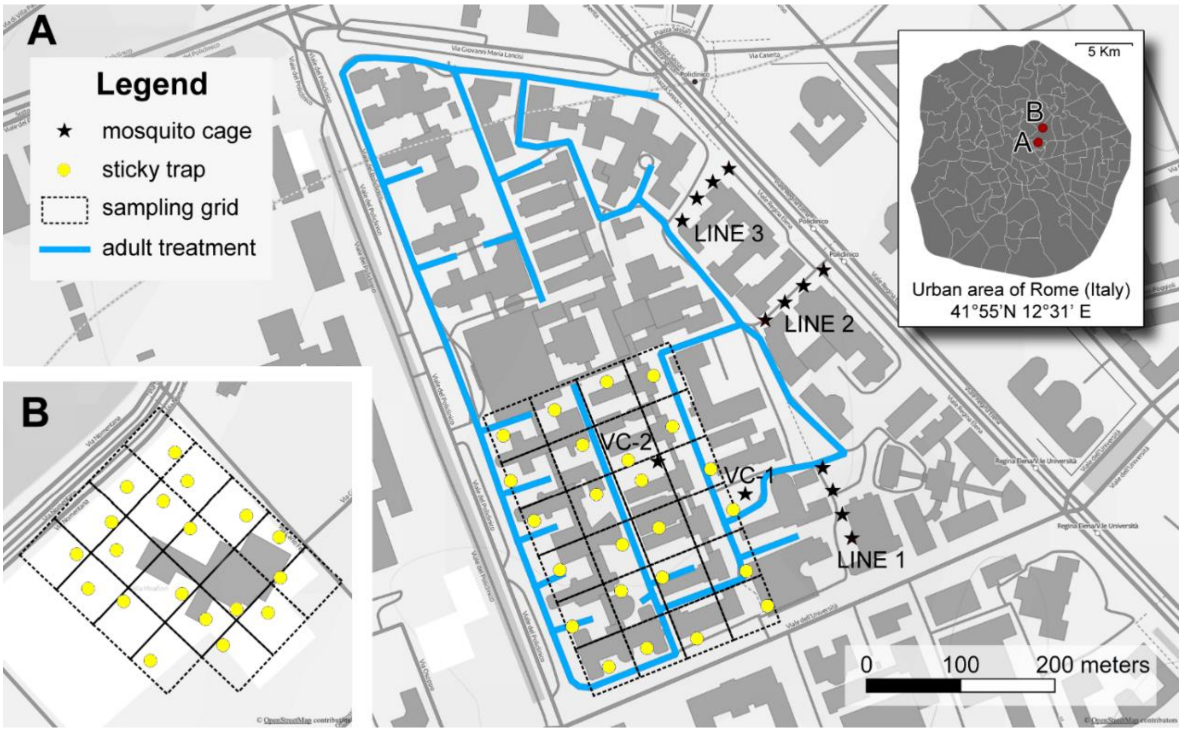

Figure 1. Map of study sites in Rome (Italy). (A) Sapienza University hospital "Policlinico Umberto I"=insecticide treated site (right panel); dark grey=buildings; light grey=open areas; blue line=itinerary of the insecticide cannon sprayer; lines of black stars=mosquito exposed cages at 10, 30, 50 and 70 m from insecticide spraying; VC-1 and VC-2=mosquito validation cages. (B) Department of Philosophy, Sapienza University=untreated site (same scale as A). Yellow dots=sticky traps.

Insecticide treatments.

Eight adulticide treatments (T1-T8) were performed in treated area by qualified technicians from a private company (SOGEA s.r.l.) from June to October 2013 by spraying 1% water diluted PERMEX 22E (BlueLine; 92% permethrin + 1.64% tetramethrin + 6.4% piperonyl butoxide) with a cannon sprayer (series "ELITE 345-400" Spray Team snc) mounted in the back of a flatbed truck. The vehicle was driven at an average speed <20 km/h. Droplet size was set up at 50/60 µM. Spraying started around midnight and lasted for about 2 hours. Moreover, all the 227 rain catch basins (i.e. drain holes in paved streets sealed by grids) within the treated area (including those not containing water to avoid risk of refilling in case of rain) were treated every two weeks from June to October by releasing tablets of an Insect-Growth-Regulators (IGR) which interferes with larval development and inhibit adult emergence (i.e. 0.5 gr pure Pyriproxyfen, PROXILAR, INDIA Industrie Chimiche).

Cage Experiments

Cages were designed and manually built following Cooperband et al. (2007) (Cooperband *et al.* 2007) and WHO guidelines (2009) (WHO 2009b). Cages - containing a Petri dishes with filter paper (Pall Corporation, 90 mm diameter) and *Ae. albopictus* adults (either 10 or 20 males and 10 or 20 females reared in the lab from wild collected eggs with ovitraps) - were positioned in the treated site at 1.5 m height. During T2-T8 treatments, cages were located as follows: i) 12 cages along 3 roads (hereafter lines) at a 10, 30, 50 and 70 m distance from the cross with the closest road where the cannon sprayer was passing (hereafter exposed cages); ii) 2 validation cages within the treated site at 13 m (VC-1) and 41 m (VC-2) from the closest road where the cannon sprayer was passing; and iii) 3 cages in the untreated site (hereafter control cages). Cages were located 1 hour before adulticide spraying and removed approximately 30 minutes afterwards. Filter papers were immediately extracted from cages and introduced in a sealed glass vial for subsequent Gas-Chromatography Mass-Spectrometry (GC-MS) analysis. Adults were transferred to paper cups and provided with cotton pads soaked with 10% sucrose solution. Mosquito mortality at 24 h post-exposure was recorded.

Gas-Chromatography Mass-Spectrometry analysis

Gas-Chromatography Mass-Spectrometry analyses were carried out by Agilent 6850 II gas-chromatograph (GC) equipped with mass selective detector (MSD) Agilent mod. 5975C and capillary column Agilent HP-5 MS (60.0 m long x 0.25 mm i.d., 0.25 µm film thickness). The column operated at 60°C (hold 1 min) to 170°C (hold 0 min) at 10°C/min, then to 280°C (hold 5 min) at 4°C/min. The split/splitless injector was maintained at 250°C, and transfer line at 280°C. Helium was used as carrier gas at 1.4 mL/min. The MSD was used in the single ion monitoring mode (SIM). Insecticides were monitored by considering two ions for each compound, with the following masses (m/z): permethrin=127 and 183; tetramethrin=123 and 164; piperonyl butoxide= 119 and 176.

After withdrawal filters left in cages during the insecticide space-spraying were transferred in a cylinder and extracted 3 times with 5, 2.5 and 2.5 mL of hexane (Sigma-Aldrich, USA), respectively. The organic extracts were collected in a vial, sealed and stored at -20°C until analysis.

Analytical determinations were carried out by GC/MS with the external standard technique. Stock standard solutions of analysed insecticides at 100.0 ± 0.5 µg/mL were obtained by Ultra Scientific, USA. Working standard solutions (w.s.s.) for calibration were prepared daily and were obtained by diluting aliquots of the stock solution with hexane, to obtain working standard concentrations of 0.01, 0.05, 0.50, 1.00, 2.50, 5.00, and 10.00 µg/mL. All the glassware was in borosilicate class A. Calibration curves were obtained by injecting five 1 µL injections of each w.s.s. and calculating the average peak area for each different concentration. Linear responses were observed in the range of concentrations considered. Analytes concentrations were determined by three 1 µL injections of each sample extract, and average peak areas were considered for quantitation. Results were expressed as µg/cm2. Whole procedure blank tests were performed, in order to assess the absence of any contamination occurring from reagents and materials. A solvent blank was analysed each five samples to check the response of chromatography.

