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**DEVELOPMENT AND APPLICATION OF MATHEMATICAL
AND STATISTICAL METHODS TO ESTIMATE RISK OF
MOSQUITO-BORNE DISEASES AND EFFECTIVENESS OF
CONTROL INTERVENTIONS**

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Microbiology XXXIV cycle

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Contents

Abstract -----	3
Introduction -----	4
Objectives of the thesis -----	11
Chapter 1: Estimation of epidemiologically relevant parameters related to the bionomics of malaria vectors by statistical methods: two case studies from Sub-Saharan Africa -----	12
Entomological Survey Confirms Changes in Mosquito Composition and Abundance in Senegal and Reveals Discrepancies among Results by Different Host-Seeking Female Traps-----	18
Is Côte D'Ivoire a new high hybridization zone for the two major malaria vectors, <i>Anopheles coluzzii</i> and <i>An. gambiae</i> (Diptera, Culicidae)? -----	19
Chapter 2: Estimation of epidemiologically relevant parameters related to <i>Aedes albopictus</i> bionomics in Italy from Mark-Release-Recapture data by mathematical and statistical methods. -----	20
<i>Aedes albopictus</i> bionomics data collection by citizen participation on Procida Island, a promising Mediterranean site for the assessment of innovative and community based integrated pest management methods -----	26
Modelling arthropod active dispersal using Partial differential equations: the case of the mosquito <i>Aedes albopictus</i> -----	27
Chapter 3: Estimation of the effectiveness of conventional and innovative mosquito control tools in Italy by Bayesian statistical methods. -----	28
Evaluation of <i>Bacillus Thuringiensis subsp. Israelensis</i> and <i>Bacillus sphaericus</i> combination against <i>Culex pipiens</i> in highly vegetated ditches -----	31
-----	31
Innovative vector control method: <i>Wolbachia</i> -----	32
Material and Methods -----	33
Results-----	35
Supplementary Materials -----	40
Conclusions -----	41
References -----	43
Acknowledgements -----	48

Abstract

Vector-borne diseases represent one of the greatest health problems worldwide, accounting for >17% of all infectious diseases. *Anopheles*, *Aedes* and *Culex* mosquitoes are the insects most involved in the transmission of vector-borne human diseases. Some species of the genus *Anopheles* are responsible for the transmission of the malaria parasite. *Aedes* and *Culex* are instead vectors of human (es. Yellow Fever, Dengue, Zika, Chikungunya) and zoonotic (e.g. West Nile, Japanese encephalitis) arboviruses, and of filarial nematodes. Mosquito-borne diseases are disproportionately transmitted in tropical regions. However, in the last years, they increase their relevance also in temperate regions, previously considered at low risk. This is due to the global invasion of Asian *Aedes* invasive vectors of human arboviruses and to climatic conditions favouring the transmission of zoonotic arboviruses by native *Culex* species.

The goal of medical entomology is to understand and define vector species bionomics under specific eco-climatic conditions in order to build a model useful to predict the patterns of pathogen's transmission, and to develop effective prevention and control interventions and methods to assess their efficacy. The latter are particularly crucial in non-endemic countries where sporadic pathogen's circulation requires assessment based on entomological parameters. Collaboration between medical entomologists, mathematicians and statisticians is instrumental to progress towards these goals. In fact, mathematical and statistical models provide a simplified representation of a complex system, which involves a variety of underlying factors, interactions, heterogeneity, random variations and fluctuations that have an impact on the prediction.

The overarching objective of this thesis was to apply my mathematical background to research questions in the field of medical entomology - with specific reference to mosquito and mosquito-borne diseases - and to complement it with advanced statistical approaches. Under the tutoring of the Medical Entomology group of the Department of Public Health and Infectious Disease of Sapienza University in Rome, I became familiar with the research field of vector ecology and vector-borne disease and acquired data from field studies in Italy and Africa, on which I based my analyses. Under the tutoring by the Applied Ecology at the Research and Innovation Centre of Fondazione Edmund Mach, I exploited my background in mathematical modelling and I learned and applied basic and advanced statistical techniques to model mosquito dynamics and mosquito-borne pathogen transmission. More in detail during the first part of my PhD I learned and applied inferential statistical techniques based on the frequentist approach to analyse field data from two studies on species of the *An. gambiae* complex from Afro-tropical malaria-endemic regions. Afterwards, I exploited my mathematical background to develop advanced mathematical tools based on Partial Differential Equations to estimate epidemiologically relevant parameters (population size, survival rate and dispersal) of *Ae. albopictus* in Italy from Mark-Release-Recapture data. Finally, I enriched my knowledge on statistical inference by studying and applying the Bayesian framework in order to evaluate the effectiveness of traditional and innovative mosquito control tools. Results are summarized in 3 chapters, each of which introduces the specific entomological/epidemiological topic(s) and briefly describes the scientific questions addressed, the experimental field designs, the analytical approaches applied (discussed in the framework of conventional analytical tools) and the results obtained. At the end of each chapter, the scientific articles related to each specific topic are provided, in order to include all details of the individual studies, for a total of 5 published papers, and a manuscript in preparation.

Introduction

Vector-borne diseases (VBDs) comprise illnesses caused by parasites, viruses and bacteria that are transmitted by hematophagous arthropods and represents one of greatest health problem worldwide, accounting for more than 17% of all infectious diseases (WHO, <https://www.who.int/>). Nearly 82 % of the global population lives in areas at risk from one vector-borne disease with >50% living in areas at risk of two or more of the major vector-borne diseases. Most of this burden affects those living in low-income countries where resources for disease control are limited. Populations from sub-Saharan Africa, south Asia, and the Americas are at risk of five or more major vector-borne diseases (Fig. 1).

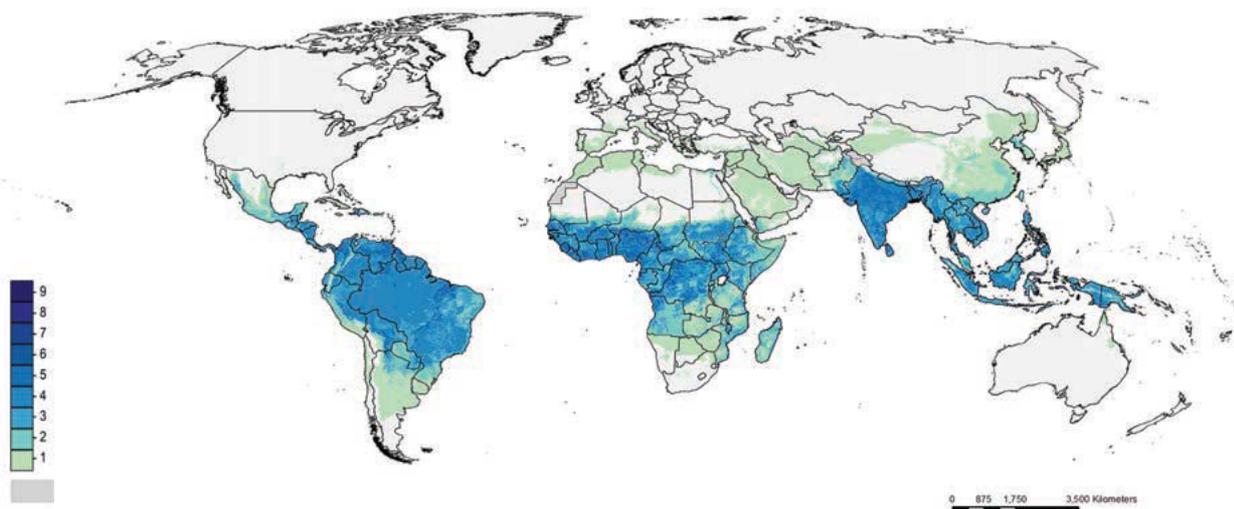


Figure 1. Combined global distribution of seven major vector-borne diseases for which integration of vector control programs may be beneficial: malaria, lymphatic filariasis, leishmaniasis, dengue, Japanese encephalitis, yellow fever, and Chagas disease. Colors indicate the number of vector-borne diseases that pose a risk at each 5×5 km grid cell

Mosquitoes are the insects most involved in the transmission of vector-borne human diseases, to which over 700,000 deaths/year are attributed. Mosquitoes of medical importance belong to the subfamilies Culicinae and Anophelinae (Order Diptera: family Culicidae). The most relevant species from a public health perspective mainly belong to three genera, *Anopheles*, *Aedes* and *Culex*.

Anopheles mosquitoes are vectors of malaria, an acute febrile illness caused by *Plasmodium* parasites. Out of more than 400 *Anopheles* species only 30 are vectors of malaria, mostly leaving in tropical and subtropical areas. According to the Malaria Report by World Health Organization (WHO, 2021), malaria case incidence globally (i.e. cases per 1,000 people at risk) was reduced from 81 in 2000 to 59 in 2015 and 56 in 2019, thanks to a large international effort to fight malaria (WHO, 2021). Over the same temporal window, malaria mortality rate (i.e. deaths per 100,000 people at risk) decreased from about 30 in 2000 to 15 in 2015 and 13 in 2019 (Fig. 2). The main anti -malaria measures of the renewed international effort in the last 20 years are the reduction of human-vector contact using insecticide-impregnated bed nets and indoor insecticide spray. Technological progress has also allowed the production of nets impregnated with long lasting insecticides that remain effective for a long time even after several washes (Long-Lasting Insecticide-treated Nets, LLIN). Globally almost 2.3 billion were distributed from 2004 to 2020 (Malaria Report), of which 2 billion (86%) were supplied in Sub-Saharan region. It is estimated that in 2019 about 43%

of the population at risk was protected during sleep from the bites of *Anopheles* vectors (Malaria Report). Despite the huge effort to implement the strategy, between 2015 and 2020 the implementation of control interventions has no longer corresponded to a further epidemiological improvement, particularly in the WHO African region. In 2020, 627,000 deaths due to malaria were reported (12% more than in 2015), with an estimate of a total of 241 million malaria cases reported from 85 malaria endemic countries, > 95% of which in Sub-Saharan Africa (Fig. 2).

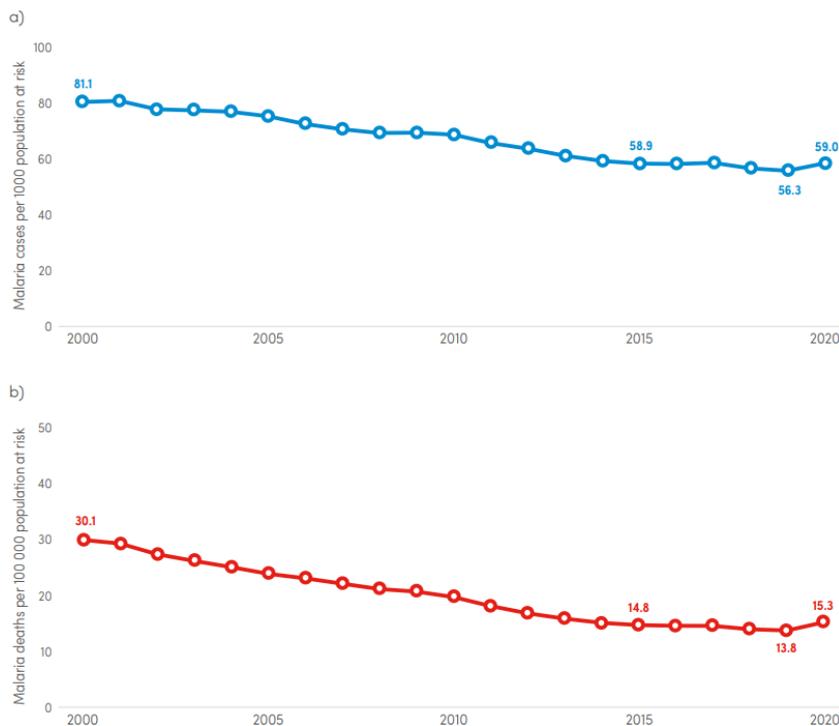


Figure 2. Global trends in a) malaria case incidence (cases per 1000 population at risk) and b) mortality rate (deaths per 100 000 population at risk), 2000–2020; and c) distribution of malaria cases and d) deaths by country, 2020 Source: WHO, 2021.

Aedes mosquitoes are vectors of human arboviruses (e.g., yellow fever, dengue, chikungunya, Zika). After effective control of yellow fever transmission by a worldwide vaccination campaign (WHO, 2017), Dengue remains the most prevalent viral infection transmitted by *Aedes* mosquitoes: >3.9 billion people – mostly from tropical and sub-tropical countries - are at risk of being infected, with an estimated 96 million symptomatic cases and 40,000 deaths every year (Bhatt et al., 2013).

Culex mosquitoes are the most relevant vectors of filarial nematodes (i.e. *Wuchereria bancrofti* and *Brugia malayi*) causing chronic suffering, life-long morbidity, disability in the tropics, and of zoonotic arboviruses (e.g. West-Nile – WNV - St. Louis encephalitis, Eastern and Western equine encephalitis, Japanese encephalitis viruses) causing encephalitis to humans and animals worldwide.

In Europe, malaria was endemic in the first half of the last century but has been eliminated from the region after the second world (Italy was declared “malaria-free” from WHO in 1970). In the same period, importation of infected *Aedes aegypti* (the main Afrotropical arbovirus vector) has caused epidemics of yellow-fever and dengue viruses in Mediterranean port cities, but these did not repeat in the second part of the century, leading to almost negligible risk of mosquito-borne disease in Europe in the last 60-70 years. In the last decades, however, risk of mosquito-borne transmission of human and zoonotic pathogens in temperate regions has changed as a consequence of human-made environmental changes (e.g. international travel and trades and climate changes) which increased the transmission of endemic viruses (mainly of WNV by *Culex pipiens*) and triggered the invasion by Asian *Aedes* invasive species (such as the Asian tiger mosquito, *Aedes albopictus*, a species competent for transmission of several arbovirus such as Dengue, Chikungunya and Zika (Di Luca et al., 2001; Gratz, 2004).

The first record of *Ae. albopictus* in Europe dates to 1979, in Albania (Adhami et al., 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) (Fig. 3).

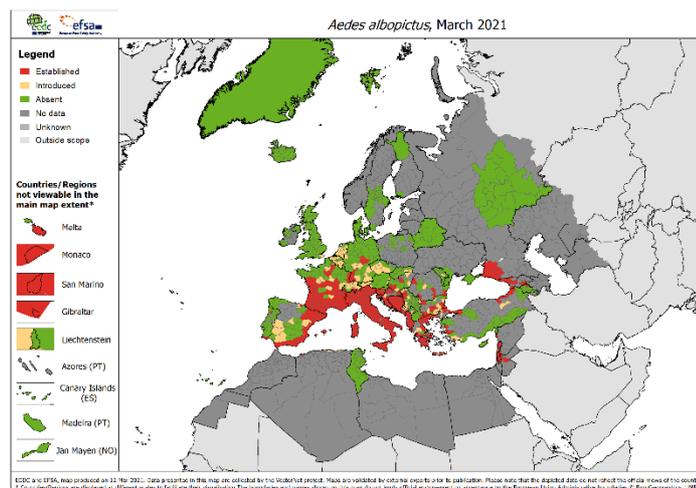


Figure 3. Map of the current known distribution of *Aedes albopictus* in Europe. The map shows the current known distribution of *Aedes albopictus* in Europe at ‘regional’ administrative level, as of March 2021 (ECDC & EFSA, 2021).

In Europe *Ae. albopictus* has been already involved in the transmission of Chikungunya virus (CHIKV) (Caputo et al., 2020), imported by travelers infected in endemic regions. However, several autochthonous transmissions of Dengue were reported in France (La Ruche et al., 2010) Croatia (Gjenero-Margan et al., 2011) and Italy (Lazzarini et al., 2020).