Aedes albopictus monitoring in the field

Aedes albopictus adult population monitoring was carried out from June 17th to October 17th 2013 in treated and untreated site. Monitoring of adults populations was conducted by means of Sticky-Trap (ST) consisting in a water container similar to a commonly used ovitrap equipped with an internal structure lined with adhesive films to which the mosquitoes approaching the trap either to lay eggs or to rest remained stuck (Facchinelli *et al.* 2007). ST number and position was established subdividing an area within the treated site into a 24-cell grid and untreated site into a 19-cell grid (each cell= 40 x 40 m) (Figure 1). One ST was located in each cell and equipped with sticky sheets and 500 ml tap water. On a weekly basis, mosquitoes stuck in ST were marked directly on sticky sheets after 72 hours (day 3); after additional 72 hours, ST were removed and stuck mosquitoes were identified and counted under a binocular stereo microscope (day-6). No ST were left in the field at day-7, during which insecticide spraying were carried out if scheduled. STs were re-located in the same position at day-1 of each week after being equipped with freshly prepared sticky sheets. Water leftover was measured concomitantly to mosquito monitoring.

Temperature and rainfall data were obtained from "Roma Macao" weather-station at 300 m distance from the treated site (http://www.idrografico.roma.it/annali/).

Statistical Analysis

All analysis were carried out using R version 3.1.0 (R Core Team 2015) and lme4, strucchange packages (Zeileis *et al.* 2002, 2003; Bates *et al.* 2014).

Assessment of effectiveness of insecticide spraying on caged *Aedes albopictus*.

Effectiveness of single treatments on caged mosquitoes was computed by using the Henderson formula (Henderson & Tilton 1955) adapted to the experimental protocol as follows:

$$\%\ Effectiveness = 100 * (1 - \frac{mosquitoes\ treated\ after * mosquitoes\ untreated\ before}{mosquitoes\ treated\ before * mosquitoes\ untreated\ after}), \quad (1)$$

where *mosquitoes treated before* [*after*] are the mean numbers of live mosquitoes in exposed cages before the treatment [after the treatment] and *mosquitoes untreated before* [*after*] are the corresponding mean numbers of live mosquitoes in control cages.

Moreover, a first binomial Generalized Linear Mixed Model (GLMM-1) was carried out to test the effect of spraying treatments on caged mosquitoes. Date of treatment was introduced in the model as random effect to take into account that the eight pseudo-replicates were characterized by different conditions exclusive of each treatment date (e.g. wind, climate). In addition, lines within date of treatments were modelled as nested random effect. Response variable was the proportion of dead mosquitoes out of the initial number in each cage, while explanatory variables were: i) exposure to insecticide treatments (exposed vs. control cages), ii) permethrin concentration in exposed cages as detected by GC-MS and iii) mosquito gender. All two-way interaction terms were included into the model.

A second binomial GLMM (GLMM-2) was carried out only for exposed cages to quantify the relationship between adult mortality and distance among cages and insecticide spraying. As in GLMM-1, lines within date of treatments were modelled as nested random effect. Random structures were selected a priori (Zeileis & Kleiber 2005; Bolker *et al.* 2009). Variance inflation factors and conditional boxplot were applied to assess collinearity. Finally, VC-1 and VC-2 (see above) were used to validate model prediction. For each cage we computed the adult mortality predicted by the model on the basis of the cage distance to the spraying. Then, given the initial number of mosquitoes in cages and using estimated mortality, we simulated the number of dead adults obtained by a random binomial sample for each of the seven treatments. Ten thousand random samples have been simulated resulting in the distribution of the expected mortality for each treatment. Observed mortality out of the 0.025 and 0.975 quantile of the expected distribution was considered statistically significant.

Assessment of effectiveness of insecticide sprayings on wild *Aedes albopictus* adults.

Effectiveness of single space-spraying treatments was evaluated by monitoring mosquito abundance by ST within the treated and untreated sites for 72 hours before and after the ground spraying. Effectiveness of each treatment was computed by using Henderson formula (1) (Henderson & Tilton 1955) here mosquitoes treated before [after] are the mean numbers of mosquitoes collected in all STs of the treated site in the latest collection date before the treatment [in the first collection date after the treatment] while mosquitoes untreated before [after] are the corresponding (measured at same collection date) mean number of mosquitoes collected in all STs in the untreated site.

Linear Mixed Models (LMM-1 and LMM-2) were carried out to evaluate whether water leftover in STs could be a reliable proxy of eco-climatic conditions at finer scale (i.e. association between overall climatic conditions and ST exposure to sun-light) and whether water leftover was different between treated and untreated sites. Model response variable was water leftover in each ST, while explanatory variables were average maximum daily temperature (for LMM-1) and daily rainfall (for LMM-2) recorded at closest weather station, sites (treated vs. untreated) and their interaction. Collection date and ST identification number were considered as random effects. The random structures were selected a priori (Bolker *et al.* 2009; Zuur, Hilbe & Ieno 2013).

A Poisson Generalized Linear Mixed Model (GLMM-3) was carried out to test whether *Ae. albopictus* abundance was different between sites, whether mosquito abundance at ST level was related to water leftover and whether this relationship changed between sites. Model response variable was mosquito counts recorded in each ST, while explanatory variables were water leftover in ST, sites (treated vs. untreated) and their interaction. Collection date and ST identification number were considered as random effects. The random structures were selected a priori (Bolker *et al.* 2009; Zuur, Hilbe & Ieno 2013).

Change point analysis (Jandhyala *et al.* 2013) was carried out to assess the impact of the control strategy adopted over time and to understand which drivers (i.e. insecticide treatments and/or eco-climatic conditions) were responsible for differences in observed mosquito abundance between treated and untreated sites. Time series of the average values of the mosquito collected at each collection date and of the corresponding water leftover in ST were compared between treated and untreated sites. Both series were pre-whitened by fitting them individually an autoregressive model ARIMA (Shumway & Stoffer 2011) to avoid distorted or misleading results as consequence of autocorrelation or common trends over time (Chatfield 2003). Afterwards, Pearson correlations between treated and untreated sites of ARIMA residuals for either mosquito or water leftover were computed. In order to evaluate whether correlation between treated and untreated sites changed during the season, correlation coefficients were computed by comparing 27 time series: the shortest series included 10 subsequent collection dates (from June 17th to July 18th), while subsequent series were obtained by adding one collection at time till the end of the sampling (i.e. 36 collections). The temporal variation of the resulting 27 correlation coefficients was then compared between treated and untreated sites. Change point analysis was applied to detect abrupt changes in the mean of either mosquito and water leftover series of correlation coefficients, to estimate the number and location of changes of the mean of each series (see (Zeileis & Kleiber 2005) for further details).

Results

Results obtained on caged mosquitoes exposed to single insecticide treatments and results on the effectiveness of the overall control strategy adopted (i.e. adulticide sprayings and larvicide treatments of street catch basins) on the wild mosquito population are as follows.

Effectiveness of insecticide sprayings on caged *Aedes albopictus* adults.

The average effectiveness of the seven monitored insecticide sprayings assessed based on Henderson's formula applied to caged mosquitoes was 77% (Confidence Interval: 93% - 61%) at 10 m, 36% (CI: 49% - 22%) at 30 m, 22% (CI: 35% - 8%) at 50 m, 1% (CI: 2% - 0%) at 70 m from spraying (Table S1). Restricting the analysis to cages located at ≤50 m distance from spraying (due to low mortality in the 70 m-distant cages), the average effectiveness of the treatments were as follows: T2=20.1%, T3=51.2%, T4=68.6%, T5=37.5%, T6=54.4%, T7=23.5%, T8=53.4%.

Results from the binomial GLMM-1 carried out to test the effectiveness of insecticide spraying on caged adult *Ae. albopictus* either exposed or not-exposed to the adulticide treatments indicated an overall higher mortality in exposed cages (Table 1; p=0.002). No differences in mortality were detected between genders. As expected, permethrin detection was positively associated with mortality (Table S2; p<0.001). However, mortality was observed also in cages where permethrin was not detected (concentration<0.0006 µg/cm2). Tetramethrin values were not taken into consideration for data elaboration as they were below the limit of detection of the analytical procedure.

Table 1. Binomial Generalized Linear Mixed Model of *Aedes albopictus* mortality in cages exposed and non-exposed to insecticide spraying.

GLMM-1 Variables	Coeff.	SE	z-value	Pr(>|z|)
Intercept	-3.642	0.506	-7.197	<0.001
Male	0.221	0.526	0.420	0.674
Perm. conc.	10.298	1.012	10.170	<0.001
Exposed	1.734	0.556	3.134	0.002
Male*Perm. conc.	-1.551	1.228	-1.265	0.206
Male* Exposed	-0.118	0.546	-0.216	0.829

Adult females in control cages set as reference (intercept). Number of observations = 184, groups = 28; treatment date = 7. Estimated random effect standard deviation: location within each treatment date = 0.9, treatment date = 0.08. SE=standard error of parameter estimate; z-value=estimate to standard error ratio; Pr(>|z|)=statistic for z-value.

Moreover, the second binomial GLMM-2 - carried out to assess mortality in cages at distinct distances from the insecticide spraying in the treated site (i.e. 10, 30, 50 and 70 m) - showed lower mortality at increasing distances (Estimated coefficient for Distance=-0.087; Z-value=-18.74; p<0.001).

Figure 2A shows expected adult mortality in treatment site modelled as a function of the distance between the cages and the insecticide spraying, as predicted by GLMM-2. Overall, adult mortality was predicted to be higher than 0.75 in 29% of the area not occupied by buildings, and higher than 0.50 in 41% of the same area (Figure 2B). Expected mortality obtained from GLMM-2 was validated by using mortality values observed in validation cages, located at 13m (VC-1) and 41m (VC-2) from spraying (Figures 2A and 2B). Mortality rates were extremely variable among treatments, ranging from 5 to 100% in VC-1 and from 0 to 80% in VC-2 (Figure 2A). In T7 observed mortality in VC-1 was even lower than in VC-2. Mortality in VC-1 (average observed value=54%, predicted=77%) and in VC-2 (average observed value=29%, predicted=22%) was outside the 0.025 and 0.975 quantile of the expected mortality distribution in 5 and 3 out of 7 monitored treatments, respectively (Figure S1). Specifically, observed mortality was underestimated in 6 out of 8 of these cases (i.e. values<0.025), overestimated in 2 cases (i.e. values>0.975).

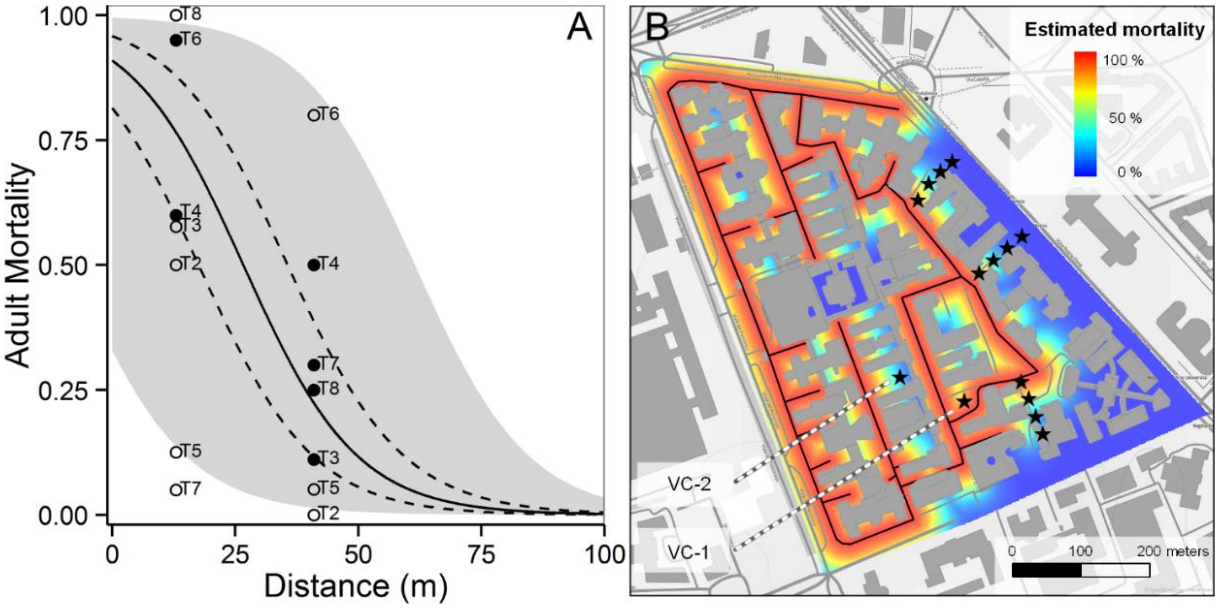

Figure 2. Expected effectiveness of insecticide sprayings in study area based on mortality observed in caged mosquitoes. (A) Expected *Aedes albopictus* adult mortality modelled as a function of the distance between the cages and the insecticide treatments (T2-T8), as predicted by GLMM-2. Central solid line=fitted values determined by the intercept and distance effect (fixed part); dashed lines=95% confidence interval; grey area=uncertainty in predicted values due to variations in random terms (date and cage locations); circles=observed mortality values in validation cages (VC-1 and VC-2, 13m and 41m distant from spraying, respectively), either statistically different (empty circles) or not-statistically different (filled circles) from values simulated by GLMM-2. (B) Spatialized expected mosquito mortality modelled as a function of distance taken from binomial GLMM-2 result (fixed part) (central solid line in panel A). Lines of black stars=mosquito cages at 10, 30, 50 and 70 m from insecticide spraying. VC-1 and VC-2=cages inside treated area used for GLMM-2 validation.

Effectiveness of insecticide sprayings on wild *Aedes albopictus* adults.

Henderson's formula computed for each single insecticide spraying showed a mosquito adult reduction only for 4 out of 8 treatments (i.e. T1=100%, T2=0%; T3=0%; T4=55.5%; T5=57.1%; T6=0%;T7=83.8%; T8=0%; Table S3).