When the environmental conditions are favorable, in areas where *Ae. albopictus* is well established, viremic travel-related cases may generate an autochthonous transmission of the virus, as demonstrated by sporadic events of CHIKV transmission since 2007 in South France (Grandadam et al., 2011) and two relevant outbreaks in Italy, with >200 infected human cases in Emilia-Romagna (North-East Italy) in 2007 (Angelini et al., 2007) and ~500 cases in Lazio (Central Italy) and Calabria (South Italy) 10 years later (Venturi et al., 2017) (Table 1). No events of autochthonous transmission were reported in EU/EEA since 2007. After the chikungunya outbreaks in the Indian Ocean in 2005–2006 and in Italy in 2007, ECDC established a network for arthropod vector surveillance for human

public health to improve the surveillance of mosquito competent vectors, and published guidelines for the surveillance of *Aedes* invasive species and areas at risk for arbovirus outbreaks, based on the current distribution of *Ae. albopictus* in mainland Europe and *Ae. aegypti* in Madeira (European Centre for Disease Prevention and Control, 2017 hereafter ECDC)

YEAR	Country	Number of Autochthonous cases	Period of circulation	Origin of the primary travel-related case	Virus	Presence E1-A226V
2007	Italy, region of Emilia Romagna	~330 suspected, probable and confirmed	July-September	India	CHIKV ECSA	Yes
2010	France, Var department, Frejus	2	September	India	CHIKV ECSA	No
2014	France, Hérault department, Montpellier	12	September-October	Cameroon	CHIKV ECSA	Yes
2017	France, Var department, Le Cannettes-Maures and Taradeau	17	July-September	Central Africa	CHIKV ECSA	Yes
2017	Italy, Lazio Region (Anzio, Latina, Roma) and Calabria region (Guardavalle marina)	270 confirmed and 229 probable	August-November	Asia (India/Pakistan)	CHIKV ECSA belonging to a branch of Indian Ocean Lineage reported from Indian subcontinent	No

Table 1. Events of chikungunya virus transmission by *Ae. albopictus* in Europe since 2007 (revised by ECDC, 2020a). The table combines information published in official reports and in the scientific literature plus information that was provided by the public health institutes and/or the ministries of health in the affected Member States. CHIKV ECSA: Chikungunya virus East/Central/South African lineage. E1-A226V: CHIKV envelope protein 1 with alanine to valine substitution at position 226

Within Europe, *Cx pipiens* is the most widely recognized urban mosquito species; it has also been reported in almost all continents and countries around the world (Knight et al., 1977). *Culex pipiens* is present in almost of European states, such as France, Italy, Greece, United Kingdom and Spain (Fig. 4)

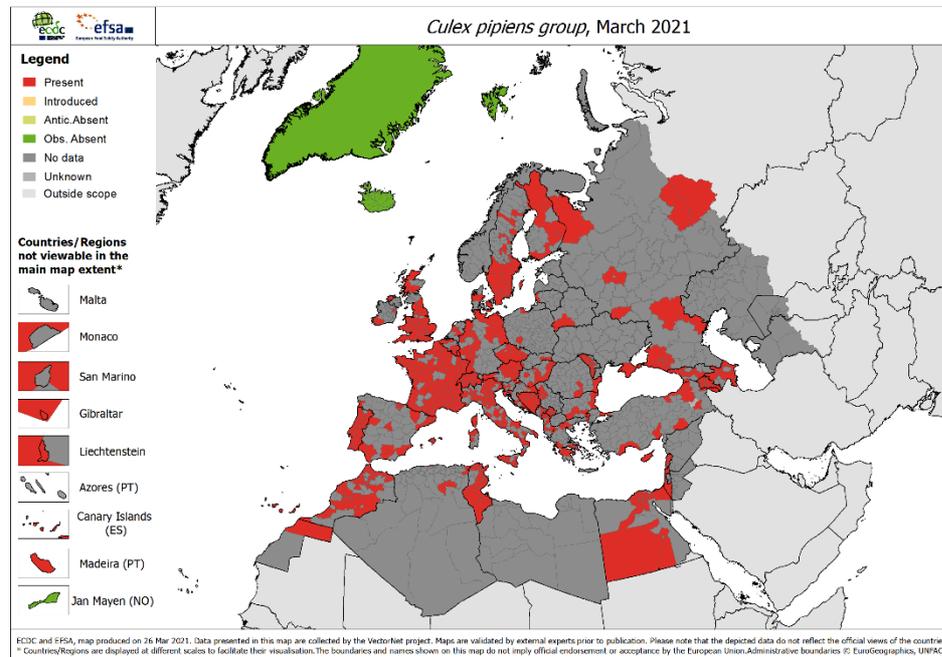


Figure 4. Map of the current known distribution of *Culex pipiens* in Europe. The map shows the current known distribution of *Culex pipiens* in Europe at ‘regional’ administrative level, as of March 2021 (ECDC & EFSA, 2021).

Cx. pipiens have been shown to be critical for the maintenance and transmission of some arboviruses, such as WNV (Brugman et al., 2018) endemo-epidemic in countries across southern, eastern, and western Europe. This arbovirus is usually transmitted among birds via the bite of infected mosquitoes, while humans and other mammals (e.g., horses) may become infected accidentally. A sharp increase in locally acquired WNV infections had been reported in the EU/EEA for 2018 (n=1 549), exceeding by far the total number for the previous four years (ECDC, 2021). In 2019, WNV infections decreased, ECDC reported 443 WNV infections, 425 (96%) of which were locally acquired. Most locally acquired infections were reported by Greece and Italy, representing 65% and 13% of EU cases, respectively (Table 2). Non-EU/EEA Member States did not report any locally acquired WNV infections. For 2019, Cyprus reported 23 locally acquired WNV infections, after previously having reported only one WNV infection in 2016 and 2018, respectively. During 2019, Slovakia and Germany reported the first mosquito-borne locally acquired WNV infections. For 2019, the EU/EEA notification rate for locally acquired infections was 0.1 cases per 100 000 people, compared to 0.3 for 2018.

Country	2015		2016		2017		2018		2019	
	Number	Rate	Number	Rate	Number	Rate	Number	Rate	Number	Rate
Austria	6	0,1	5	0,1	6	0,1	21	0,2	4	0
Belgium	0	0	0	0	0	0	0	0	0	0
Bulgaria	2	0	2	0	1	0	15	0,2	5	0,1
Croatia	1	0	2	0	5	0,1	58	1,4	0	0
Cyprus	0	0	1	0	0	0	1	0,1	23	2,6
Czechia	0	0	0	0	0	0	5	0	1	0
Denmark	-	-	-	0	-	-	-	-	-	-
Estonia	0	0	0	0	0	0	0	0	0	0
Finland	0	0	0	0	0	0	0	0	0	0
France	1	0	0	0	2	0	27	0	2	0
Germany	-	-	-	0	-	-	1	0	5	0
Greece	0	0	0	0	48	0,4	315	2,9	227	2,1
Hungary	18	0,2	44	0,4	20	0,2	215	2,2	36	0,4
Iceland	-	-	-	-	0	0	0	0	0	0
Ireland	0	0	0	0	0	0	0	0	0	0
Italy	61	0,1	76	0,1	53	0,1	610	1	54	0,1
Latvia	0	0	0	0	0	0	0	0	0	0
Liechtenstein	-	-	-	-	-	-	-	-	-	-
Lithuania	0	0	0	0	0	0	0	0	0	0
Luxembourg	0	0	0	0	0	0	0	0	0	0
Malta	0	0	0	0	0	0	0	0	0	0
Netherlands	0	0	0	0	0	0	0	0	0	0
Norway	0	0	0	0	0	0	0	0	0	0
Poland	0	0	0	0	0	0	0	0	0	0
Portugal	1	0	0	0	0	0	0	0	0	0
Romania	32	0,2	93	0,5	66	0,3	227	1,4	67	0,3
Slovakia	0	0	0	0	0	0	0	0	1	0
Slovenia	0	0	0	0	0	0	4	0,2	0	0
Spain	0	0	3	0	0	0	0	0	0	0
Sweden	0	0	0	0	0	0	0	0	0	0
United Kingdom	0	0	0	0	0	0	0	0	0	0
EU/EEA	122	0	226	0,1	201	0,1	1549	0,3	425	0,1

Table 2. Distribution of locally-acquired West Nile virus infection cases and rates per 100 000 population by country and year, EU/EEA, 2015–2019

The dramatic situation of malaria in Africa and the new epidemiological scenario of mosquito-borne diseases in Europe claim for additional efforts and tools, which need to be based on a better knowledge of the bionomics, distribution, evolution and genomics of mosquito species, and of improved estimates of the transmission intensity/risk and of the effectiveness of vector control interventions. The complexity involved in the transmission dynamics of mosquito-borne disease, distribution and genomics of mosquitoes urgently demands a collaboration among the experts in epidemiology and medical entomology and scientists that have other skills to obtain an interdisciplinary approach. One of the most crucial interdisciplinary approaches needed is the collaboration between vector biologists, epidemiologists, mathematicians, and statisticians. In essence, mathematical, and statistical models provide a simplified representation of a complex system, which involves a variety of underlying factors, interactions, heterogeneity, random variations, and fluctuations that have an impact on the predictions. Models allow to improve the understanding of how a certain pathogen spreads and how to reduce disease incidence (Brauer et al., 2015; Keeling et al., 2008) and to predict epidemiologically relevant entomological parameters, the levels transmission of mosquito-borne disease and the effectiveness of vector control. For their development, multiple techniques (Reiner et al., 2013) and variables have been historically used, including climatological, environmental, entomological, demographic and socioeconomic data (Aswi et al., 2019; Johansson et al., 2019; Manica et al., 2016; Massaro et al., 2019). The results obtained, have shown the potential of such models to highlight key factors for public health interventions and to guide public policy, thus helping allocate human and economic resources more efficiently for the fight against mosquito-borne pathogens (Norris et al., 2018).

Objectives of the thesis

The overarching objective of this thesis was to apply my mathematical background to research questions in the field of medical entomology - with specific reference to mosquito and mosquito-borne diseases - and to complement it with advanced statistical approaches, under the tutoring of the Applied Ecology at the Research and Innovation Centre of Fondazione Edmund Mach (S. Michele All 'Adige, Trento) – with a specific expertise in the application of advanced statistical and mathematical model of vector and vector-borne disease as well as statistical methods for the assessment of surveillance and control strategies- while acquiring a deep knowledge of the research field, thanks to the tutoring of the Medical Entomology group of the Department of Public Health and Infectious Disease of Sapienza University in Rome (MedEntSap) - with a longstanding experience on the bionomics, genomics and vectorial role of Afrotropical malaria vectors and on the bionomics and monitoring of autochthonous and invasive mosquito vectors in Italy.

More in detail, the specific thesis' objectives were:

1. The estimation of epidemiologically relevant parameters related to the bionomics of malaria vectors (host-seeking behaviour, seasonal dynamic and malaria transmission intensity) in two case studies in Sub-Saharan Africa (in Senegal and in Ivory Coast) by statistical methods (Chapter 1).
2. The estimation of epidemiologically relevant parameters related to *Aedes albopictus* bionomics (survival rate, population size and dispersal) from Mark-Release-Recapture data in two sites in Italy (Procida Island and Padua Province) by mathematical and statistical methods (Chapter 2).
3. The estimation of the effectiveness of mosquito control tools in Italy - i.e. larvicides against *Culex pipiens* in rural sites in Padua Province, and of a novel non-chemical approach to reduce *Ae. albopictus* fertility in a study site in Rome - by Bayesian statistical methods (Chapter 3).

Each of the 3 chapters introduces the specific entomological/epidemiological topic(s) and briefly describes the scientific questions addressed, the experimental field designs, the analytical approaches applied (discussed in the framework of conventional analytical tools) and the results obtained. At the end of each chapter, the scientific articles related to each specific topic are provided, in order to include all details of the individual studies, for a total of 5 papers published or already accepted for publication, and a summary of the methodological approaches and results of the second study mentioned in Chapter 3.

Chapter 1: Estimation of epidemiologically relevant parameters related to the bionomics of malaria vectors by statistical methods: two case studies from Sub-Saharan Africa

Mathematical and statistical models are useful tools to better understand the dynamic of epidemics, to evaluate epidemic control interventions and to determine efficient combinations of these interventions. The first exploitation of mathematical modelling in the field of epidemiology is the “vectorial capacity” model developed for malaria by Ross more than a century ago (Ross, 1911) and extended by McDonald in the 1950s and 1960s (Macdonald, 1957; Smith et al., 2012). The vectorial capacity formula is based on a quantitative description of the mosquito and pathogen (*Plasmodium falciparum*) life cycle. The parameters are shown in Figure 1 and are summarized in Table 1. The concept defined by McDonald’ formula was later generalized to all infectious diseases with the definition of the “basic reproductive number R_0 ” defined as the number of secondary cases per case in a naïve population. R_0 measures the success of the establishment of a pathogen and provides an index of transmission intensity, establishing threshold criteria. If the value of R_0 is greater than >1 , an outbreak of the infectious agent is possible, whereas if R_0 is less than <1 , the infection is going to die out (Diekmann et al., 2000).

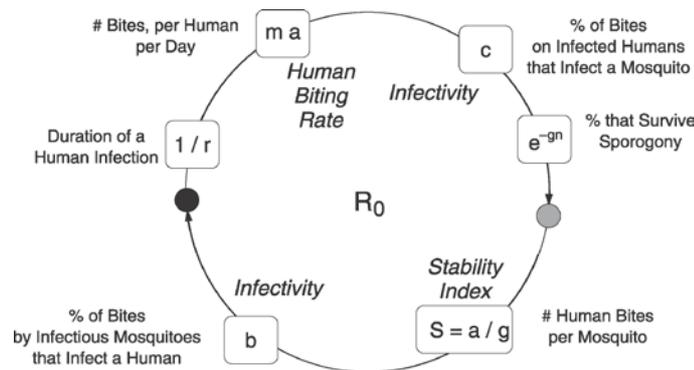


Figure 1. The Life Cycle Model and R_0 (figure modified from Smith et al., 2007)

Parameters	Description
a	Human feeding rate: the number of bites on a human, per mosquito, per day
b	Infectivity of mosquitoes to humans: the probability that a human becomes infected from a bite by an infectious mosquito.
c	Infectivity of humans to mosquitoes: the probability that a mosquito becomes infected from a bite on an infected human.
g	Death rate of mosquito
m	Number of mosquitoes per human.
n	Number of days required for a mosquito to complete sporogony
1/r	Expected waiting time to naturally clear a simple infection.

Table 1. Parameters included in The Life Cycle Model and R_0 .

In McDonald's formula which over time has been improved and integrated, R_0 is computed as the product between the number of infectious mosquitoes generated from an infectious human (R_0^{HV}) and the number of infectious humans generated by the infectious mosquitoes surviving the extrinsic incubation period (R_0^{VH}), as follows (Smith et al., 2012)

$$R_0 = R_0^{VH} * R_0^{HV} = \frac{a}{g} * b * \frac{1}{r} c * ma * e^{-gn} \quad [1]$$

The value $ma * e^{-gn}$ is called Entomological Inoculation Rate (EIR) and it is an indication of malaria transmission intensity, i.e. the average number of infectious bites received by a person in given time frame. At the computational level it is possible to calculate EIR multiplying ma (human biting rates i.e. the number of mosquito bites per person per day) and *Plasmodium* species sporozoite infection rate (the fraction of infectious mosquitoes, i.e., with sporozoites in their salivary glands).

In this chapter is presented is the application of statistical models (i.e. Generalized linear model, Generalized Additive Model) to predict host-seeking behaviour, seasonal dynamic of main malaria vector species (*An. gambiae*, *An. coluzzii* and *An. arabiensis*) and analyse the malaria transmission intensity (estimating *P. falciparum* sporozoite rate, hereafter PfSR, and *P. falciparum* Entomological Inoculation Rate, hereafter PfeIR) in two malaria endemic countries in West Africa (Senegal and Ivory Coast). The results have been published in two scientific articles (Ngom & Virgillito et al., (2021); Caputo et al., 2022) embedded at the end of the chapter.

In both studies, mosquito collections were carried out using host-seeking adult traps (i.e. CDC-light traps and BG-sentinel traps). Species identification and sporozoite detection was carried out by PCR and Real time PCR, respectively, using as template DNA extracted from head and thorax of single specimens (to exclude mosquito carriers of oocyst and ookinete in abdomen). Collections were carried out in 10 randomly selected houses in a coastal and in an inland village, by two traps of the same type located in each house, the first in a human sleeping room (indoor trap) and the second in the house courtyard (outdoor trap) (in Ivory coast only CDC-light traps were located inside and outside the dwellings). The traps were located nearby a sleeping person under a bed net to protect the volunteers from being bitten (Fig. 2, left Senegal, right Ivory coast). When sample sizes were appropriate, PfeIR was estimated from trap collections using a mean mosquito per traps per night (m/t/n), as proxy of mean mosquitoes per person per night (m/p/n), as suggested in Kilama et al., (2014). In the following paragraphs, the main results of two studies are discussed with particular reference to PfeIR and to the level of malaria transmission with respect to vector control intervention, eco climatic conditions and human malaria parasitaemia.

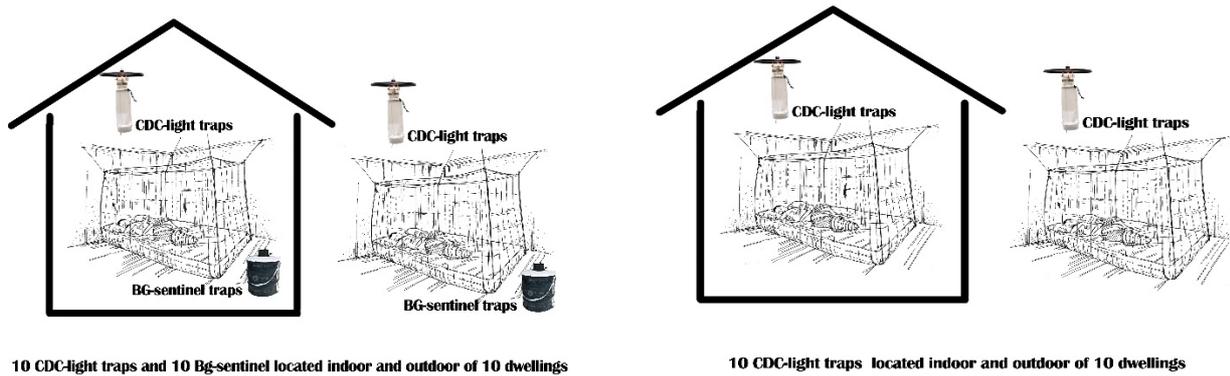


Figure 2. Host-seeking mosquitoes collections in Senegal (Left) and Ivory coast (Right).