However, the objective of the study was not only to evaluate effectiveness of single adulticide spraying, but to assess the impact of the overall control strategy adopted (i.e. adulticide sprayings and larvicide treatments of street catch basins) taking into account the eco-climatic conditions in the two study sites. In order to achieve this objective, water leftover inside ST was taken as a proxy of the specific eco-climatic conditions at ST level (i.e. association between overall climatic conditions and ST exposure to sun-light). This was based on LMM results showing a negative relationship of water leftover in ST with temperature (LMM-1; Table S4; Figure S2A) and a positive relationship with rainfall (LMM-2; Table S5; Figure S2B).

Table 2. Poisson Generalized Linear Mixed Model of *Aedes albopictus* counts in sticky traps in insecticide treated and untreated sites.

GLMM-3 Variables	Coeff.	SE	z-value	Pr(>|z|)
Intercept	1.358	0.659	2.062	0.0392
Treated	-4.976	0.777	-6.405	<0.0001
Water leftover	-0.400	0.134	-2.986	0.0029
Water leftover* Treated	0.848	0.167	5.091	<0.0001

The reference level is untreated site. Water leftover = water leftover in STs during 72 hours. Number of observation = 1523, number of collections = 36, ST number = 43. Estimated random effect standard deviation: collection = 0.73, ST = 0.42. SE=standard error of parameter estimate; z-value=estimate to standard error ratio; Pr(>|z|)=statistic for z-value.

Afterwards, measures of water leftover were included as explanatory variables in the Poisson GLMM-3 carried out to test how mosquito counts varied between treated and untreated sites. The result showed that mosquito counts were significantly higher in the untreated site (N in treated site=231; N in untreated site=552; p<0.001). However, while in the untreated site higher mosquito counts were observed in ST with lower values of water leftover, unexpectedly no relationship between mosquito counts and water leftover was observed in the treated site (Table 2; Figure 3).

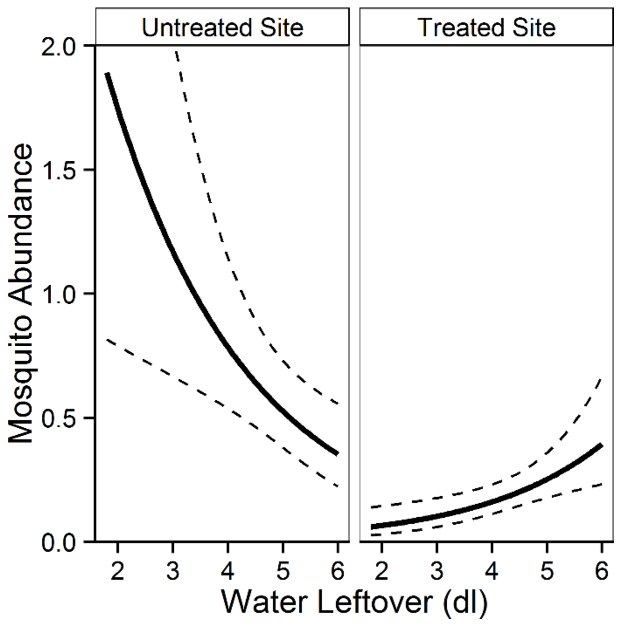

Figure 3. Plots of predicted mean *Aedes albopictus* abundance as a function of water leftover in sticky traps. Predictions in untreated and treated sites based on GLMM-3. X-axis=water leftover after 72 hours (5 dl initial water level; values>5 dl due to rainfall and/or artificial watering); Y-axis=predicted mean abundance in sticky traps; solid lines=predicted mean value; dashed lines=95% confidence intervals.

Finally, change point analysis was carried out to assess temporal variations of the impact of the control strategy adopted on the seasonal mosquito population dynamic (Figure 4A). Results showed a sharp decrease in correlation (Pearson's coefficient from 0.77 to 0.47) between time series of adult mosquito mean counts in the treated and in the untreated site after T3 (collection 15, August 5th; Figure 4B). This change occurred when population in the untreated site was reaching its peak; afterwards, correlation between the two time series remained stable (Figure 4B). Change point analysis was also applied to water leftover between ST-time series in treated and untreated sites to understand whether eco-climatic conditions was a major determinant of differences observed in mosquito abundance between the two sites. Results showed a sharp decrease in correlation coefficients between the two sites at collection 19 (August 19th, after T4). Afterwards, an increase of correlation along the season was observed (Figure 4C and 4D).