In Ngom & Virgillito et al., (2021) host-seeking mosquitoes collections were carried out in 3 sampling periods (one during rainy season, one in the late rainy season and one during dry season) for 4 consecutive nights each, in a coastal and in an inland Senegalese village, at 300 km distance from each other. GLMs have been carried out to predict mosquito vector's host-seeking behaviour and temporal dynamics, as well to assess differences in the performance of the two type of host-seeking traps considering several explanatory variables (i.e. position of traps, period of year of collections, CDC-light and BG traps) in a single model. This assessment would have not been possible by applying classical statistical methods (chi-squared test, anova, Fisher-test) that are commonly used in the entomological studies. In the GLM model, interaction terms was also considered to test possible interaction effects among independent variables. Significant interaction effects indicate that a third independent variable influences the relationship between another independent and the dependent variable. This type of effect makes the model more complex, but more reliable and realistic. In addition, GLM allows to quantify the goodness of model fit (i.e., residuals, R^2 , confidence intervals). The results show that the two traps depict a different patterns of species abundance, temporal dynamics, and host-seeking activity, raising concern on their indiscriminate use for the assessment of these epidemiologically relevant parameters. Moreover, results show low malaria vector abundance, with a peak depicted during the rainy season in both villages (Figure 2 in the manuscript).

Here we describe the statistical method used to estimate abundance of malaria vectors and results are discussed with reference to malaria transmission level indicated in Malaria Operational Plan FY 2019 of Senegal (hereafter MOP) and Plan Strategique National de Lutte Contre le Paludisme Au Senegal, 2016-2020 (hereafter PNLP) reports. Poisson and/or Negative Binomial distribution are usually applied to analyse aggregated count data obtained from monitoring experiments. We choose to apply, a Negative Binomial distribution in the calculation of “m/p/n” by GLM to account for the variance of response variable larger than the mean (this phenomenon is called overdispersion). The equations used to estimate “m/p/n” from host-seeking trap data are:

$$Y_{i,j} \sim NB(\mu_{i,j}, \vartheta)$$

$$E(Y) = \mu_{i,j} \quad VAR(Y) = \mu_{i,j} + \frac{\mu_{i,j}^2}{\vartheta}$$

$$\log(\mu_{i,j}) = \alpha + Traps_i * \beta + \varepsilon \quad \text{whit } \varepsilon \sim Norm(0, \sigma^2)$$

Where $Y_{i,j}$ is the response (or dependent) variable, in our case the number of mosquitoes collected in each trap i at time j . $Traps_i$ is the independent variable defined as categorical variable with two levels representing the trap methods (i =CDC light traps, BG-sentinel trap). The unexplained variation is captured by the residuals ε , and these are assumed to be normally distributed with mean 0 and variance σ^2 , α is the intercept (that it is represented by BG traps) and β is the slope of statistical model.

Although low sample size did not allow to calculate reliable and unbiased PfSR, we used PfSR values from MOP (PfSR: coastal village=0.009, inland village=0.02) and the estimated average of *An. gambiae* s.l. females/person/night (coastal village=0.96; 95% CI 0.60-1.5, inland village=0.55; 95% CI 0.34-0.90), to calculate the annual mean PfeIR values in the two villages (2.92 and 3.65, respectively). Despite a higher value of m/p/n found in the coastal village, the annual mean of PfeIR is higher in inland, due to a higher sporozoite rate. According to MOP, highest infection rates and PfeIR were found in the southeast of Senegal where the inland village is settled. Moreover, we had the opportunity to investigate the abundance of *An. gambiae* at monthly level and our predictions are in agreement with malaria transmission seasonality in the two settings. According to MOP the country is divided in two epidemiological zones: the tropical zone in the south and southeast, with a year-round transmission peaking during the rainy season (we estimate a m/p/n of ~1.97) and lower transmission during the rest of the year (we estimated a m/p/n of <1.); and the Sahelian zone in the north, with higher transmission toward the end of the rainy season and very low transmission during the rest of the year. A low value of PfeIR found here is consistent to those reported in MOP, has disseminated long-lasting insecticidal-treated bed nets (hereafter LLINs) in the whole population, reaching a coverage of at least 80% of the population sleeping under the bed-net and at least 90% of the population is protected with Indoor Residual Spraying (hereafter IRS). Recent scientific papers (Sougoufara et al., 2016, 2014) as well as reports (MOP, PNLP) have demonstrated that these, vector control interventions: LLINs-IRS, accurate diagnosis and prompt treatment with artemisinin-based combination therapies have contributed to the decrease of malaria transmission.

In Caputo et al., (2022) host-seeking mosquitoes collections were carried out by CDC-light traps in 3 samplings periods (one during the late rainy, one in early rainy season, and one in dry season) for 4 consecutive nights each, in a coastal and in an inland village in Ivory Coast at 250 km distance from each other. GAMs and GLMs have been applied to predict: (i) the relationship between the relative frequency of *An. coluzzii* and *An. gambiae ss* and the relative frequency of hybrid genotypes; (ii) *An. coluzzii*, *An. gambiae ss* and hybrid genotypes indoor/outdoor and the seasonal dynamics; (iii) the average number *An. gambiae sl* (and its members)/ person/ night; iv) PfSR for *An. gambiae sl* and its members; (v) PFEIR for *An. gambiae sl* and its members.

In order to study the first point a GAM model was developed, the same approach already applied in Pombi et al., (2017). In fact, GAMs are able to estimate non-linear relationships, through smoother functions, between the response variable (in our case the prevalence of hybrids) and the predictors (frequency of parental taxa).

The second point was investigated using GLMMs (for the same reasons above highlighted). Specifically, tree statistical model were developed separately for each members of the *An. gambiae* complex, in this case we incorporated a random structure to account for the individual variability of each house of collection. The main results show that prevalence of hybrids between *An. coluzzii* and *An. gambiae* is frequency dependent both at a spatial (as previously reported in Pombi et al., (2017)) and at temporal scale. Moreover, no differences between hybrid and parental taxa in host-seeking behaviour and temporal dynamic was found, with peak of abundance during rainy season in the coastal village and late rainy season in the inland one.

Given the large number of sample collected, we had the opportunity to estimate both the parameters inside the equation of PFEIR. To explore the third point, as in Ngom & Virgillito et al., (2021), we estimated “m/p/n” using counts of *An. gambiae s.l.* (and its members). The approach conducted gave us the possibility not only to estimate *An. gambiae sl* PfSR, but also those of *An. gambiae ss*, *An. coluzzii* and for the first time those of hybrids, separately. The year-round collections permit us to estimate also PfSR along the different sample period, revealing large seasonality on malaria transmission. The model applied to estimate “m/p/n” was in this case a Generalized Linear Mixed Models (GLMMs), in which we incorporated a random structure to account for the individual variability of each house of collection. In this case, we used a Negative Binomial distribution as the response variable (see previous paragraph).

To model presence or absence of *Plasmodium* infection in malaria vectors we choose Generalized Linear Mixed Models with Binomial distribution. Precisely, two separated models were fitted for *An. gambiae sl* and its members: a GLMM model considering as covariates the villages, the months of collection and their interaction; a GLMM considering as covariates the species, the village, and their interactions. A random effect structure was included in both models to account for the individual variability among sampled houses.

As shown in Table 1 in Caputo *et al.*, (2022), estimated value of PfSR for *An. gambiae sl* is higher in the coastal area than the inland village. The large dimension of sample permit us to estimate PfSR for each month collections, and the result shows that PfSR ranging from 13.6 % in early rainy season (95% CI 8.1-22.0) to 4.1% in late rainy season (95% CI 0.06-24.3) in the coastal village, and from 16 % in early rainy season (95% CI 5.8-36.8) to 2% (95% CI 0.07-5.5) in late rainy season in the inland village. On the other hand, no significant differences in m/p/n/ (0.8-1.3) and PfSR (6-9%) was

observed between hybrids and the two species. Overall, PfSR were in the range of those reported by Adja et al., (2011), Zogo et al., (2019), Zoh et al., (2020)

Finally, both average estimates derived from GLMM models of m/p/n and PfSR were used to compute PfEIR and compared them to literature data (Malaria Operational Plan FY 2019 of Ivory Coast ;2019; Zogo et al., 2019; Zoh et al., 2018). In *An. gambiae* sl we found lower average annual PfEIR (coastal= 27,74 and inland=10,95 infected bites per person per year) compared to values found in another study carried out in Korhogo area located in northern Ivory Coast (PfEIR = 897,9 data Zogo et al., 2019; infected bites per person per year). In addition, for inland village we obtained lower average annual PfEIR for *An. coluzzi* (10,95) and *An. gambiae* ss (18,25) compared to values found in a previous study carried out in the same village (219 and 186 ib/p/yr for *An. coluzzi* and *An. gambiae* ss respectively Zoh et al., 2020).

The lower PfEIR estimations obtained in this study could be due to samples biases (for instance in our case estimates are based on CDC light traps and in the literature derived from Human Landing Collections). As hypothesized by Kilama et al., (2014) the estimation of PfEIR should be carried out not only using CDC- light trap but also using other collection methods in order to approximate the values derived from HLC. However, it is important to point out that also HLC is just an approximation of the biting rates, as humans usually do not expose themselves to mosquito bites without any protection and this could lead to an overestimation of the biting rates.

Based on our results about PfEIR and PfSR, low risk of malaria transmission occurs both in the coastal and in the inland villages. The apparent decrease in risk of malaria transmission compared to previous data is consistent to the recent distribution of LLINs conducted in 2017-2018 (MOP) in both regions.

Entomological Survey Confirms Changes in Mosquito Composition and Abundance in Senegal and Reveals Discrepancies among Results by Different Host-Seeking Female Traps

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Article

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Simple Summary: Mosquito-borne diseases such as malaria, arbovirolosis and lymphatic filariasis are major public health issues, particularly in Africa. In order to predict the risk of transmission of these diseases and plan optimal mosquito control interventions, it is vital to have updated information of the mosquito species present, as each of them has a different capacity to transmit different pathogens, and to monitor how these species vary over time, also in relation to environmental and climatic changes. This is achieved by entomological monitoring carried out by various types of traps, whose collection efficacy may vary depending on the mosquito species and the ecological and climatic situation. We carried out collections in two villages in Senegal and showed evidence of a decline of malaria vector species and an increase of lymphatic filarial vectors. Moreover, we showed that using different traps to collect female mosquitoes may provide different estimates of mosquito species composition over time, depending on geographical setting and season. This is very relevant for a correct planning of mosquito monitoring and for appropriate interpretation of the results.

Abstract: Mosquitoes-borne diseases are major public health issues particularly in Africa. Vector control interventions and human-made environmental/climatic changes significantly affect the distribution and abundance of vector species. We carried out an entomological survey targeting host-seeking mosquitos in two different ecological contexts—coastal and inland—in Senegal, by CDC-light and BG-sentinel traps. Results show high predominance of *Culex quinquefasciatus* (90%) and of *Anopheles arabiensis* within malaria vectors (46%), with mean numbers of females/trap/nights =8 and <1, respectively, reinforcing previous evidence of changes in species composition and abundance, highlighting thus increasing risk of transmission of filariasis and emerging arboviruses in the Senegambia region. From the methodological perspective, results show a higher specificity of BG traps for *Cx. quinquefasciatus* and of CDC traps for *An. gambiae* s.l. and highlight that, despite both traps target the host-seeking fraction of the population, they provide different patterns of species abundance, temporal dynamics and host-seeking activity, leading to possible misinterpretation of the species bionomics. This draws attention to the need of taking into account trapping performance, in order to provide realistic quantification of the number of mosquitoes per units of space and time,

the crucial parameter for evaluating vector–human contact, and estimating risk of pathogen transmission.

Keywords: *Culex quinquefasciatus*; *Anopheles gambiae* complex; *Anopheles arabiensis*; *Anopheles coluzzii*; CDC-light traps; BG-sentinel traps

1. Introduction

Mosquito-borne diseases such as malaria, arbovirosis and lymphatic filariasis are major public health issues particularly in Africa. Vector control interventions, as well as human made environmental and climatic changes, are significantly affecting the distribution and abundance of vector species [1]. In the Senegambia region, at the western extreme of west Africa, massive implementation of long-lasting insecticidal bed nets (LLINs) and indoor residual spraying (IRS) has been associated with: (i) a shift in malaria vector species [2,3]; (ii) a decline of mosquito biting rates [3]; (iii) a rapid evolution of insecticide resistance [4]; (iv) an increase of outdoor biting behavior of the local vectors [2]; (v) a high rate of hybridization between two main malaria vector species, *Anopheles coluzzii* and *An. gambiae* s.s. [5] possibly leading to a novel hybrid form resilient against introgression of medically-important loci and traits as hypothesized by Vicente et al. [6]. Moreover, the exponential increase of human population and a consequent increase in deforestation and rapid desertification as occurring in Senegal [7] are favoring species more adapted to polluted and arid environments, such as *Culex quinquefasciatus* among *Culicinae* [8], and *An. arabiensis* among the members of *An. gambiae* complex [9,10].

The careful monitoring of these major changes in mosquito vector populations is crucial to understand disease transmission and to plan/optimize anti-vector measures. Notably, the assessment and prediction of key entomological parameters (such as species abundance and dynamics, and host-seeking activity) relies on entomological monitoring which may be carried out by different trap devices.

The most widespread method to collect host-seeking mosquito females is the CDC-light trap, originally designed to collect *Anopheles* mosquitoes attracted by light [11,12] and later shown to be also effective for collecting *Culex* species [13]. A few studies highlighted that CDC-light trap is very efficient in collecting malaria vectors when located indoors close to a person sleeping under a net, while the performance of the trap outdoors is poorer [14,15]. Recently, CDC-light traps have been proposed as a reasonable alternative to human landing catches (HLC) for estimating *Anopheles* biting rates indoors [16].

Other traps have been developed in order to collect host-seeking females by releasing an artificial odor blend simulating that produced by a human host [17]. The BG-sentinel trap was initially designed to attract *Aedes albopictus* in temperate areas, as well as *Aedes aegypti* in tropical areas [18,19] and was later applied to collect host-seeking malaria vectors, as well as *Cx. quinquefasciatus* and other *Culicinae* species [20–22].

Few studies have been carried out to compare the performance of CDC and BG traps indoors and outdoors. In Burkina Faso, BG and CDC baited with the same chemical lure were compared, and CDC were shown to collect significantly higher numbers of *An. gambiae* s.l. females indoors, while the opposite occurred outdoors [22]. In Brazil, BG-malaria traps (BG with an upward airflow) were shown to be more efficient than CDC-traps in collecting *An. darlingi* outdoors [20].

We here present the results of an entomological survey carried out in two different ecological contexts—coastal and inland—in Senegal, and confirmed evidence of changes in species abundance and composition in the region. Results also highlight significant differences in the performance of CDC-light and BG-sentinel traps in collecting *Cx. quinquefasciatus* and *An. gambiae* s.l. (and its member species) females and in depicting their sea-

sonal dynamics, raising concern on the use of entomological data to predict mosquito densities and evaluate vector–human contact necessary to feed epidemiological models and estimate the risk of pathogen transmission.

2. Materials and Methods

2.1. Study Areas

Mosquito collections were carried out in two sampling sites in Senegal about 300 km distant from each other. Madina Djikoye (13°38′59.55″ N, 16°19′36.52″ W; hereafter referred to as coastal village) is a rural village within the so call Peanut Basin, immediately north of the Gambian border and about 25 km from the coast. The ground is sandy, and the original wooded savannah has been almost entirely cleared by firewood, bushfires, drought or deforestation for agricultural activities. The climate is influenced by vicinity to the coast; highest temperatures are observed in April–May (40 °C) and October (35 °C), and lowest temperatures (<10 °C) in December–January. Two distinct seasons are present: a 7–8 month-long dry season (from November to May–June) and a 4–5 month-short rainy season (from June–July to October). The village is situated at 12 km distance from the field research station of Dielmo, which in 1990 was selected for a (still ongoing) longitudinal survey to investigate the determinants of malaria transmission, because of its high malaria prevalence [23,24]. Djinkore Mafing (13°42′23.35″ N, 13°39′14.9″ W; hereafter referred to as inland village) is located in Tambacounda region, in south-east Senegal, approximately 300 km from the coast. The region consists of land cover characterized by sandstone plateaus of the continental sedimentary basin with savannah woodlands, areas of agricultural parkland, and thin sections of gallery forest near river and bed streams. The region's climate ranges between Sudan-Sahelian and Sudan-Guinean with an overall annual rainfall of 500 mm. The east of Senegal is one of the warmest regions of the country during the dry season. During the rainy season, the temperature decreases significantly due to land surface cooling associated with larger precipitation. The climate is characterized by two seasons: a 4–5 month-long rainy season (from May–June to October, with a peak in August–September, when rainfall can reach up to 200 mm) and a dry season, from November to May [10,25]. The main breeding sites around the villages are represented by rainwater puddles, well dug, riversides, borrow pits. Septic tanks often not sealed are also present in both villages.