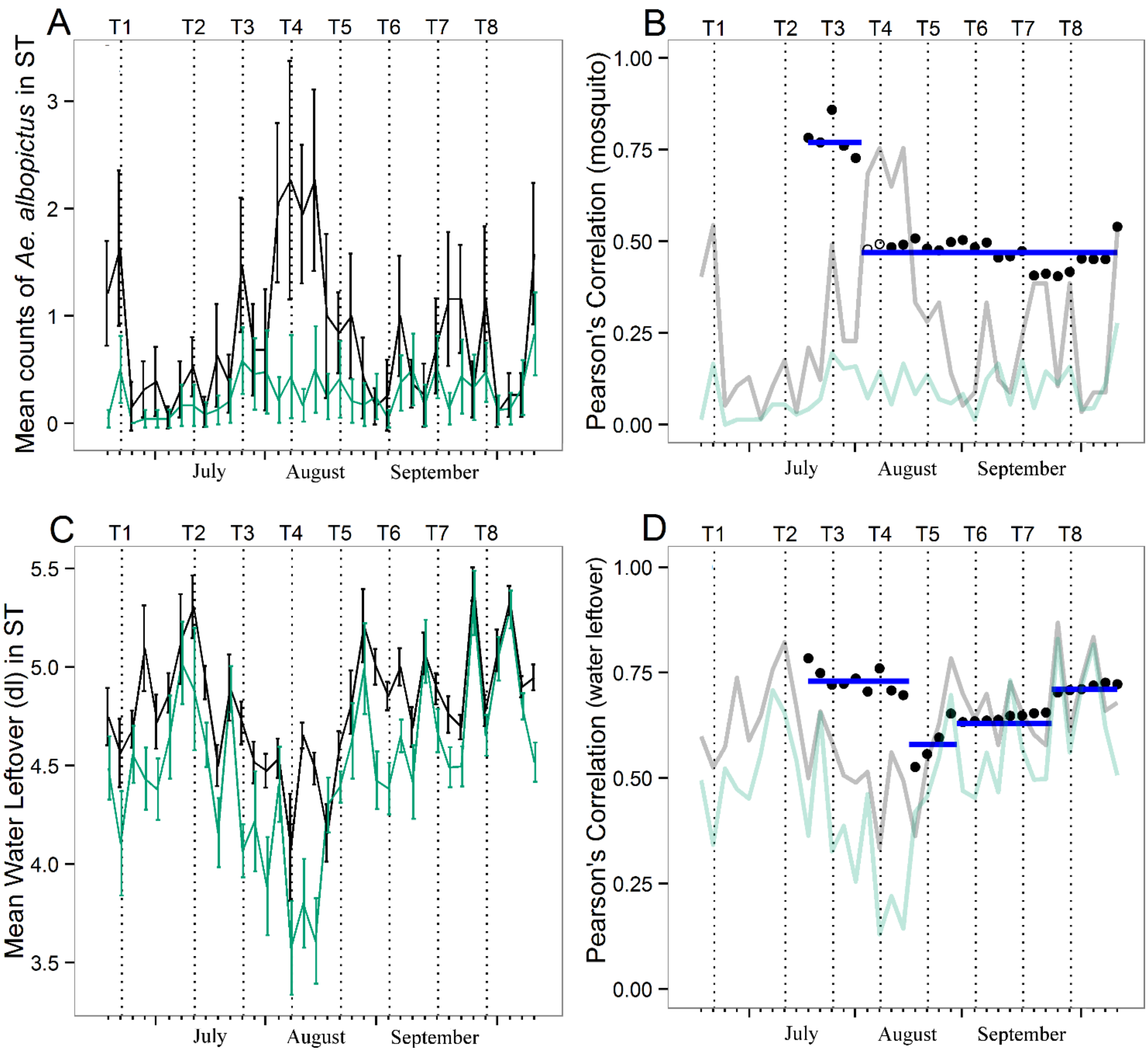

Figure 4. **Change point analysis of *Aedes albopictus* abundance and of water leftover in study sites**. (A) Seasonal pattern of mosquito abundance in insecticide-treated (green line, N=24) and untreated (black line, N=19) sites. (B) Correlation of residual of mosquito time series between treated and untreated sites. (C) Seasonal pattern of water leftover in sticky traps in insecticide-treated (green line, N=24) and untreated (black line, N=19) sites. (D) Correlation of residual of water leftover time series between treated and untreated sites. Filled circle=significant correlation estimate (p-value < 0.05). Empty circle=non-significant correlation estimate (p-value > 0.05). Blue horizontal line in B and D panels=fitted mean in each sequence; each break identifies a statistically

significant change in mean. Vertical bars in A and C panels =95% confidence intervals. X-axis=2013 collection dates. Vertical dotted lines=dates of insecticide sprayings.

Discussion

The results obtained clearly show that a proper detection of the effectiveness of sequential insecticide treatments on *Ae. albopictus* population dynamics can be achieved by coupling an intensive seasonal spatio-temporal monitoring of mosquito population dynamics and eco-climatic variations in treated vs untreated sites with the use of advanced statistical methods. These are necessary to disentangle the effect of the treatments from those of eco-climatic inter-site differences on mosquito population patterns. Thus, the proposed approach may allow to overcome the need to have information on mosquito populations in treated and untreated sites in seasons/years before the effectiveness assessment and the difficulty in attributing inter-site differences in population patterns to the insecticide treatments rather than to site-specific eco-climatic variations. In fact, results of the temporal analysis showed that mosquito seasonal patterns were initially comparable in the two sites, diverged in the absence of diverging eco-climatic conditions and remained stable afterwards. This led us to attribute the lack of *Ae. albopictus* population expansion in the area of the main University hospital in Rome to the combined effect of multiple adulticide sprayings and larvicide treatments regularly carried out during the whole season. In fact, a clear population expansion was observed in August in the untreated control site and is known to typically occur in the same period in Rome (Toma *et al.* 2003; Romi *et al.* 2009). The conclusion would have been very different if we would have speculated on the effectiveness of the treatments only based on Henderson's formula results from caged mosquitoes and/or from field ST-collections before and after single sprayings in treated vs untreated sites. These results were variable and inconsistent. In the case of cage experiments, mortality was found negatively associated to distance from vehicle spraying and positively associated to Permethrin concentration, as expected. However, high variability in mortality was observed among cages within single treatments, as well as among treatments. Based on these results adult mortality was predicted to be higher than 50% only in 41% of the treated area. The high variability observed in results on caged mosquitoes was most likely due to variations in wind direction and/or strength (not measured), as suggested by the variable concentrations of Permethrin detected in cages. In the case of the assessment based on ST-collections of wild mosquitoes after single insecticide sprayings, results showed an adult reduction with respect to the untreated area only after 4 out of 8 treatments. This high variability could be at least partially due to the fact that we did not sample the sites immediately before and after the insecticide spraying (as implied by Henderson's formula), but for 3 days before and 3 days after each treatment, thus introducing the confounding factor of freshly adult emergence. Other factors intrinsic to field experiments may account for the inconsistency between results based on ST-collections and those based on cage experiments: e.g. i) "controls" are affected by the mosquito population dynamics in the field, but not in the cages; ii) mortality in cages is measured immediately after the treatment, thus reflecting the rapid knock-down effect, while assessment of treatment effectiveness in the field is based on ST-collection in the 72h following the treatment, thus reflecting both rapid knock-down and residual effect.

The methodological approach here proposed to assess the effectiveness of seasonal-long mosquito control strategies can be applied to assess the effectiveness of various control methods, under the assumption that the major forces determining mosquito population dynamics are eco-climatic factors. The approach relies on the possibility to compare mosquito population dynamics in treated sites and in untreated control sites by sticky trap collections, even in the absence of previous information on mosquito abundance and eco-climatic situation in these sites. In fact, water leftover in sticky trap was shown to be correlated with temperature (negatively) and rainfall (positively) and can thus be taken as a good proxy of the eco-climatic conditions at sticky trap level, synthetizing the association between overall climatic conditions and sticky trap exposure to sun-light. Notably, water leftover can be easily measured during routine sticky trap monitoring activities without significant additional efforts in term of time and costs. This allowed us to compare with great resolution changes in correlation between time series of adult mosquito mean counts and seasonal changes of eco-climatic conditions in the treated and in the untreated sites and to reach the conclusion that the lack of *Ae. albopictus* population expansion in the treated site was due to the insecticide treatments rather than to eco-climatic factors. In theory, the methodological approach here proposed could be carried out by ovitrap collections, a widely used method to indirectly assess adult abundance. However, complete water evaporation is frequently observed in ovitraps after <3 days in very hot sites/seasons, such as in Rome in August (BC, personal observation), but not in ST which are supplied with a top lid. Moreover, ovitrap exploitation for assessing adult abundance based on number of collected eggs has been questioned (Focks 2004). On the other hand, it should be noted that monitoring is more laborious by ST that by ovitrap, due to the need to manipulate sticky-sheets.

Overall, our results suggest that the combined effect of adulticide sprayings and larvicide treatments carried out in the study site had an effect in reducing *Ae. albopictus* abundance – and likely its nuisance - during the seasonal peak of the species. Larvicide treatments seem to have had a major role in determining the observed lack in the mosquito population expansion, as suggested by the apparent low impact of single adulticide sprayings assessed based on caged and wild mosquitoes. The latter could be due, among other factors, to the execution of sprayings during night time (*when Ae. albopictus* is believed to be less affected due to its diurnal activity), in order to decrease risk associated to human exposure to insecticides. However, it should be mentioned that single night-time ULV adulticiding were shown to result in a significant percent of reduction in *Ae. albopictus* abundance in treated vs. untreated sites in the US (Farajollahi *et al.* 2012; Fonseca *et al.* 2013)

The results of the present work are consistent with preliminary indications on the effectiveness of a combined intervention based on IGR-treatments of catch basins and two insecticide sprayings carried out at the beginning of the major population expansion in Sapienza University campus in Rome (Caputo *et al.* 2015). This may suggest that intervention based on the combination of larvicide and adulticide treatments may have an effect even if sprayings are carried out only during the phase of population expansion, thus allowing to reduce and better focus the use of insecticide ground spraying. Other studies are needed to confirm this hypothesis and to shed light on the relative contribution of larvicide and adulticide treatments.