2.2. Mosquito Sampling and Processing

Host-seeking *Culicidae* collections were carried out during 3 samplings (September—hereafter, the rainy season, October—hereafter, the late rainy season, and November/December (2018)—hereafter, the dry season) for 4 consecutive nights each (from 6.00 p.m. until 7.00 a.m.) in 9 and 10 randomly selected houses in the coastal and inland village, respectively. During each sampling night, collections within each house either were carried out by CDC-light traps (John W. Hock Ltd., Gainesville, FL, USA; hereafter CDCs) or by BG-sentinel traps baited with BG lure (without CO₂) (BioGents, Regensburg, Germany; hereafter BGs), two traps of the same type were located in each house, one in a human sleeping room (indoor trap) and the second in the house courtyard (outdoor trap). In both cases, the traps were located close to a person sleeping under a bed-net. CDC and BG traps were rotated daily among houses in order to avoid sampling biases. Ethical approval for the study was granted by Ministère de la Santé et de l'Action Sociale (Comité National d'Ethique pour la Recherche en Santé, N0000049 MSA/DPRS/CNERS, Dakar, Senegal, 27 July 2018).

Anophelinae and *Culicinae* mosquitoes were identified at species level using morphological taxonomic keys [26]. *Anophelinae* samples were labeled and stored for molecular analyses in Eppendorf tubes containing silica gel desiccant.

Genomic DNA of *An. gambiae* complex specimens was extracted from single mosquitoes (legs or head + thorax) following [27]. Extracted DNA was then used as template for the PCR-based species identification [28].

2.3. Statistical Methods

Species diversity was calculated for each trap type in the two villages by Simpson's Index:

$$D = \frac{\sum_{i=1}^S ni (ni - 1)}{N (N - 1)} \quad (1)$$

where S = number of *Culicinae* or *Anophelinae* in the area, ni = number of individuals in species- i and n = total number of *Culicinae* or *Anophelinae* in the area. The Simpson's index gives the probability without replacement that two individuals taken at random from a sample are of the same species. It ranges between 0 (greater diversity) and 1 (lower diversity).

To evaluate the performance of the two trap types, we assessed by regression analysis the relationship between abundance of either *Cx. quinquefasciatus* or *An. gambiae* s.l. females and the following covariates: trap type (CDC and BG traps), trapping location (indoor vs outdoor), and month of collections (from rainy to dry season). We used the number of *Cx. quinquefasciatus* or *An. gambiae* s.l./trap/night as response variable and assumed that it followed a negative binomial distribution (using a Poisson distribution resulted in overdispersion). Initially, we included in the full model the three covariates and all their interactions to check whether the sampling is influenced by trapping location and months of collections. We tested both generalized linear models (GLM-1) and generalized linear mixed effect models (GLMMs-1) approach by considering houses as random effect. We decided whether to include or not the random effect by comparing the models by the Akaike information criteria (AIC). Then, after selecting the appropriate random structure we performed variable selections by fitting all possible sub-models and ranking them by AICc. Finally, we discuss in the result section, the best parsimonious model among the subset of models having $\Delta AICc < 4$. The model was fitted for each village separately.

The above described model without random effects was also used to estimate the mean number of mosquitoes/person/night in each village (assuming that this corresponds to the number of collected mosquitoes/trap/night [16]) considering only trap type as covariate (GLM-2).

For *An. gambiae* s.l., we computed the relative frequencies of *An. arabiensis*, *An. gambiae* s.s., *An. coluzzii*, hybrids and *An. melas* in each village and in each month of collection. We analyzed the performance of trap type, in terms of the probability of detection of *An. arabiensis*, *An. gambiae* s.s. and *An. coluzzii* females, as a function of period of collections and trapping location, assuming that the response variable (i.e., presence/absence of mosquitoes/trap/night) followed a Bernoulli distribution. We tested both generalized linear models (GLM-3) and generalized linear mixed models (GLMM-3) approach by considering house as a random effect. The same selection procedure described above was applied to select and discuss the best parsimonious model.

We used R statistical software version 3.6.3 [29] and lme4, MuMIn, MASS packages for all statistical analysis. To avoid convergence problems, the maximum number of iterations of the glmer.nb function in the lme4 package was increased to 10,000.

3. Results

3.1. Species Composition and Descriptive Statistics

A total of 4192 (3792 females and 400 males) *Culicidae* were collected by CDC (1557 females, 338 males) and BG (2235 females; 62 males) traps in 24 sampling nights carried out in the coastal and inland village from rainy to dry season 2018 (Tables S1 and S2).

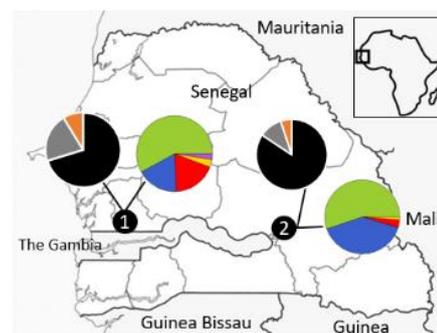
Overall, *Culicinae* largely prevails (90%) over *Anophelinae* in both villages (Figure 1, left panel).

As expected in case of nocturnal collections, *Culicinae* are represented by *Culex* (87% and 95% in the coastal and inland, respectively), *Aedes* (7% and 5%) and *Mansonia* (5% and 0.1%). Highest *Culicinae* species diversity is observed in BG collections (coastal village: D-BG = 0.18, D-CDC = 0.69; inland village: D-BG = 0.17, D-CDC = 0.39). *Cx. quinquefasciatus* is the most abundant *Culicinae* species in both villages (80% and 88% in the coast and inland, respectively). Other species found at frequencies >1% are *Culex nebulosus* (6%), *Cx. policilipes* (1.4%), *Ae vexans* (5%) and *Mansonia uniformis* (4%) in the coastal village, and *Ae vexans* (4%), *Cx tritaeniorhynchus* (1%) and *Ae aegypti* (1.5%) and *Cx tigris* (1%) in the inland village (Tables S1 and S2).

A sharp temporal dynamic of *Cx. quinquefasciatus* is observed in the inland village where 9% of the specimens were collected in the rainy season and 53% in the dry season, but not in the coastal one (28% in the rainy season and 37% in the dry season).

Anopheles gambiae s.l. represents 83% and 96% of the total *Anophelinae* captured in the coastal and inland village, respectively. Few individuals of *An. funestus* and other less public health relevant anopheline species (i.e., *An. ziemanni*, *An. rufipes*, *An. domicola*, *An. nili*) are also found. As opposed to *Culicinae*, the highest *Anopheline* species diversity is observed in CDC-collections (coastal village: D-BG = 0.64, D-CDC = 0.42; inland village: D-BG = 0.25, D-CDC = 0.19). Among the 207 *An. gambiae* s.l. females successfully genotyped out of the 214 collected (Table S3; Figure 1), *An. arabiensis* represents 55% and 52% of the coastal and inland collected samples, respectively. *Anopheles coluzzii* and *An. gambiae* are both found at frequencies around 20% in the coastal village, while the latter species largely prevails (42%) in the inland one. *Anopheles gambiae/coluzzii* hybrids represent 3% and 1% of the total samples collected in the coastal and inland village, respectively. *Anopheles melas* is found only in the coast (3%), as expected due to its salt-water habitat preferences. Relative frequencies of *An. gambiae* s.l. species in the four collection periods are shown in Figure 1 (right panel).

Given the strong prevalence of *Cx. quinquefasciatus* among *Culicinae* and *An. gambiae* s.l. among *Anophelinae*, as well as their relevant role as vectors of filarial worms and malaria in Senegal respectively, comparisons of trap performances were carried out for these species only.



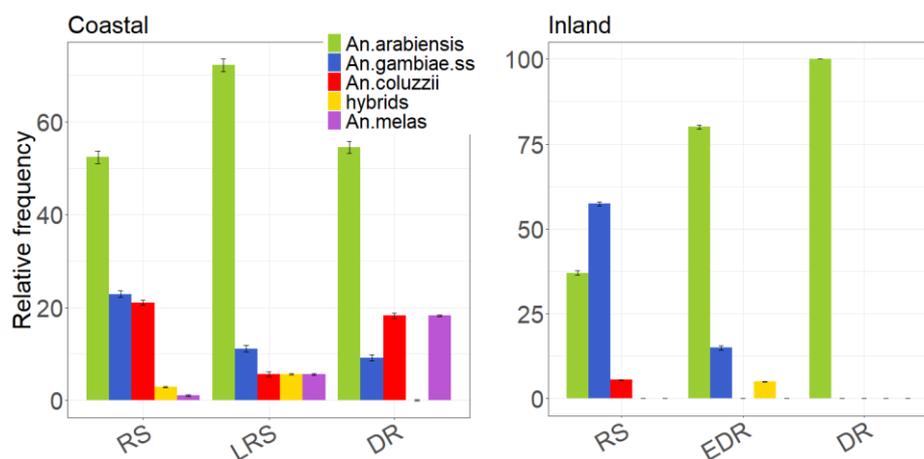


Figure 1. Relative frequency of *Culicidae* species in a coastal and in an inland village of Senegal (pies) and of Anopheline species in the four collection periods (histograms). Upper panel: *Culex quinquefasciatus* ($n = 2899$; black), Other *Culicinae* species ($n = 624$; grey), Anopheline ($n = 266$; orange). *Anopheles arabiensis* (green) *Anopheles coluzzii* (red), *An. gambiae* (blue) their hybrids (yellow) and *An. melas* (purple). Coastal village: RS (Rainy Season; 1–4 September 2018), LRS (Late Rainy Season; 10–13 October 2018), DS (Dry Season; 29–30 November–1–2 December 2018). Inland village: RS (Rainy Season; 27–30 September 2018), EDS (Early Dry Season; 4–7 November 2018), DS (Dry Season; 17–20 November 2018).

3.2. Performance of CDC and BG Traps in Collecting *Culex quinquefasciatus* Females as a Function of Month of Collection and Trapping Location

BG traps collected 57% and 86% of the overall *Cx. quinquefasciatus* sample in the coastal ($n = 1493$) and inland village ($n = 1406$), respectively. Within the BG-trap overall sample, 57% and 77% of the females were collected outdoors, respectively, while the opposite trend is observed for CDC-traps (44% and 47%, respectively).

Results of model selection show lower AIC for GLMM-1 (coastal village: AIC = 1186, inland village AIC = 1119) than for GLM-1 (coastal village AIC = 1194, inland village = 1128), indicating the need to include house of collections as random effect.

In the coastal village, the covariate selection indicates that the mean abundance of *Cx. quinquefasciatus* females correlates with trap type, period of collection and with the interactions between trap type and period of collection, but not with the indoor/outdoor location of the traps (Table S4). The interaction between trap type and period of collections is statistically significant, indicating that CDC and BG traps depict a different temporal pattern, i.e., the mean predicted abundance per night decreases from rainy to dry season when estimated based on CDC collections, but increases when estimated based on BG ones (Figure 2). Overall, the BG performance is higher for all periods of collection (except in rainy season) compared to CDC.

In the inland village, the mean abundance of *Cx. quinquefasciatus* host-seeking females correlates with all variables and with the interactions between trap type and period of collection and between trap type and trapping location (Table S5). The interaction between trap type and period of collection is statistically significant and follows the same patterns observed in the coastal village (Figure 2). Moreover, the interaction between trap type and trapping location is statistically significant, i.e., higher values are predicted for BGs located outdoors vs indoors, while the opposite is observed for CDC. A summary of GLMM-1 models results in both village was reported in Table S6.

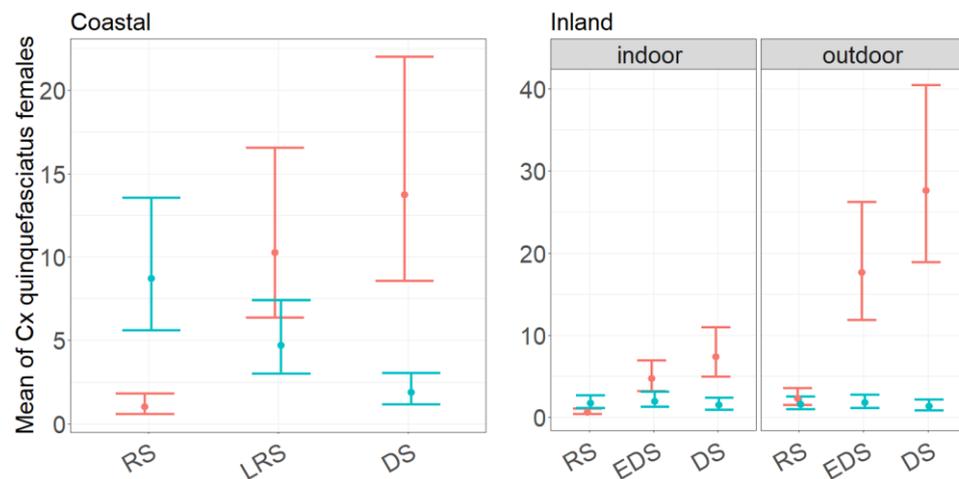


Figure 2. Mean estimated abundance of *Culex quinquefasciatus* host-seeking females/night in a coastal (Left) and inland (Right) village in Senegal in 2018, as predicted by GLMM-1 model. Red and blue lines = predicted regression lines from models fit on data collected in BG and CDC, dots = mean estimated abundance of *Cx. quinquefasciatus* females. Vertical lines = 95% confidence intervals. Coastal village: RS (Rainy Season; 1–4 September 2018), LRS (Late Rainy Season; 10–13 October 2018), DS (Dry Season; 29–30 November–1–2 December 2018). Inland village: RS (Rainy Season; 27–30 September 2018), EDS (Early Dry Season; 4–7 November 2018), DS (Dry Season; 17–20 November 2018).

3.3. Performance of CDC and BG Traps in Collecting *Anopheles gambiae* s.l. Females as Function of Month of Collection and Trapping Location

CDC traps collected 96% of the overall *An. gambiae* s.l. sample in the coastal village ($n = 150$) and 79% in the inland one ($n = 82$). Within CDC coastal and inland village samples, 59% and 81% of the females were collected indoors, respectively, while BG collections show an opposite trend (34% and 42%, respectively).

Results of the model selection applied to GLM-1 and GLMM-1, developed to test the performance of the two trap types to collect *An. gambiae* s.l. host-seeking females as function of period of collection and trap type, show no effect in including houses of collections as random effect (coastal village: AIC GLM-1 = 397, AIC GLMM-1 = 398; inland village AIC GLM-1 = 301, AIC GLMM-1 = 303).

In the coastal village, the covariate selection indicates that the mean abundance of *An. gambiae* s.l. host-seeking females per night correlates with trap type and period of collection, but not with the indoor/outdoor location of the traps (GLM-1, Table S7). No interaction among variables is observed. Mean abundance of *An. gambiae* s.l. is significantly higher in CDC-collections (Figure 3) and in rainy season, independently from the trap type (Figure 3).

In the inland village, covariate selection indicates that the mean *An. gambiae* s.l. abundance correlates with all variables and includes the interaction between trap type and period of collection, as well as trap type and trapping location (Table S8). The significance of the interaction of trap type and period of collection highlights a peak of abundance in rainy season and a decrease afterwards in the case of CDC collections, while no statistical difference is observed among months of collection for BGs (p -value in early dry season = 0.19; p -value in dry season = 0.69; Table S8; Figure 3). In addition, CDC depicts higher abundances indoors rather than outdoors during the whole sampling period (p -value = 0.027), while no statistical difference is observed between indoor and outdoor BG collections (p -value = 0.52) (Table S8). A summary of GLM-1 models results in both village was reported in Table S9.

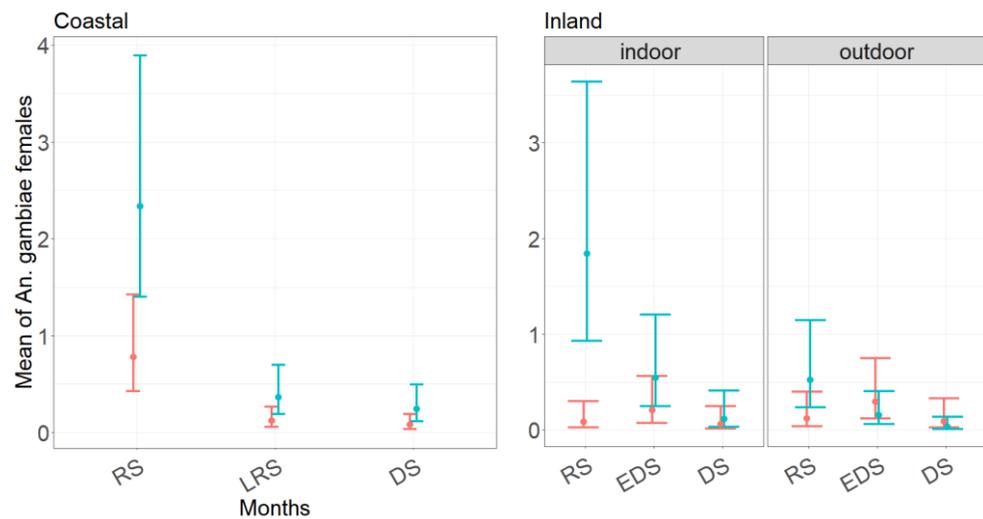


Figure 3. Mean estimated abundance of *Anopheles gambiae* s.l. host-seeking females/night in a coastal (Left) and inland (Right) village in Senegal in 2018, as predicted by GLMM-1 model. Dots = mean of *An. gambiae* s.l. females. Red and blue lines = predicted regression lines from models fit on data collected in BG and CDC, respectively. Lines = 95% confidence intervals. Coastal village: RS (Rainy Season; 1–4 September 2018), LRS (Late Rainy Season; 10–13 October 2018), DS (Dry Season; 29–30 November–1–2 December 2018). Inland village: RS (Rainy Season; 27–30 September 2018), EDS (Early Dry Season; 4–7 November 2018), DS (Dry Season; 17–20 November 2018).

3.4. Performance of CDC and BG in Assessing the Probability to Detect Members of *Anopheles gambiae* Complex

Due to low number of *An. gambiae* s.l. collected despite the relevant sampling effort, we modelled the probability of detection of *An. arabiensis* (AR), *An. gambiae* (GA) and *An. coluzzii* (CO) (the latter one only in coastal village, due to low sample size in inland one) host-seeking females in each village, rather than their mean abundance. Results of the model selection show a lower AIC for GLM-3 (coastal village: AR = 208, GA = 108, CO = 113; inland village: AR = 165, S = 100) than for GLMM-3 (coastal village AR = 210, GA = 110, CO = 111; inland village: AR = 166, CO = 102), indicating no need to include house of collections as random effect.

In the coastal village, results of covariate selection procedure indicate that the only statistically significant covariate to explain the probability to find a specimen is the month of collection (Table S10). A reduction of the predicted probability to collect the three species is observed from the first to the last sampling period (Figure 4).

In the inland village, results of covariate selection procedure indicate that the probability to find a specimen depends instead on trap type only (Table S11), i.e., a higher probability to collect *An. arabiensis* and *An. gambiae* with CDC than with BG traps. The odds to collect *An. arabiensis* and *An. gambiae* through CDC traps is 2.6 (95% CI 1.13–6.63) and 5.1 (95% CI: 1.63–22.78) times higher compared to BG traps, respectively (Figure 4).

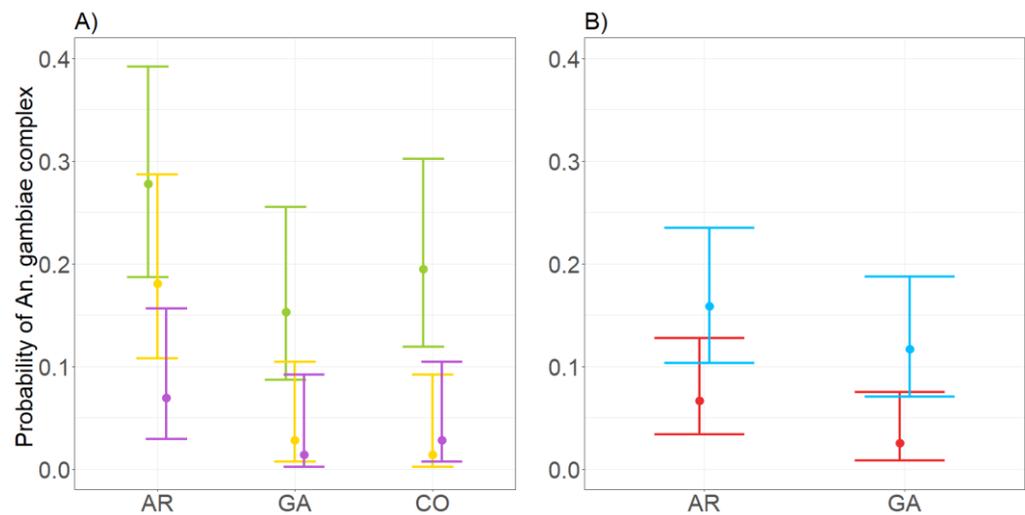


Figure 4. Probability to collect *Anopheles gambiae* complex species in coastal (A) and inland (B) village in Senegal as modelled by GLM-3. Left panel (A): green (September), yellow (October), and purple (November–December) dots = probability to collect *Anopheles gambiae* complex species. Right panel (B): blue dots = probability to collect *An. arabiensis* and *An. gambiae* with CDC-light. Red dots = probability to collect *An. arabiensis* and *An. gambiae* with BG-sentinel traps. Lines = 95% confidence intervals. AR = *An. arabiensis*, GA = *An. gambiae* s.s., CO = *An. coluzzii*.

4. Discussion

4.1. Performance of CDC and BG Traps in Collecting *Culex quinquefasciatus* Host-Seeking Females

BG-traps were found to trap larger *Culicinae* species diversity than CDC-traps and to be significantly more performant in collecting the most abundant species, *Cx. quinquefasciatus*, (except in the collection carried out in the coastal village in rainy season, when the species abundance was lowest). A slightly higher species diversity indices for BG trap was also reported in a comparison among four trapping devices (including BG and CDC) carried out in Germany [30]. Consistent results on relative trap performance were found in a study comparing BG-traps baited with octenol and CO₂ with CDC-traps baited with CO₂ in a 3 × 3 Latin-square experiment in the Samoan Islands [31]. An opposite result was instead obtained when using our same approach in China [32], suggesting that trap relative performance may be affected by eco-climatic conditions and/or by genetic traits of the target populations, as well as use of different attractants.

When exploiting the data to predict the number of collected females in relation to different variables, the trap type variable was always retained in the statistical models by the variable selection procedure and always interacted with the rest of covariates. The statistically significant interaction effect between trap type and month of collection reflects an opposite temporal dynamic depending on type of trap used for the sampling. The significant interaction between trap type and trap location observed in the inland village indicates that the two traps depict opposite host-seeking patterns, (i.e., a preference for outdoor and indoor biting depicted by BG and CDC traps, respectively), suggesting that the capture rate of the two trap types is affected by the indoor vs. outdoor location.

To our knowledge, this represents the first study addressing the performance over time of CDC and BG traps to depict *Cx. quinquefasciatus* temporal dynamics and host-seeking behavior. If the differences highlighted with reference to season, as well as to the indoor/outdoor location of the traps will be confirmed in other settings, this will need to be taken into careful consideration when trap data are exploited to plan vector control interventions and to predict absolute species abundance or risk of disease transmission.

4.2. Performance of CDC and BG Traps in Collecting Species of the *Anopheles gambiae* Complex

CDC traps are shown to be the preferable trap to samples *Anopheline* species compared to BG-trap. They yielded a higher species diversity and were significantly more performant in collecting *An. gambiae* s.l. females both indoors and outdoors in both villages. CDC also showed to have a higher probability than BGs in detecting *An. gambiae* s.s. and *An. arabiensis* (in the inland village). In the only study comparing the performance of the two traps (both baited with the same lure), CDC traps consistently collected more *An. coluzzii* females than BGs when traps were located indoors, but BG collections massively outperform CDC ones in the outdoor environment, leading the authors to strongly recommend BG traps for outdoor sampling [22]. This recommendation is weakened by present results, which may suggest that, as in the case of *Cx. quinquefasciatus*, the relative performance of the two traps may be affected by eco-climatic conditions and/or by genetic traits of the target populations. However, it should be noted that in Pombi et al. [22] both traps were baited with lure (used in the present study only in BG traps) and with a source of CO₂ produced by sugar-fermenting yeast (not used in present study).

4.3. *Culex quinquefasciatus* Female Abundance, Seasonality, and Indoor/Outdoor Preferences in the Coastal and Inland Village

Our experimental design (i.e., traps located close to a single person sleeping under the bed net either indoors or outdoors) was aimed to allow to approximate the number of females/trap/night to the number of females/person/night (as suggested by [16,22]). Under this assumption, the average of *Cx. quinquefasciatus* females/person/night in the study period (based on BG collections which performed better than CDC for this species, see above) can be estimated to range between 8.7 (95% CI 6.5–11.8) and 5.5 (95% CI 4.2–7.2) in the coastal village and between 10 (95% CI 7.9–12.7) and 1.7 (95% CI 1.3–2.3) in the inland village (see M&M Statistic Method – GLM-2; Figure S1; Table S12–S13). If we consider the mean negative binomial distribution, we can estimate that in the sampling period of highest *Cx. quinquefasciatus* abundance, the females/person/night in 5% of human host population can reach up to 44 and 34 in coastal and inland village, respectively. These estimates of high mosquito–human contact are likely associated to presence of anthropogenic polluted waters rich in organic matter in both study villages (e.g., septic tanks), and suggest high risk of disease transmission with particular reference to lymphatic filariasis, which is endemic in Senegal [33]. Results from the inland village are consistent with recent report of high *Cx. quinquefasciatus* abundance from south-eastern Senegal [8]. Results do not highlight any clear indoor/outdoor preference in *Cx. quinquefasciatus* host-seeking behavior, i.e., the estimated species abundance is higher outdoors based on BG trap collection, and indoors based on CDC traps. Overall, this suggests that in our sampling sites *Cx. quinquefasciatus* does not show the strong endophagic behavior reported from other West African sites [8,34]. It is, however, to be reminded that the species is known to exhibit different biting habits worldwide [34], indicating a high behavioral plasticity. It is possible to hypothesize that the lack of clear endophagy observed in this study could be due to species adaptation to the increased number of people protected by LIINs and/or IRS in the indoor environment in the frame of national malaria control plans.

4.4. *Anopheles gambiae* Complex Species Abundance, Seasonality, and Indoor/Outdoor Preferences in the Coastal and Inland Village

The average number of *An. gambiae* s.l. females/trap/night (i.e., females/person/night) in the coastal village is shown to be constantly < 1 (Figure S2, Table S14–S15). This value is consistent with the significant reduction in *An. gambiae* s.l. biting rate observed from 2006 to 2016, following a large scale campaign of LLINs distribution in the coastal region [3,35]. Our results suggest a possible even greater decrease, as estimates are based on trap collections rather than HLCs, which are known to be more efficient in collecting human biting Anophelines [36,37]. No previous data are available from the inland village/region, where numbers of *An. gambiae* s.l. females/trap/night are even lower than in the coast (0.55,

95% CI 0.34/0.90) (Figure S2). However, the lower average of mosquitoes/traps/night observed in the inland compared to the coastal village is not in agreement with the higher malaria incidence reported in south-eastern regions of Senegal [38] and Gambia [39]. The temporal dynamic of *An. gambiae* s.l. in the two sampling villages (as depicted by CDC collections) is consistent with previous data showing a peak of abundance in rainy season and a sharp decrease towards the end of the rainy season and the beginning of dry season [3].

Anopheles arabiensis prevails in both villages over the other members of the complex (except that in the inland village at the beginning of the survey). The high prevalence of the species in the coastal village is consistent with recent observations from the nearby village of Dielmo and neighboring coastal villages [2,35]. Furthermore, our results confirm a range expansion for *An. arabiensis*, from north to south, likely as a result of increasing drought and/or human activities (such as deforestation and urbanization), as suggested by [10].

Anopheles coluzzii and *An. gambiae* are found with similar frequencies in the coastal village—where both rain-dependent and semi-permanent breeding sites associated to small permanent rivers (Djikoye River and Nema River) are present—while *An. gambiae* predominates in the inland one, where only rain-dependent breeding sites are present. These observations are in agreement with previous reports from coastal [40] and south-eastern Senegal [10,25,40]. The report of *An. coluzzii* and *An. gambiae* hybrids at frequencies of 3% and 1% in the coastal and inland village, respectively, reflects a level of hybridization higher than in the rest of the species' sympatric range in West-Africa [41], but overall in the range of other observations from Senegal and neighboring Gambia [10,39,40]. However, more in the details, hybrid frequency reported from the coastal village appears to be lower than in previous reports from coastal Senegalese region [40], but higher than those reported inland regions [10], suggesting that the breakdown in the reproductive isolation between the two species may be not restricted to the coastal region and/or expanding inland.

Results do not highlight strong preference of the three *An. gambiae* complex members for indoor vs outdoor host-seeking in both coastal and inland village, as trap location does not explain the variability of the data in the models. Lack of endophagic/exophagic preference was already observed in *An. arabiensis* in Dielmo and in other areas of eastern Senegal [27].

5. Conclusions

Our study is focused on mosquito species most relevant from the public health perspective due their closely association to humans, as it reports results from collections carried out inside or close to human habitations. Results reinforce previous evidence of an overall decline of malaria vector species in coastal and inland southern Senegal and of a parallel increase in *Cx. quinquefasciatus* abundance, highlighting risk of transmission of endemic pathogens, such as *Wuchereria bancrofti*, and emerging pathogens such as Rift Valley Fever and West Nile in the country. This reflects an opposite effect of selective pressures of human-made origin (e.g., decreased presence of unpolluted breeding sites due to urbanization and desertification) on different vector species. Results also confirm predominance of *An. arabiensis* over other members of the *An. gambiae* complex not only in the coastal area, where this was already reported, but also inland, likely as a result of increasing drought and anthropogenic environmental changes, including extensive LLIN and IRS exploitation.

From the methodological perspective, results highlight a higher specificity of BG traps for *Cx. quinquefasciatus* and of CDC traps for Anopheline vectors. Moreover, results also unexpectedly showed that the relative performance of two traps varies in relation to the month of collection and to the trap indoor/outdoor location, despite both traps target the host-seeking fraction of the population. This implies that predictions of the mean numbers of mosquito/person/night, as well as the of the species temporal dynamic and host-

seeking behavior, may vary depending on the trapping device used, thus questioning the use of entomological data to feed epidemiological models or to plan and assess the results of control interventions. Future works should address this weakness in malaria vector monitoring, for example by applying mathematical/statistical models able to account for different trapping performance in order to provide realistic quantification of mosquito density (i.e., number of mosquitoes in units of space and time), the crucial parameter for the evaluation of vector–human contact and for the development of epidemiological models to estimate the risk of pathogen transmission.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/insects12080692/s1, Table S1, Culicidae females collected in a coastal and in an inland village in Senegal by CDC-light and BG-sentinel traps (n= 20 trap-night for both traps) located indoors and outdoors from September to November 2018; Table S2, Culicidae males collected in a coastal and in an inland village in Senegal by CDC-light and BG-sentinel traps (n= 20 trap-night for both traps) located indoors and outdoors from September to November 2018; Table S3, Females of *Anopheles gambiae* s.l. species collected indoors and outdoors in a coastal and an inland village in Senegal by CDC-light and BG-sentinel traps from September to November 2018; Table S4, Result of best parsimonious of GLMM-1 of *Culex quinquefasciatus* female abundance in a coastal village in Senegal; Table S5, Result of the best parsimonious of GLMM-1 of *Culex quinquefasciatus* female abundance in an inland village in Senegal; Table S6, Summary of the mean of *Culex quinquefasciatus* in the coastal and inland village predicted by GLMM-1; Table S7, Result of best parsimonious of GLM-1 model of *Anopheles gambiae* s.l. female abundance in the coastal village; Table S8, Result of the best parsimonious of GLM-1 of *Anopheles gambiae* s.l. female abundance in an inland village in Senegal; Table S9, Summary of the mean of *Anopheles gambiae* s.l. in a coastal and inland village in Senegal predicted by GLM-1; Table S10, Result of the best parsimonious of GLM-3 of *Anopheles arabiensis*, *An. gambiae* and *An. coluzzii* probability in a coastal village in Senegal; Table S11, Result of the best parsimonious of GLM-3 of *Anopheles arabiensis* and *An. gambiae* probability in an inland village in Senegal; Table S12, Result of GLM-2 of the mean of *Culex quinquefasciatus*/trap/night in a coastal village in Senegal; Table S13, Result of GLM-2 of the mean of *Culex quinquefasciatus*/trap/night in an inland village in Senegal; Table S14, Result of GLM-2 of mean *Anopheles gambiae* s.l. females/trap/night in a coastal village in Senegal; Table S15, Result of GLM-2 of mean *Anopheles gambiae* s.l. females/trap/night in an inland village in Senegal; Figure S1, Average number of *Culex quinquefasciatus* host-seeking females/trap/night collected by BG-sentinel and CDC-light traps in a coastal (Left) and in an inland (Right) village in Senegal as estimated by GLM-2. Dots=average number mosquito females/person/night. Black vertical lines=95% confidence intervals; Figure S2, Average number of *Anopheles gambiae* s.l. host-seeking females/trap/night collected by BG-sentinel and CDC-light traps in a coastal (Left) and in an inland (Right) village in Senegal, as estimated by GLM-1. Dots=average number mosquito females/trap/night. Vertical lines=95% confidence intervals.

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Is Côte D'Ivoire a new high hybridization zone for the two major malaria vectors, *Anopheles coluzzii* and *An. gambiae* (Diptera, Culicidae)?

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ABSTRACT

Anopheles gambiae and *An. coluzzii* are very closely related and recently differentiated species representing the main malaria vectors in the Afrotropical region and responsible of up to >3 infective bites/person/night in Côte D'Ivoire, where prevention and control has stagnated in recent years. The aim of the present study was to genetically and ecologically characterize *An. gambiae* and *An. coluzzii* populations from two villages of Côte D'Ivoire, lying in the coastal forest belt and 250 km inland in the Guinean savannah mosaic belt, respectively. Results reveal high frequencies of both species in both study sites and high frequencies of hybrids (4–33%) along the whole year of sampling. Consistently with observations for the well-known high hybridization zone at the far-west of the species range, hybrid frequencies were higher in the coastal village and highest when the two species occurred at more balanced frequencies, supporting the “frequency-dependent hybridization” ecological speciation theory. Pilot genotyping revealed signatures of genomic admixture in both chromosome-X and -3. Coupled with previous reports of hybrids in the region, the results point to the coastal region of Côte D'Ivoire as a possible regions of high hybridization. Preliminary characterization of parameters relevant for malaria transmission and control (e.g. possibly higher sporozoite rates and indoor biting preferences in hybrids than in the parental species) highlight the possible relevance of the breakdown of reproductive barriers between *An. gambiae* and *An. coluzzii* not only in the field of ecological evolution, but also in malaria epidemiology and control.