It is relevant to remind that despite there is an overall agreement that integrated control strategies – mostly based on public education, source reduction and larvicide application, with insecticide spraying restricted to specific situations - are needed to significantly reduce *Ae. albopictus* abundance and associate nuisance (Baldacchino *et al.* 2015), this is very rarely implemented. In fact, an integrated control strategy requires high level of public cooperation among local authorities, private companies, organized society, and communities and a continued support from both local authorities and communities. In practical terms, multiple calendar based adulticide sprayings associated to larvicide activities are offered by private companies to citizens in high *Ae. albopictus* infested areas, at least in Italy. Studies as the present one are thus extremely important to provide information needed to better focus treatments along the species reproductive season (for instance restricting insecticide sprayings to the beginning of the season, as suggested by present results) and definitively assess their actual cost-benefits, also taking into account the environmental impact of adulticide ground spraying.

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Supplementary Material

Table S1. Effectiveness (%) of single insecticide sprayings on mosquitoes in exposed cages based on Henderson's formula.

Positive values indicate a reduction in treated site after adjusting with control site reduction. Row=Road along which cages were located at various distance from insecticide spraying (see Figure 1).

		Distance from insecticide spraying				
Treatment	**Row**	**10 m**	**30 m**	**50 m**	**70 m**	**Total**
T2	1	37.93	1.72	12.07	-3.45	12.07
	2	1.72	12.07	1.72	-3.45	3.02

	3	100.00	1.72	1.72	6.90	27.59
	Total	46.55	5.17	5.17	0.00	14.22
T3	1	94.74	15.79	10.53	-	40.35
	2	100.00	31.58	21.05	-	50.88
	3	100.00	73.68	57.89	-	77.19
	Total	98.25	40.35	29.82	-	56.14
T4	1	100.00	57.45	62.77	-	73.40
	2	100.00	68.09	46.81	-	71.63
	3	100.00	57.45	41.49	-	66.31
	Total	100.00	60.99	50.35	-	70.45
T5	1	1.77	-0.88	9.73	7.08	4.42
	2	94.69	52.21	28.32	1.77	44.25
	3	86.73	81.42	49.56	1.77	54.87
	Total	61.06	44.25	29.20	3.54	34.51
T6	1	94.83	100.00	94.83	1.72	72.84
	2	100.00	12.07	-3.45	1.72	27.59
	3	6.90	12.07	6.90	1.72	6.90
	Total	67.24	41.38	32.76	1.72	35.78
T7	1	-1.79	8.93	8.93	3.57	4.91
	2	100.00	-1.79	-7.14	-1.79	22.32
	3	100.00	35.71	-7.14	-1.79	31.70
	Total	66.07	14.29	-1.79	0.00	19.64
T8	1	100.00	64.10	7.69	-2.56	42.31
	2	100.00	23.08	12.82	-2.56	33.33
	3	100.00	58.97	-2.56	2.56	39.74
	Total	100.00	48.72	5.98	-0.85	38.46

Table S2. Concentration of Permethrin detected in each exposed cage after single insecticide sprayings.

nd = Permethrin concentration under detection threshold (< 0.0006 µg/cm^2); na = no exposed cages available. Row=Road along which cages were located at various distance from insecticide spraying (see Figure 1).

		Distance from insecticide spraying				
Treatment	**Row**	**10 m**	**30 m**	**50 m**	**70 m**	**Total**
T2	1	0.001682827	nd	nd	nd	0.001682827
	2	nd	nd	0.001359008	nd	0.001359008
	3	0.06454743	0.000794418	nd	nd	0.065341848
	Total	0.066230257	0.000794418	0.001359008	nd	0.068383683
T3	1	0.003272307	0.001362227	nd	na	0.004634534
	2	0.085726332	nd	nd	na	0.085726332
	3	0.021510445	0.001600424	0.002191409	na	0.025302278

	Total	0.110509084	0.002962651	0.002191409	na	0.115663144
T4	1	0.002228104	0.001169095	0.001218022	na	0.004615221
	2	0.022383404	0.126931778	0.001490339	na	0.150805521
	3	0.036653958	0.003057286	0.003838829	na	0.043550073
	Total	0.061265466	0.131158159	0.00654719	na	0.198970815
T5	1	nd	0.000733259	nd	nd	0.000733259
	2	0.010346104	nd	nd	nd	0.010346104
	3	0.003798915	0.001121456	nd	nd	0.004920371
	Total	0.014145019	0.001854715	nd	nd	0.015999734
T6	1	0.017361961	0.002483039	nd	0.000653431	0.020498431
	2	0.003509216	nd	nd	nd	0.003509216
	3	nd	nd	nd	nd	nd
	Total	0.020871177	0.002483039	nd	0.000653431	0.024007647
T7	1	nd	nd	nd	nd	nd
	2	0.031839811	nd	nd	nd	0.031839811
	3	0.006144186	0.002258362	nd	nd	0.008402548
	Total	0.037983997	0.002258362	nd	nd	0.040242359
T8	1	0.008507483	0.000885834	nd	nd	0.009393317
	2	0.003855567	nd	nd	nd	0.003855567
	3	nd	nd	nd	nd	nd
	Total	0.01236305	0.000885834	nd	nd	0.013248884

Table S3. Effectiveness (%) of single insecticide sprayings on wild mosquitoes based on Henderson's formula.

Positive Effectiveness values indicate a reduction in treated site after adjusting with control site reduction. Zero percentage values indicate a minor reduction in treated site compared to control site or no reduction post treatment at all. ST = Number of active STs pre/post insecticide treatment.

	Treated site			**Control site**			
Treatment	ST	Pre-treatment Mean (± SE)	Post-treatment Mean (± SE)	ST	Pre-treatment Mean (± SE)	Post-treatment Mean (± SE)	Effectiveness (%)
T1	24/23	0.50 ± 0.16	0.00 ± 0.00	19/19	1.63 ± 0.37	0.16 ± 0.12	100%
T2	24/24	0.17 ± 0.10	0.08 ± 0.06	19/19	0.53 ± 0.14	0.11 ± 0.07	0%
T3	24/24	0.58 ± 0.16	0.46 ± 0.17	19/19	1.47 ± 0.32	0.68 ± 0.22	0%
T4	23/24	0.43 ± 0.20	0.17 ± 0.08	19/19	2.26 ± 0.57	1.95 ± 0.33	55.5%
T5	22/24	0.41 ± 0.18	0.21 ± 0.10	19/19	0.84 ± 0.19	1.00 ± 0.30	57.1%
T6	24/24	0.04 ± 0.04	0.38 ± 0.13	19/19	0.26 ± 0.17	1.00 ± 0.29	0%
T7	21/22	0.52 ± 0.15	0.14 ± 0.07	18/19	0.72 ± 0.23	1.16 ± 0.32	83.8%

T8	19/ 24	0.47 ± 0.14	0.12 ± 0.07	19/ 19	1.16 ± 0.34	0.11 ± 0.07	0%	

Table S4. **Linear Mixed Model of water leftover in sticky traps located in treated and untreated site as a function of temperature.**

The reference level is untreated site. Number of observation = 1523, number of collections = 36, number of trap = 43. Estimated random effect standard deviation: collection = 0.27, trap = 0.18

LMM-1	**Coeff.**	**Std. Error**	**t-value**	**Pr(>|t|)**
Intercept	5.141	0.101	47.685	<0.0001
Treated	-0.149	0.066	-2.252	0.027
Temperature	-0.044	0.012	-3.768	0.0005
Temperature*Treated	-0.020	0.005	-4.616	<0.0001

Table S5. Linear Mixed Model of water leftover in sticky traps located in treated and untreated site as a function of rainfall.