1. Introduction

Malaria is a leading cause of morbidity and mortality in Côte D'Ivoire (PMI 2018-2019, 2019). Progresses in malaria prevention and control has stagnated in recent years in the country, as well as in other neighboring west African countries, with the estimated number of cases increasing 15.8% between 2015 and 2018 (from 260 to 300 cases per 1000 population) (World Health Organization, 2020). Among factors responsible of the high malaria burden in the region is the very efficient vectorial system accountable for up to >3 infective bites/person/night (PMI 2018-2019, 2019; PMI, 2020). This system is largely constituted by

the two most synanthropic species of the *An. gambiae* complex (Diptera, Culicidae), i.e. *An. coluzzii* Coetzee & Wilkerson, 2013 and *An. gambiae* Giles, 1900. The latter strongly predominates in northern forest-savannahs and Sudanese savannahs, while *An. coluzzii* predominates in the south-western forested region (Edi et al., 2014). The two species are found at more balanced frequencies in south eastern evergreen and deciduous forested region (PMI 2018-2019, 2019; PMI, 2020). Extensive insecticide resistance is observed throughout the country, with populations showing resistance to all 4 classes of insecticides (Oumtoubke et al., 2020).

Anopheles gambiae and *An. coluzzii* are very closely related and

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recently differentiated species, yet they present distinct ecological adaptations, particularly at the pre-imaginal stage, with *An. gambiae* dominating in temporary rain-dependent and *An. coluzzii* in human-made permanent breeding sites, (Lehmann and Diabate, 2008). They were first recognized based on fixed differences in the chromosome-X linked rDNA region (still currently used for molecular identification) and named as molecular forms M and S (Della Torre et al., 2001) for which different mechanisms promoting reproductive isolation have been proposed (Diabaté et al., 2009; Pennetier et al., 2010). Later, despite evidence of imperfect assortative mating, these were raised to formal species (Coetzee et al., 2013), based on evidence showing that their genomes contain regions of differentiation resulting in exclusive taxonomic clustering across much of their shared geographical range (Turner et al., 2005; Lawniczak et al., 2010; Neafsey et al., 2010; White et al., 2010; Reidenbach et al., 2012; Weetman et al., 2012;). Since then, results from genomic and ecological studies have raised *An. gambiae* and *An. coluzzii* to models of ecological speciation, revealing a rich mosaic of different ancestries in the two species, shaped by geography, ecology and speciation and highlighting evidence of strong intra-specific sub-structuring at the western and eastern extremes of their range (The Anopheles gambiae 1000 Genomes Consortium, 2017, 2020). Paracentric chromosomal inversions, mostly shared by the two species, further complicate the scenario, and contribute to adaptation to marginal niches and to intra-specific sub-structuring (Coluzzi et al., 2002). Despite their major role in malaria transmission in the sub-Saharan region and the potential epidemiological consequences (White et al., 2011), the intra-specific sub-structuring and the degree of hybridization and introgression between *An. gambiae* and *An. coluzzii* are still not clearly depicted in large part of their range.

Even though only few putative adult hybrids between the two species are being detected continent-wide, several examples of periodic breakdowns of pre- and/or post-mating barriers, resulting in extensive hybridization and in detectable levels of adaptive introgression and current gene flow, have been reported (Pombi et al., 2017). Very importantly from the perspective of malaria control, introgressive hybridization from *An. gambiae* to *An. coluzzii* of alleles associated to resistance to pyrethroid insecticides has been shown to have occurred multiple times in the last decades, rapidly increasing target site resistance in the recipient species, *An. coluzzii* (Pinto et al., 2007; Hanemaaijer et al., 2018).

According to the “frequency-dependent hybridization” ecological speciation theory (Kirkpatrick, 2001; Seehausen, 2004; Stankowski, 2013), the prevalence of hybrids along their sympatric range is expected to be higher where their degree of inter-specific contact is maximal. This is certainly the case for *An. coluzzii* and *An. gambiae* in the coastal region from The Gambia and Guinea Bissau at the western extreme of the species’ range (hereafter referred to as far-west) (Pombi et al., 2017), where the two species were predicted to occur at relatively high frequencies (Tene Fossog et al., 2015). This region of apparently high hybridization was first revealed by the finding of individuals characterized by heterozygous X-linked IGS-diagnostic markers (hereafter IGS-hybrids) at frequencies significantly higher (>20%; Caputo et al., 2008; Oliveira et al., 2008; Niang et al., 2014) than those usually observed in the rest of the range (<2%; Pombi et al., 2017). Further studies exploiting also other genomic markers suggest a stable breakdown of reproductive isolation leading to massive introgression and intra-specific genomic partitioning of far-west coastal populations (Caputo et al., 2011; Marsden et al., 2011; Lee et al., 2013; Nwakanma et al., 2013; Caputo et al., 2014). Genomic studies on field populations have revealed a sub-structuring of *An. gambiae* populations in the region (The Anopheles gambiae 1000 Genomes Consortium, 2017, 2020), leading to the hypothesis of the existence of a coastal putative “hybrid form” (carrying an *A. gambiae* chromosome-X centromere and *An. coluzzii*-like autosomes) separated from inland *An. gambiae* by a central region dominated by *An. coluzzii* (Vicente et al., 2017). Data also highlighted potential for spatio-temporal stability of the putative coastal

hybrid form and evidence of resilience against introgression of medically important loci and traits, as well as of greater zoophilic tendency.

In addition to the far-west, the other region in West Africa predicted to harbour relatively high frequencies of *An. coluzzii* and *An. gambiae*, and thus possibly representing a second high- hybridization zone, is the forested region between south-east Côte D’Ivoire and south-west Ghana (Tene Fossog et al., 2015). Intriguingly, multiple records of IGS-hybrids have been reported in Côte D’Ivoire since 2012: in the evergreen forested coastal region (hybrid frequency = 2.5–11%; Edi et al., 2017; PMI 2018-2019, 2019; Mouhamadou et al., 2019; Meiwald et al., 2020) and in central western forested region (4.3% Man area region, at the borders with Guinea; Assogba et al., 2018), as well as hundreds of kilometers northwards, i.e. in rice fields in Yamoussoukro area (H = 1.8–7.7%; Chouaïbou et al., 2017; Assogba et al., 2018; PMI 2018-2019, 2019) and in urban/peri-urban sites in the forest-savanna mosaic belt in Bouakè area (H = 0.6–5.4%; Assogba et al., 2018; Oumbouke et al., 2020) ~210 km and ~300 km from the coast, respectively. Moreover, an overall frequency of 2.1% of IGS-hybrids was reported in 10 sites across the different eco-climatic zones (Fodjo et al., 2018). Noteworthy, all the above reports refer to adult females emerged from larvae collected in the field in order to assess susceptibility to insecticide by in vivo bioassays. To the best of our knowledge, the only report of an adult IGS-hybrid collected in the country dates back to 1998, when the two species were still referred to as M and S molecular forms (H = 1.1%, Bouake area; Della Torre et al., 2005).

The aim of the present study was to characterize *An. gambiae* and *An. coluzzii* adult populations from a coastal and an inland village of Côte D’Ivoire where both species were expected to occur at relatively high frequencies, and to identify possible signatures of genomic admixture. Results highlight frequencies of adult *An. gambiae/An. coluzzii* hybrids along the whole year even higher than those observed in the far-west high hybridization region, strongly supporting the “frequency-dependent hybridization” hypothesis, and open the opportunity to preliminarily characterize the hybrids with respect to parameters relevant for malaria epidemiology and control.

2. Materials and method

2.1. Entomological collections

Mosquito collections were carried out in two villages in Côte D’Ivoire (Fig. 1A), where both *An. coluzzii* and *An. gambiae* are reported to be present (PMI 2018-2019, 2019; PMI, 2020).

Ayamé village Piste IV (GPS: 5°28' N 3°12'W, hereafter coastal village) is located in the proximity of a large artificial lake and hydro-electric basin in the south-eastern region of Aboisso, 100 km east of the capital city Abidjan and 50 km from the coast. The region lies within the evergreen forest mosaic belt and is characterized by an equatorial transition climate, with annual rainfall ranging between 1300 and 2400 mm, with a long rainy season in March–July and a shorter one in September–December. The region is hilly and covered with dense moist forest and cacao or coffee plantations.

Petessou village (GPS: 8°6' N 5°28'W; hereafter inland village) is situated at about 300 asl, 11 km south from the city of Bouaké and > 250 km from the coast. The village is located nearby a permanent watercourse maintaining a large area of shallows used for rice farming and market gardening, representing suitable breeding sites for mosquitoes (Zogo et al., 2019). The region lies within the Guinean savannah mosaic belt and is characterized by a tropical humid climate, with annual rainfall ranging between 1000 and 1600 mm. Dry season lasts from November to February and rainy season from March to October.

Mosquito collections were carried out in December 2018, March, May and October 2019 for four consecutive nights/month by CDC-light traps located inside and outside 10 randomly selected houses. Traps were located nearby a person sleeping under bed-net both indoors and outdoors. Ethical approval for the study was granted by Ministère de la

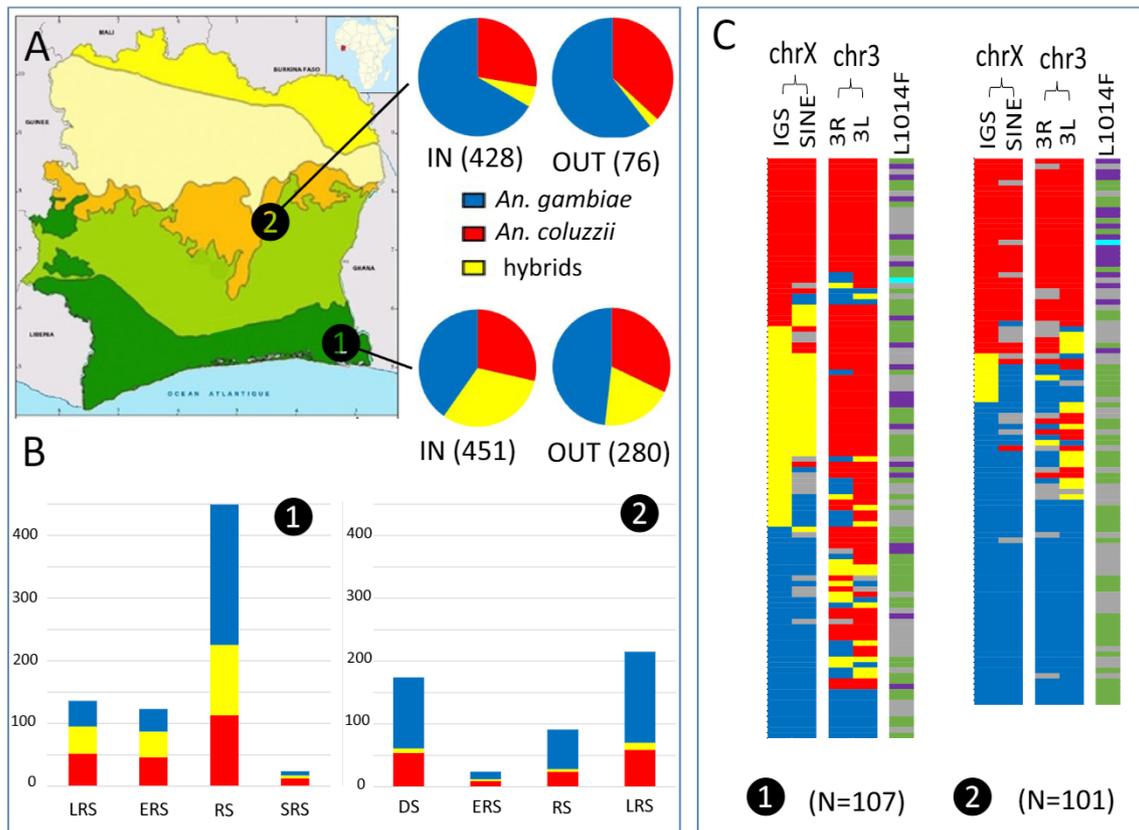


Fig. 1. *Anopheles coluzzii* (red), *An. gambiae* (blue) and IGS-hybrids (yellow) in indoor and outdoor collections in two villages in Côte D'Ivoire (A) and in the monthly collections between December 2018 and October 2019 (B). Coastal village: LRS = Late Rainy Season (5–8 December 2018), ERS = Early Rainy Season (6–9 March 2019), RS = Rainy Season (15–18 May 2019), SRS = Short Rainy Season (9–12 October 2019). Inland village: DS = Dry Season (12–15 December 2018), ERS = Early Rainy Season (12–15 March 2019), RS = Rainy Season (26–29 May 2019), LRS = Late Rainy Season (16–19 October 2019). Dark green in the map = evergreen forest; green = deciduous forest; orange = forest-savannah mosaic bet; Yellow = Sudanese savannah (modified from [Edi et al. 2017](#)). C = Genotyping results of the coastal and inland populations, relative to: two X-centromeric diagnostic markers (IGS and SINE), two Ancestry Informative Markers (*sensu* [AG1000G 2017](#)) on chromosome-3 (3R and 3 L), and the L1014F mutation in the *vgsc* gene on chromosomal arm 2 L (light blue = 1014 L homozygous susceptible; green = 1014F homozygous resistant; purple = 1014 L/1014F heterozygous; grey = unsuccessfully genotyped locus). Only specimens for which at least 2 of the 4 species-specific markers were successfully genotyped are included. Each row represents an individual mosquito and columns represent genotyped markers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Santé et de l'Hygiène Publique (Comité National D'Ethique de la Recherche, CNER, 023–18/1VISHP/CNER, Abidjan 3/4/2018).

Sampled mosquitoes were morphologically identified as *An. gambiae* *sensu lato* (s.l.) based on [Gillies and Coetzee \(1987\)](#) and were preserved in tubes with silica gel.

2.2. Molecular identification and genotyping

DNA was extracted from head and thorax of *An. gambiae* s.l. females following the protocol by [Rider et al., 2012](#) and used as template for molecular analyses. All collected specimens were genotyped for diagnostic SNPs in the IGS-rDNA region by [Santolamazza et al. \(2004\)](#) or [Wilkins et al. \(2006\)](#) PCR-approaches. Individuals showing a IGS-heterozygous genotype are referred hereafter as IGS-hybrids. *Plasmodium falciparum* (Welch, 1897) (Apicomplexa, Plasmodiidae) presence was assessed by a TaqMan approach ([Bass et al., 2008](#)).

A subset of random selected specimens was also genotyped for:

- 1) presence of *An. coluzzii*-specific insertion of a Short Interspersed Nuclear Element (SINE) in the centromeric region of chromosome-X ([Santolamazza et al., 2008](#); hereafter referred to as SINE-X).
- 2) two Ancestral Informative Markers (*sensu* [Ag1000G, 2017](#)) on chromosomal arms 3R (3R:42848) and 3L (3L:129051) by the Tetra-arms-PCR protocol developed by [Caputo et al. \(2021\)](#).

- 3) L1014F (*kdr*-West) mutation in the voltage-gated-sodium-channel (*vgsc*) gene, associated with insecticide-resistance ([Bass et al., 2007](#)).
- 4) 2Rb, 2Rc and 2La paracentric chromosomal inversions by the molecular karyotyping PCR-approaches developed by [Montanez-Gonzalez et al. \(2020\)](#); [Montanez-Gonzalez et al. \(2021\)](#) and [White et al. \(2007\)](#), respectively.

2.3. Statistical analyses

The mean number of mosquito/person/night (m/p/n) was estimated based on Generalized Linear Mixed Models (GLMMs) assuming that this corresponds to the number of collected mosquitoes/trap/night ([Kilama et al., 2014](#)). A negative binomial distribution for the response variable (i.e. counts of mosquitoes per night), with mean μ and dispersion parameter θ was chosen to predict m/p/n. Two models were fitted: GLMM-1 considers as covariates the villages, the months of collection and their interaction. GLMM-2 considers as covariates the species, the village, and their interaction. A random effect structure was included in both models to account for the individual variability of each house.

Plasmodium falciparum sporozoite rate (SR) was estimated by GLMM. Assuming that the response variable (presence and absence of *P. falciparum* DNA in the head+thorax of processed specimens) follows a binomial distribution, two models were fitted: GLMM-3 considers as covariates the villages, the months of collection and their interaction. GLMM-4 considers the species, the village and their interaction. A

random effect structure was included in both models to account for the individual variability among sampled houses.

The Entomological Inoculation Rate (EIR) was computed as the product of the estimated $m/p/n$ and the estimated SR, under the assumption that collected specimens represent the biting fraction of the mosquito population.

The relationship between the relative frequency *An. coluzzii* and *An. gambiae* and the relative frequency of IGS-hybrid genotypes in each village was assessed by Generalized Additive Models (GAM-1). Assuming that the frequency of IGS-hybrids follows a binomial distribution, GAM-1 was fitted with the relative frequency of hybrid genotypes as response variable and the relative frequency of *An. coluzzii* and *An. gambiae* taxa and the villages as covariates.