The reference level is untreated site. Number of observation = 1523, number of collections = 36, number of trap = 43. Estimated random effect standard deviation: collection = 0.29, trap = 0.18.

LMM-2	**Coeff.**	**Std. Error**	**t-value**	**Pr(>|t|)**
Intercept	4.725	0.070	67.227	<0.0001
Treated	-0.348	0.058	-5.998	<0.0001
Mm of rain	0.011	0.004	2.994	0.0049
Mm of rain *Treated	0.006	0.001	4.744	<0.0001

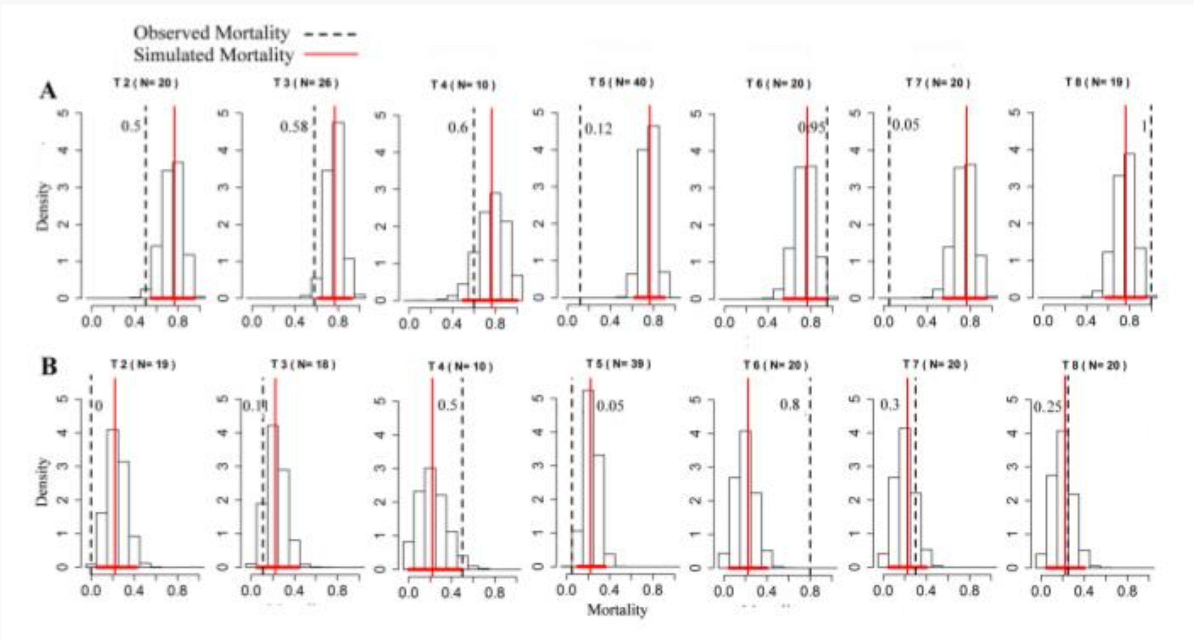

Figure S1. Distribution of expected *Aedes albopictus* mortality in validation cages after adulticide treatments. A=VC-1 (13 m distant from spraying); B=VC-2 (41 m distant). N=number of initial mosquito adults in cages in each treatment (T2-T8). Dashed black line=observed mosquito mortality (values reported in each graph); red vertical line at distribution mean=predicted mortality based on GLMM-2 (VC-1: 77%, VC-2: 22%); red segment at the bottom=95% credible interval. X-axis=mosquito mortality; Y-axis=probability density.

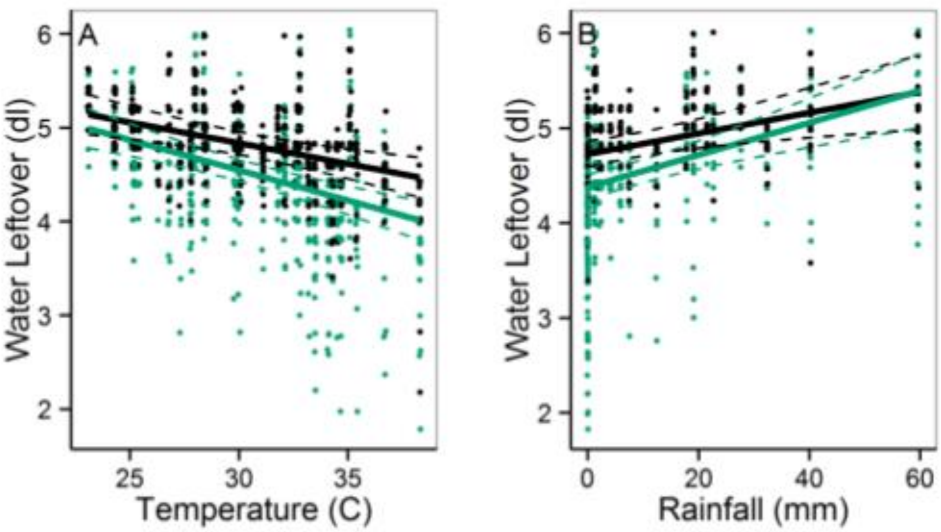

Figure S2. Result of Linear Mixed Model for relationship between water leftover in sticky trap and temperature (A) or rainfall (B) in treated and untreated site.

Initial values of water leftover= 5 dl; values >5 dl are due to rainfall or artificial watering. Lines=predicted mean value of water leftover; dashed line=95% confidence intervals. Green line=treated site; black line=untreated site.

Conclusion

The 2017 CHIKV epidemics in central Italy had definitively demonstrated that the public health burden associated to the stable presence of *Ae. albopictus* in Italy (and Europe) goes far beyond the strong nuisance associated to the species aggressive biting behaviour and could be high and costly (e.g. hospitalization, emergency control activities, suspension of blood donation, …). Therefore, actions have to be made to reduce the hazard. Solid scientific data on the biology, epidemiology and response to control interventions of Italian populations of the species can greatly contribute in making these actions more effective.

During my PhD, I have exploited my background in statistical and mathematical modelling and the long standing entomological expertize of the Medical Entomology group of the DSPMI of Sapienza University to: i) contribute to clarify some relevant information for a better knowledge on the species distribution and temporal dynamics in Lazio region (where the 2017 chikungunya outbreak occurred) (chapter 1); ii) estimate CHIKV importation time, transmission dynamic, magnitude of the outbreak and associated health costs during the 2017 CHIKV outbreak (chapter 2); iii) assess whether the most widely used approach to monitor adult female adult densities (i.e. collections of eggs by ovitraps) allow precise estimations of mosquito-human contact (i.e. the most relevant parameter for epidemiological models) (chapter 3); and iv) assess the effectiveness of insecticide-based control interventions on the mosquito seasonal dynamics and abundance (chapters 4, and 5). At each chapter corresponds a scientific publication.