Anopheles coluzzii, *An. gambiae* and IGS-hybrid genotypes indoor/outdoor abundance was estimated by GLMM-5 including as covariates the village, the month of collection, the indoor/outdoor trap location, and their interaction. We fitted a model for each species separately. Since *An. coluzzii* and *An. gambiae* abundance data using a Poisson distribution resulted over-dispersed, we considered a Negative Binomial distribution with mean μ and dispersion parameter θ , and the count of specimens/night/house and trapping location (indoor/outdoor) in the two villages as response variable. On the other hand, overdispersion was not detected for IGS-hybrid genotypes when assuming a Poisson distribution with mean λ , where the response variable was the number of hybrid genotypes/night/house and indoor/outdoor location in the two villages. Initially, the full model included village, month, and trap location, and all their interaction as independent variables. A random effect structure was included in both models to account for the individual variability of each house. A model selection procedure was applied to all GLMM-5 (Burnham and Anderson, 2004). Subsequently, the Akaike Information Criteria (AICc) was used to rank all submodules, then the best parsimonious model was considered between the subset of models having a difference (delta) in AICc < 4. Due to convergence problem, the maximum number of iterations of the glmer.nb function in the lme4 package has been increased to 10.000.

Frequency of paracentric chromosomal inversions was estimated by GLM-6 only in the inland village, due to low inversion frequencies in the coastal village. Assuming that the response variable “inversion frequency” follows a binomial distribution, the full model considers as covariates *An. coluzzii* and *An. gambiae* (no hybrid genotypes were considered, due to low sample), location and their interaction. We decided whether to include or not the interaction terms by comparing the models by AIC. The model was fitted for each paracentric chromosomal inversion separately.

R statistical software version 3.6.3 (R Core Team, 2019) and packages lme4 (Bates et al., 2015), MuMIn (Bartoń, 2019), mgcv (Wood et al., 2016), tidyverse (Wickham, 2017) and MASS (Venables and Ripley, 2002) were used for all statistical analysis.

3. Results

Seven-hundred forty-four *An. gambiae s.l.* females were collected in the coastal village, with a mean number of $m/p/n$ ranging from 3.4 in May (95% CI 2.2–5.3, $\theta = 0.45$) to 0.2 in October (95% CI 0.1–0.3, $\theta = 0.45$). The total number of *An. gambiae s.l.* females collected in the inland village was 505, with an $m/p/n$ ranging from 1.3 in October (95% CI 0.8–2.05, $\theta = 0.45$) to 0.15 in March (95% CI 0.005–0.27, $\theta = 0.45$) (GLMM-1, Table S0).

Plasmodium falciparum detection was successful for 729 and 476 specimens from the coastal and the inland village, respectively. *Plasmodium falciparum* SR ranged from 13.6% in March (95% CI 8.1–22.0) to 4.1% in October (95% CI 0.06–24.3) in the coastal village, and from 16% in March (95% CI 5.8–36.8) to 2% (95% CI 0.07–5.5) in October in the inland village. No significant differences were observed between the two villages (except in the inland village where SR was significantly lower in October than in December 2018; p -value < 0.001 and March 2019; p -

value = 0.026) (GLMM-3; Table S1, Fig. S1). The average EIR ranged between 0.16 (March)–0.007 (October) in the coastal village, and between 0.05 (December)–0.02 (March) in the inland one. Overall, the estimated average of EIR was 0.07 and 0.03 infectious bites/person/night in the coastal and inland village, respectively.

Overall, 1235 specimens were successfully genotyped for species-specific SNPs in the IGS-rDNA region: *An. coluzzii* ($N = 368$), *An. gambiae* ($N = 641$) were found at frequencies of 30.4% and 42.1% in the coastal village and 29% and 66.1% in the inland one, respectively (Fig. 1A; Table S2). IGS-hybrids ($N = 226$; Fig. 1B) were found at overall frequency of 27.5% in the coastal village (where frequencies ranged between 20.8% in October and 33.3% in March), and of 5% in the inland village (where frequencies ranged between 4% in Dec and 12.5% in March).

In the coastal village, results of GAM-1 show a curvilinear relationship between IGS-hybrid frequencies and *An. coluzzii* and *An. gambiae* relative frequencies (p -value < 0.0001; Table S3; Fig. 2), indicating a higher IGS-hybrid prevalence when the two taxa occur at balanced frequencies. No statistical association is observed in the inland village.

3.1. Phenotypic characterization of *Anopheles coluzzii*, *An. gambiae* and of IGS-hybrids

3.1.1. Temporal dynamics and indoor/outdoor preferences

According to covariates selection in GLMM-5 the mean predicted abundance of *An. coluzzii*, *An. gambiae* and IGS-hybrids is correlated to village, month of collection and indoor/outdoor trapping location and includes the interactions between village and month of collection, as well as between village and indoor/outdoor trapping location (Fig. 3; Tables S4, S5, S6).

The analysis of the interaction between villages and months of collection highlights significant differences in species seasonality in the two villages (p -value = 0.002) (Table S4; S5), with peaks of abundance at the peak of the rainy season in the coast (May: *An. coluzzii* = 2.8; *An. gambiae* = 8.1) and at the end of the rainy season inland (October: *An. coluzzii* = 1.9; *An. gambiae* = 4.2). The same pattern is predicted for IGS-hybrids in the coastal village (peak in May = 3.8), although the predicted mean abundance of hybrids is neglectable in the inland village except in October (0.54; p -value = 0.009) (Table S6).

On the other hand, the analysis of the interaction between villages and indoor/outdoor trapping location highlights significantly higher mean predicted abundance of the two species in indoor vs outdoor collections in the inland villages (p -value < 0.0001). In the coastal one only the mean predicted abundance of IGS-hybrids is predicted to be significantly higher indoors (p -value = 0.0002) (Table S6). IGS-hybrids abundance in indoor vs outdoor trapping location collections in the inland village was not computed due to the very low sample size.

3.1.2. Mean females/person/night

According to GLMM-2 model, the mean number of *An. coluzzii*, IGS-hybrids and *An. gambiae*/person/night ($m/p/n$) is 0.90, 0.85 and 1.26 in the coastal, and 0.59, 0.10 and 1.28 in the inland village, respectively (Table 1). In addition, the model highlights higher IGS-hybrids/ p/n than *An. coluzzii*/ p/n ($p < 0.0001$) in the coastal village and lower IGS-hybrids/ p/n than mean numbers of both species in the inland one ($p < 0.001$; Table S7).

3.1.3. Sporozoite and entomological inoculation rates

According to GLMM-4, *P. falciparum*-SRs *An. coluzzii* and *An. gambiae* are 9% and 6% in the coastal village and 6% and 4% in the inland one (Table 1), with no significant differences between the two species. On the hand, SR is higher in IGS-hybrids than in the two species in the inland village (SR = 16% in 23 hybrids), while no significant differences are shown in the coastal village (Table S8, Fig. S2).

EIR values range between 0.06 and 0.08 and 0.001–0.05 infective bites/night in the coastal and inland village, respectively (Table 1).

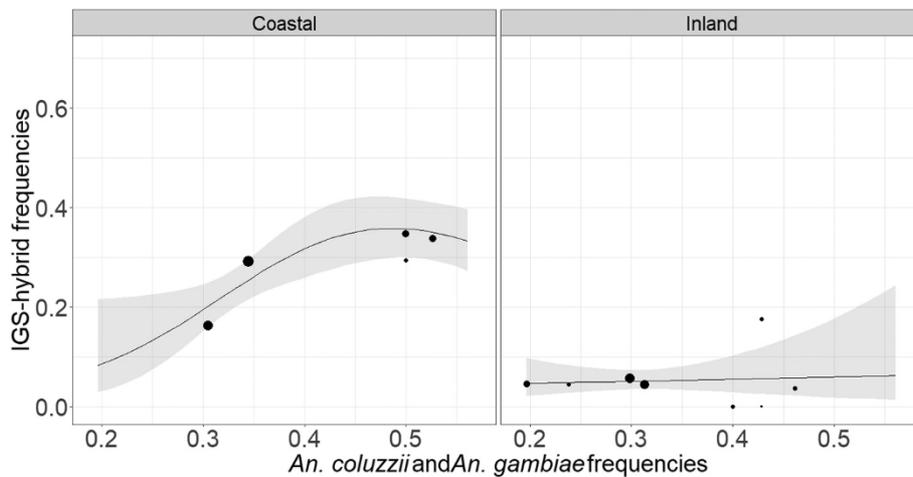


Fig. 2. Predicted relative frequency of IGS-hybrids (lines) as a function of *Anopheles coluzzii* and *An. gambiae* frequencies in a coastal (left) and inland (right) village in Côte D'Ivoire (GAM-1). Shaded areas = 95% confidence intervals. Dots = sample size classes from 50 to 250 mosquito/collection.

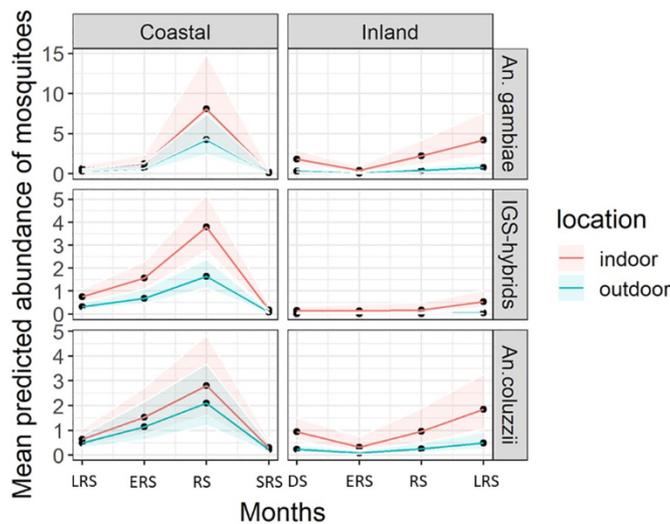


Fig. 3. Mean predicted abundance of *Anopheles coluzzii*, *An. gambiae* and IGS-hybrid females collected in a coastal and inland village in Côte D'Ivoire between December 2018 and October 2019. Coastal village: LRS = Late Rainy Season (5–8 December 2018), ERS = Early Rainy Season (6–9 March 2019), RS = Rainy Season (15–18 May 2019), SRS = Short Rainy Season (9–12 October 2019). Inland village: DS = Dry Season (12–15 December 2018), ERS = Early Rainy Season (12–15 March 2019), RS = Rainy Season (26–29 May 2019), LRS = Late Rainy Season (16–19 October 2019). Black dots = mean number of specimens/collection. Red and blue lines connect the predicted fit from the regression models on data collected indoors and outdoors, respectively. Shaded areas around the predicted lines = 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Genotypic characterization of *An. coluzzii*, *An. gambiae* and IGS-hybrids

3.2.1. Chromosome-X SINE genotyping

A total of 241 specimens were genotyped for the presence of the *An. coluzzii*-specific SINE-X insertion in the centromeric region of chromosome-X (Santolamazza et al., 2008). The concordance between IGS and SINE-X genotyping is 75% in the coastal (N genotyped = 139) and 88% in the inland sample (N genotyped = 102) (Table S9). The most frequently observed mismatch (i.e. 20 out of 47 specimens with mismatching genotyping results) involved IGS-hybrids identified by SINE-X

Table 1

Mean number of mosquito/person/night (m/p/n), *Plasmodium falciparum* Sporozoites Rates (SR) and Entomological Inoculation rate (EIRs) in members of the *Anopheles gambiae* complex in a coastal and inland village in Côte D'Ivoire. N = total numbers of mosquitoes examined for the presence of *Plasmodium falciparum* over the whole sampling season; 95% CI = confidence intervals. Θ = dispersion parameter in the GLMM-2 0.35.

Village	Species	N	m/p/n (95% CI)	SR (95% CI)	EIR
Coastal	<i>An. gambiae</i>	303	1.3 (0.8–1.9)	0.06 (0.03–0.1)	0.07
	IGS-hybrids	199	0.8 (0.3–1.4)	0.07 (0.04–0.13)	0.06
	<i>An. coluzzii</i>	222	0.9 (0.6–1.4)	0.09 (0.05–0.15)	0.08
Inland	<i>An. gambiae sl</i>	729	1 (0.7–1.5)	0.07 (0.05–0.11)	0.076
	<i>An. gambiae</i>	315	1.3 (0.8–1.9)	0.04 (0.02–0.8)	0.05
	IGS-hybrids	23	0.1 (0.1–0.2)	0.16 (0.5–0.37)	0.001
	<i>An. coluzzii</i>	137	0.6 (0.4–0.9)	0.06 (0.03–0.12)	0.03
	<i>An. gambiae sl</i>	476	0.6 (0.4–0.9)	0.05 (0.03–0.09)	0.035

genotyping as *An.gambiae*. SINE-X heterozygous genotypes were detected in the coastal village only (35/139); of these 11.4%, 20% and 68.6% have been identified as *An. coluzzii*, *An. gambiae* and IGS-hybrid specimens, respectively.

3.2.2. Chromosome-3 genotyping

A total of 188 specimens (out of 218 processed ones) were successfully genotyped for the two Ancestry Informative Markers (AIMs, sensu (The *A. gambiae* 1000 Genomes Consortium, 2017), one on chromosomal arm-3R (3R: 42848) and one on -3L (3L: 129051) (Fig. 1C; Table 2 and S10) (Caputo et al., 2021). In the coastal village, 61.2% of the

Table 2

Characterization of chromosome-3 genotype in *Anopheles coluzzii* (CO), *An. gambiae* (GA) and hybrids (IGS-H) from a coastal and an inland village of Côte D'Ivoire. Chr X (IGS) = diagnostic SNPs in the IGS rDNA region (Wilkins et al., 2006, and Santolamazza et al., 2011); CO-Chr3, GA-Chr3 = specimens with concordant homozygous *An. coluzzii* or *An. gambiae* chromosome-3 genotypes; Chr3-H = specimens with chromosome-3 markers either discordant or concordantly heterozygotes.

	CHR X (IGS)	CO-Chr3	Chr3-H	GA-Chr3	Total
Coastal	<i>An. coluzzii</i>	25	4	2	31
	IGS-H	27	10	0	37
	<i>An. gambiae</i>	11	15	9	35
	Total	63	29	11	103
Inland	<i>An. coluzzii</i>	27	3	0	30
	IGS-H	1	2	4	7
	<i>An. gambiae</i>	3	9	36	48
	Total	31	14	40	85

specimens (63/103) carry *An. coluzzii*-specific alleles at both chromosome-3 loci and 10.7% (11/103) the *An. gambiae*-specific alleles. In the inland village, 36.5% (31/85) and 47.1% (40/85) specimens carry either *An. coluzzii* or *An. gambiae*-specific alleles, respectively. Individuals characterized by a heterozygous genotype for both chromosome-3 loci were only found in the coastal village (3/103). Genotypes characterized by discordant 3R/3 L genotypes were found at frequencies of 25.2% (26/103) and 16.5% (14/85), respectively. These were mostly characterized by a homozygous 3R-GA genotype.

3.2.3. Association between chromosome-X and -3 genotypes

Inconsistent IGS and chromosome-3 genotypes (non in bold in Table 2) are significantly more frequent in the coastal (57%; 59/103) than in the inland village (23.5%; 20/85) ($p < 0.001$). In the coastal village, 80.6% (25/31) of *An. coluzzii* and only 25.7% (9/35) of *An. gambiae* show consistent association between IGS-species diagnostic marker and both chromosome-3 markers ($p < 0.0001$) (Fig. 1C; Table 2). In the inland village, 90% (27/30) of *An. coluzzii* and 75% (36/48) of *An. gambiae* show consistently associated CO-Chr3 and GA-Chr3 genotypes ($p = n.s$) (Fig. 1C; Table 2).

The overall picture does not change substantially if specimens are identified either by SINE-X or by both IGS and SINE-X diagnostics (Table S11). However, it is interesting to note that in the coastal village 18 out of 19 individuals characterized by an IGS and SINE-X heterozygous genotype, indicative of F1 hybrids (Santolamazza et al., 2011), are characterized by a “*coluzzii*-like” chromosome-3 genotype (Fig. 1C).

3.2.4. L1014F genotyping

Mutation L1014F was genotyped in 120 and 115 specimens from the coastal and the inland village respectively. Frequency of the 1014F allele was >90% in *An. gambiae* in the coastal (N alleles = 65/72) and in the inland (121/122) village, respectively, and > 80% in hybrids (i.e. 69/80 and 12/12, respectively) (Table S12). In *An. coluzzii* higher frequency of the 1014F is observed in the coastal (83%, 73/88) than inland (68%, 65/96) village ($\chi^2 = 4.907$, p -value = 0.02). Only 5 *An. coluzzii* (3 from the inland and 2 from the coastal village) and 1 *An. gambiae* (from the coastal village) specimens carry the susceptible 1014 L allele in homozygosity. The frequency of 1014F allele is significantly higher in *An. gambiae* than in *An. coluzzii* in the inland village (Fisher exact test, p -value < 0.0001).

3.2.5. Paracentric chromosomal inversion molecular karyotyping

Paracentric chromosomal inversions were molecularly karyotyped in 120 and 209 specimens in the coastal and inland village respectively, with 94%, 98% and 98% success rate for 2Rb, 2Rc and 2La inversions, respectively (Table S13). Higher frequencies of 2Rb and 2La inversions are observed in the inland village compared to the coastal one in either species, as well as in IGS-hybrids (p -value < 0.005) (Table S13, Fig. S3). The 2Rc inversion polymorphism is found at frequencies up to 9% in the three groups in the two villages, with no significant differences between groups or villages. Probability of occurrence of 2Rb and 2La inversion frequencies in the inland village, as estimated by GLM-6, is statistically higher in *An. gambiae* than in *An. coluzzii* both indoors and outdoors (Table S14, S15 and Fig. S4).