In Manica et al. (Manica, M., Filipponi, F., D'Alessandro, A., Screti, A., Neteler, M., Rosà, R., Solimini, A., della Torre, A. & Caputo, B. (2016). Spatial and Temporal Hot Spots of *Aedes albopictus* Abundance inside and outside a South European Metropolitan Area. PLoS Neglected Tropical Diseases) we reported the results obtained applying generalized additive mixed models to entomological capture data showing that *Ae. albopictus* is more abundant in strongly urbanized areas compared to more rural ones. Interestingly, at smaller scale inside the metropolitan Rome only, small green areas within the most heavily urbanized settings, such as private gardens or public recreational areas represent host-spots of high adult mosquito densities. These hot-spots should thus be the first target of anti-mosquito activities. The temporal dynamic of the species in Rome area pictured by the data available was shown to follow a bimodal pattern rather than a bell shaped curve, opening new question on the mechanism causing the midseason population drop. Speculatively, the particular meteorological condition of Rome, where high temperature and almost absent rainfall is common during summer months, could decrease the availability of breeding sites then impacting negatively the population growth. On the other hand, the second peak of density observed in October seems to be mostly triggered by the early September rains followed by weeks of novel increase in temperature – a common pattern in Rome in the last decades – raising the issue of the need to continue routine control interventions up to end of October, as well as of the risk of arbovirus transmission even beyond the summer season. These results have been exploited for the "Rapid Risk Assessment: Clusters of autochthonous chikungunya cases in Italy (September 2017)" carried out by the European Centre for Disease Prevention and Control (ECDC).

In Manica et al., (Manica M, Guzzetta G, Poletti P, Filipponi F, Solimini A, Caputo B, della Torre A, Rosà R, Merler S, Transmission dynamics of the ongoing chikungunya outbreak in Central Italy: from coastal areas to the metropolitan city of Rome, summer 2017) accepted for publication in *Eurosurveillance*) we reported the results obtained applying mathematical modelling techniques in order to estimate CHIKV importation time, transmission dynamic, magnitude of the outbreak and associated health costs during the period of the actual virus outbreak in Lazio region in 2017. The results highlight the power of joining entomological data and analytical assessment for the correct forecast and characterization of transmission risk and consequent planning of timely responses. First, the R_0 in Anzio (the first focus of the outbreak) was shown to be lower, but comparable to R_0 associated with the 2007 CHIKV outbreak in Emilia Romagna and other outbreaks worldwide. Second, perhaps counter-intuitively, the highest transmission potential was predicted in coastal and rural areas, as opposed to metropolitan ones (due to the higher mosquito to human ratio compared in less densely populated areas), consistently with the higher incidence of CHIKV observed in Anzio compared with Rome. This may have prevented a major secondary outbreak inside Rome. Third, the model estimated the health and economic burden related to the outbreak, which are instrumental to evaluate cost–benefits of preventive interventions aimed to reduce mosquito vector densities. In fact, availability of information on insecticide treatments carried out after CHIKV notifications would also allow predicting their effect on mosquito population dynamics. Fourth, the model predicted a risk of autochthonous transmission in Lazio region up to mid-November, because of the expected persistence of favourable climatic conditions in the area. Finally, the model estimated the probable introduction time of the index case between May 21st and June 18th 2017, considerably earlier than September 7th when Italian authorities reported through the Early Warning and Response System the presence of autochthonous cases in Anzio.

The above results highlight the need to investigate patterns of human movement in spreading arbovirus disease, both locally and at national or international level, as well as the need to improve the detection/assessment of *Ae. albopictus* population at the local scale and its spatial heterogeneity and to provide abundance threshold to precisely identify risk levels. The results presented in Manica et al. (Manica, M., Rosà, R., della Torre, A. & Caputo, B. (2017). From eggs to bites: do ovitrap data provide reliable estimates of *Aedes albopictus* biting females? PeerJ) deal with the latter aspect, contributing to the debate on the best monitoring tool to assess mosquito presence/abundance and risk of arbovirus transmission, as effort which is challenged by high cost of entomological monitoring and by the intrinsic complexity of the biology of the species (e.g. is highly esophilic behaviour). By carrying out a generalized least square analysis of the relationship between collection of eggs carrier out by ovitraps and collections of adult biting females collected by Human Landing Catches carried out in hot-spots of *Ae. albopictus* abundance in Rome along a whole reproductive season and taking into account meteorological parameters, we found a significant positive relationship between the two sets of data and estimated an increase of one biting female/person every five additional eggs found in ovitraps. However, wide confidence intervals of estimates of biting females based on eggs were observed. The patterns of exotic arbovirus outbreak probability obtained by introducing these estimates in risk models were similar to those based on females/HLC. Moreover, the model predicted that in this case-study scenario an $R_0 > 1$ for CHIKV is also to be expected when few/no eggs/day are collected by ovitraps. Although the large confidence intervals in the model predictions represent a caveat regarding the reliability of monitoring schemes based exclusively on ovitraps, the results obtained provided the first evidence of the possibility to predict mean number of adult biting *Ae. albopictus* females based on mean number of eggs and to compute the threshold of eggs/ovitrap associated to epidemiological risk of arbovirus transmission in the study area.

Since the introduction of *Ae. albopictus* in Italy in 1990 – with the single exceptions of the 2007 CHIKV outbreak in Emilia Romagna – insecticide-based control interventions have been carried out by public authorities or private citizen in order to reduce nuisance due to the species aggressive biting behaviour. During the large 2017 CHIKV outbreak in Lazio these interventions were carried out around resident areas of infected cases as the only method to contain the epidemics, as recommended by E-CDC and ISS guidelines. However, the effectiveness of these interventions has not be assessed and few data are available in this respect. Manica et al. (Manica M, Cobre P, Rosà R, Caputo B (2017) Not in my backyard: effectiveness of outdoor residual spraying from hand-held sprayers against the mosquito *Aedes albopictus* in Rome, Italy. Pest Management Science) and Caputo et al. (Caputo B, Manica M, D'Alessandro A, Bottà G, Filipponi F, Protano C, Vitali M, Rosà R, della Torre A (2016) Assessment of the Effectiveness of a Seasonal-Long Insecticide-Based Control Strategy against *Aedes albopictus* Nuisance in an Urban Area PLoS Neglected Tropical Diseases) deal with the assessment of the effectiveness of insecticide-based control innervations carried out in Rome in the year preceding the outbreaks. We applied a novel methodological approach to monitor the effectiveness of seasonal-long calendar-based control interventions and showed that were effective in reducing the mosquito abundance in the months when highest densities and nuisance are known to occur, although the effectiveness of single adulticide spraying were very variable in space and time. The approach proposed facilitates the assessment of the actual effectiveness of control strategies against mosquitoes, which are very rarely assessed due to technical difficulties, high costs and lack of commitments, but are instrumental to optimize control strategies. Results of these two papers were cited in the reports "Vector control with a focus on *Aedes aegypti and Aedes albopictus* mosquitoes - Literature review and analysis (October 2017)", underlying the relevancy of the subject studied and its importance for public health.

Research on invasive mosquito ecology is an expanding field, where both multidisciplinary approaches and high specializations are needed. The daily endeavour of field technicians and entomologists is essential as well as advanced analytical tools combined with geographic information system technology and statistical and mathematical modelling. This thesis is my humble contribution to the prevention of health threat caused by *Aedes albopictus*.

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