4. Discussion

This study confirms expectation of relatively balanced frequencies of *An. coluzzii* and *An. gambiae* in the forested coastal region of Côte D'Ivoire, as well as in the south-east Guinean forest-savannah mosaic belt, 250 km inland (Fig. 1). The chromosomal polymorphism pattern of the coastal population shows the expected “forest” karyotype (i.e. almost complete standard karyotype), while the inland population is characterized by a “savanna-like” karyotype (i.e. frequencies 2Rb and 2La inversion up to 32 and 55%, respectively). The latter are higher in *An. gambiae*, confirming a different role of inversions in ecological

adaptation in the two species (Coluzzi et al., 2002).

High frequencies of IGS-hybrids are observed during the whole year, ranging from 21% up to 33% in the coastal site and from 4% to 12.5% in the inland one. These data - coupled with other IGS-hybrid reports in larval samples [(from 0.6% to 10.9% since 2012; (Marsden et al., 2011; Lee et al., 2013; Nwakanma et al., 2013; Caputo et al., 2014; Tene Fossog et al., 2015)]- suggest that reproductive isolation between *An. coluzzii* and *An. gambiae* is not complete in Côte D'Ivoire, neither in the forested southern region, nor in the forest-savannah mosaic belt. A similar pattern has been reported so far only at the western extreme of the two species range (i.e. in the “far- west”, Caputo et al., 2011) where, consistently with present data, hybridization was higher in the coast than inland.

Previous and present reports of relatively high frequencies of both species and hybrids in Côte D'Ivoire are fully consistent with the predictions of high frequencies of the two species in the forested region between south-east Côte D'Ivoire and south-west Ghana (Tene Fossog et al., 2015), as well as with expectations to find high prevalence of hybrids where their degree of inter-specific contact is maximal (Pombi et al., 2017). Present data add a temporal perspective to the spatial scenario. In fact, the frequency of IGS-hybrids in the coastal site is significantly higher during the rainy season when *An. coluzzii* and *An. gambiae* frequencies are more balanced (Fig. 2). Lower absolute abundance of IGS-hybrids did not allow highlighting the same trend in the inland village.

Temporary break-downs of reproductive barriers leading to introgressive hybridization of adaptive insecticide resistant *kdr* alleles has been shown to have occurred multiple times out of the far-west region. This did not produce any discernable long term impact on the species' genomes, other than in regions associated to genes involved in insecticide resistance, e.g. the VGSC gene region on chromosome-2 and a dramatic increase in the resistant *kdr* allele frequency in most west African *An. coluzzii* populations (Lee et al., 2013; Clarkson et al., 2014). In Côte D'Ivoire, introgressive hybridization of the 1014F allele from *An. gambiae* to *An. coluzzii* likely predates 2004 (Edi et al., 2014) and the allele is today reported at very high frequencies in both species in the whole country (PMI 2018-2019, 2019; PMI, 2020), as well as in both species in both study sites ($\geq 68\%$). This suggests that the observed pattern of genomic admixture is not associated to recent adaptive introgression of 1014F allele.

The relatively large IGS-hybrid sample size and the balanced frequency of *An. coluzzii* and *An. gambiae* in the coastal village allowed a preliminary characterization of parameters relevant for malaria transmission and control in the three groups. No significant differences between IGS-hybrids and the two species are observed with regard to Human Biting Rates (0.8–1.3 mosquitoes/person/night) and Sporozoite Rates (6–9%). The only exception refers to the significantly higher Sporozoite Rate observed in IGS-hybrids from the inland site (16%), but this should be taken with caution, due to the limited sample size ($N = 26$). Overall, Sporozoite Rates are in the range of those reported by Adja et al., 2011; Zogo et al., 2019; Zoh et al., 2020, while Human Biting Rates and Entomological Inoculation rates are lower in our study compared to assessments based on Human Landing Catches (HLC), possibly due to lower efficacy of trap collections compare to HLC [e.g. (Adja et al., 2021)]. In addition to these epidemiologically relevant parameters, we investigated the three groups' indoor/outdoor biting preferences. *Anopheles coluzzii* and *An. gambiae* exhibit a strong endophagy only under the inland eco-climatic conditions, while in the coastal village only IGS-hybrids are collected significantly more indoors.

It is important to remind that in the far-west region heterozygous patterns of IGS-diagnostic markers were shown not to be appropriate proxies of F1 hybrids, as commonly assumed for the rest of the species range (Santolamazza et al., 2011). Previous studies on far-west male populations showed that a heterozygous IGS pattern may be the product of recombination and gene conversion within the multicopy ribosomal region (Caputo et al., 2016). To investigate whether this is also the case

in Côte D'Ivoire, we used additional species-specific PCR-based assays for further characterizing the collected samples. First, we genotyped the SINE-X insertion (in proximity of the IGS ribosomal region on chromosome-X centromere) which is *An. coluzzii*-specific in most of the two species range (Santolamazza et al., 2008). Results show presence of specimens heterozygous for SINE-X insertion only in the coastal village. Most of these specimens (69%) are characterized by an IGS-heterozygous pattern and may represent putative F1-hybrids (Caputo et al., 2016; Santolamazza et al., 2008). Second, we genotyped two Ancestry Informative Markers on either arms of chromosome-3 (3R and 3L), which were shown to be capable to ascertain admixture similarly to a multilocus approach (Caputo et al., 2021). Results show low frequency of autosomal admixture in *An. coluzzii* IGS-identified specimens in both villages (15%), and a higher one in *An. gambiae* (46%). Highest admixture is observed in coastal *An. gambiae* (74%), where "pure" *An. gambiae* individuals (i.e. 9 specimens with concordant IGS and chromosome 3 markers) are largely outnumbered by individuals apparently characterized by an *An. gambiae*-like chromosome-X genotype and an admixed chromosome-3 one ($N = 26$). This pattern suggests asymmetric autosomal introgression from *An. coluzzii* to *An. gambiae*, as already hypothesized in the far-west region (Marsden et al., 2011). However, while in The Gambia and Guinea Bissau most putative F1-hybrids are characterized by polymorphic chromosome-3 loci (Caputo et al., 2021), in the coastal village individuals characterized by IGS/SINE-X heterozygous patterns are apparently characterized by *An. coluzzii*-like autosomes.

5. Conclusions

The present results based on longitudinal field sampling in two sites in east Côte D'Ivoire fits with previous observations and lead to identify this area as a putative area of high hybridization between the two main malaria vectors in the country, *An. coluzzii* and *An. gambiae*. Hybridization appears to be higher in the coastal evergreen forested region, but is substantial also in the Guinea-savannah belt, at least up to 250 km inland. Interestingly, south-east Côte D'Ivoire and south-west Ghana were predicted to be the larger area in the Gulf of Guinea where *An. coluzzii* maintains high relative frequencies with respect to *An. gambiae* (Tene Fossog et al., 2015). Overall, the scenario very well fits the hypothesis that extrinsic post-mating selection against hybrids is lower and hybrid fitness is higher under environmental conditions where/when neither of the parental taxa predominates, as predicted by the ecological speciation theory (Kirkpatrick, 2001; Seehausen, 2004; Stankowski, 2013).

Further studies are needed to characterize the biological basis of the break-down of interspecific reproductive barriers (both pre-mating, e.g. swarm segregation and mate recognition, and post-mating, e.g. larval selection) in the region and the eco-geographic distribution, seasonality, ecology and behavior of hybrid individuals. These studies, however, are hampered by the difficulty in defining the taxonomic units to be studied. Here we analyzed epidemiologically relevant phenotypic characters (i.e. seasonality, human biting rate, sporozoite rate, biting behavior) based on IGS-PCR diagnostics. However, preliminary genomic characterization of the target populations provides evidence of a complex pattern of admixture on both chromosome-X and -3, which deserves deeper investigations.

The existence of a high hybridization area in Côte D'Ivoire does not only have a great interest for those interested in the evolution of the *An. gambiae* complex and, more in general, on ecological evolution, but may also have implications in malaria epidemiology and control. Preliminary phenotypic characterization of IGS-hybrids highlights sporozoite rates comparable to (or possibly higher than) those of *An. coluzzii* and *An. gambiae*. Data are clearly insufficient to assess their vectorial capacity, but suggest that that longevity is not reduced in adult hybrids, further supporting their high fitness. Finally, high levels of gene-flow between the two species - as preliminary revealed by the signatures of admixture

in the populations analyzed - are very relevant from the perspective of assessing the outcomes of gene-drive based vector control strategies. Future epidemiological assessment and malaria control plans in Côte D'Ivoire - and in other regions in the Gulf of Guinea where both species are predicted to coexist at relatively high frequencies - will need to take into careful account the results of this (and, hopefully) further deeper studies.

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Beniamino Caputo: Conceptualization, Formal analysis, Funding acquisition, Investigation, Writing – original draft. **Naminata Tondosoma:** Formal analysis, Investigation, Writing – review & editing. **Chiara Virgillito:** Formal analysis, Writing – original draft. **Verena Pichler:** Formal analysis, Writing – original draft. **Paola Serini:** Formal analysis. **Maria Calzetta:** Formal analysis. **Mattia Manica:** Formal analysis, Writing – review & editing. **Zanakoungou Ibrahim Coulibaly:** Conceptualization, Formal analysis, Investigation, Writing – review & editing. **Ibrahima Dia:** Conceptualization, Funding acquisition, Writing – review & editing. **Maurice Akre:** Conceptualization, Writing – review & editing. **Andre Offianan:** Conceptualization, Funding acquisition, Writing – review & editing. **Alessandra Alessandra della Torre:** Conceptualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Chapter 2: Estimation of epidemiologically relevant parameters related to *Aedes albopictus* bionomics in Italy from Mark-Release-Recapture data by mathematical and statistical methods.

In the context of medical entomology, mathematical and statistical modelling can play an important role in the quantification of parameters such as population size, survival, and dispersal that characterize the ecology of vectors and affect the epidemiology of related vector-borne diseases. Knowledge about these parameters in the area exposed to the risk of transmission, whether the disease is already endemic or emerging in the considered area, is valuable to understand disease transmission dynamics and to guide the control strategy necessary to curb pathogen transmission. Parameters characterizing the vector eco-biology are estimated from field and/or laboratory experiments.

Mark-Release-Recapture (hereafter MRR) experiment is the most widely used technique to quantify parameters such as dispersal, population size and survival. Briefly, in MRR experiments collected or reared mosquitoes which are previously marked are released in the field and recaptured through traps placed at different distances in each study area (typically divided into annuli) (Pollock et al., 1990; Silver, 2008). Conventionally MRR data are analysed to estimate epidemiologically relevant entomological parameters: population size of wild insects, survival and dispersal of marked insects.

The present chapter reports the application of conventional and innovative mathematical and statistical approaches to estimate entomological parameters from MRR data collected in Procida island (Gulf of Naples) and in a town in Padua Province. The first study was carried in order to estimate survival, population size and dispersal of *Ae. albopictus* males, while the second aimed to estimate dispersal and survival rate of *Ae. albopictus* females. The results have been published in two scientific articles included at the end of this chapter (Caputo et al., 2021; Virgillito et al., 2021, respectively).

In both studies, eggs were collected in the field by ovitraps, larvae were reared to adults under semi-field conditions, marked with fluorescent dusts (after blood feeding in the case of females) and released in the field. In Procida, male recaptures were performed by BG-Sentinel traps and Human Landing Catches (HLC), while in Padua released females were collected by Sticky traps. In order to have a homogeneous sampling effort across the study areas, this was subdivided in annuli of equal trap density (i.e. more traps were positioned in outer areas than closer to the release site). In Virgillito et al., (2021), batches of marked females were kept under semi-field conditions during the MRR experiments in order to estimate their survival rate.

In the following paragraphs, the statistical and mathematical methods used to estimate survival, population size and dispersal are described, with a focus on advantages and disadvantages of each approach. Each paragraph addresses one entomological parameter and includes the mathematical/statistical methods used to predict them in the two studies.

2.1 Survival Rate

The conventional approach to estimate mosquito survival from MRR data has been to regress the log of the number of the recaptured individuals (usually after adding 1 to eliminate problems related to 0 recapture) as a function of time in days after release and to estimate the survival rate by exponentiating the resulting slope.

In Caputo et al., (2021) a GLM with Binomial distribution was used to estimate the survival rate of marked *Ae. albopictus* males, which is useful to predict the population size of the wild population (Buonaccorsi et al., 2003). GLM was applied on the series of recaptured mosquito males out of the total number of collected males, with the following equation:

$$\log\left(\frac{\pi}{1-\pi}\right) = -\log(N) - \log[S_m(t)] + \log(M), (1)$$

where π is the population fraction of marked mosquitoes, N is the population size, M is the number of mosquitoes released and $S_m(t)$ is a survival function. Assuming that the survival function is an exponential function:

$$S_m(t) = e^{-\lambda t} (2)$$

The equation (1) becomes

$$\log\left(\frac{\pi}{1-\pi}\right) = -\log(N) - \lambda t + \log(M), (3)$$

Where λ is the rate of survival function and t is the time (days) between release and recapture of marked mosquitoes as in Cianci et al., (2013). In our analyses we chose an exponential function, but this can be changed to another function should more information about the survival function of mosquitoes be available for the field situation being studied. The estimates of daily survival of marked males obtained from equation (3) ranges from 0.8 to 0.95 according to the sampling area (200 m and 50 m from the release point) and the trap type (ie BG-traps and HLC collections) considered in the estimation. Our estimates are in the range of estimates for not-marked males in Switzerland = 0.88 Vavassori et al., (2019), even if another statistical method has been used (ie Kaplan Meir). The Kaplan Meir method is based on the comparison between survival curves from the unmarked and the marked cohorts by the log-rank test. In our analysis we chose not to use Kaplan Meir as it allows to assess the effect of one factor at the time and cannot be used for multivariate analysis.

In Virgillito et al., (2021) mosquito mortality was calculated on results of an assessment carried out on marked mosquitoes under semi-field conditions in parallel with the MRR experiment carried out in Padua (see Material and Method section in the manuscript for further details). Empirical data were fitted with several functions: (i) $M_0 e^{-\sigma t}$ exponential, (ii) $e^{-\frac{A}{B}(1-e^{Bt})}$ Gompertz, (iii) $e^{-\frac{c}{d}t^d}$ Weibull, (iv) $\frac{e^{a+bt}}{1+e^{a+bt}}$ GLM; where t is days of experiment, M_0 is the initial number of mosquitoes in the cages σ , A , B , c , d are free-parameters estimated through the Non-Linear Square Levenberg-Marquardt (hereafter nlsLM) method, finally a is the intercept and b the slope estimated through the GLM using Binomial distribution. For each function we computed the likelihood of daily mosquito mortality, which is assumed to follow a binomial distribution, for each tested function. Therefore, the likelihood of the observed data was:

$$L(p, x, n) = \prod_t p_t^x (1 - p_t)^{n-x}$$

where t is the days of semi-field experiments, x is the number of observed marked mosquitoes dead, n is the total number of mosquitoes in the cages and p is the probability of marked mosquitoes dead (in our case is the fitted values obtained from the exponential, Gompertz and Weibull function and GLM model). In addition, we evaluated the Root Mean Square Error (RMSE) for each function to quantify differences between predicted and observed values. Finally, we selected the best function for mortality rate considering both measures, we chose the function that presented the maximum value of likelihood and minimum value of RMSE. The Levenberg-Marquardt (LM) algorithm is an iterative technique that locates the minimum of a function that is expressed as the sum of squares of nonlinear functions. We choose to use LM algorithm since we treated the mortality of marked mosquito as nonlinear problem and LM is a standard technique for nonlinear least-squares and can be thought of as a combination of steepest descent and the Gauss-Newton method (Bates et al, 2008).

2.2 Population size

The basic reproduction number, R_0 , combines, in a weighted way, the factors that determine whether a pathogen can become established in an area where it is introduced. One of the factors included in the R_0 formula, to which the numerical value is very sensitive, is the ratio of vectors to hosts (Hartemink et al., 2011, 2009). The more precise the estimate of the population size, the better the estimate of R_0 .

In Caputo et al., (2021), we predicted the population size of wild *Ae. albopictus* male using Fisher-Ford's (Dowdeswell et al., 1940; Fisher and Ford, 1947). This method is not probabilistic and does not treat sampling uncertainty properly. The Fisher-Ford's equation, modified for low recapture rate (Bailey, 1952) is the following:

$$N = \frac{\varphi^t(n+1)(M+1)}{m+1}, \quad (4)$$

where N is the population size (the unknown value that we want to calculate), φ is the marked male survival function, t is the time between release and capture, n is the number of both marked and unmarked mosquitoes captured, m is the number of marked mosquitoes recaptured and M is the number of marked mosquitoes released. To calculate the confidence intervals related to the estimates we calculated it with the method of percentile bootstrap (Efron et al., 1993) based on 1000 bootstrap replicates at 95% level.

Fisher-Ford's deterministic equations has the limitation that the value of survival rate of marked male (φ) is taken from the literature. To overcome this limitation, in Caputo et al., (2021) we applied GLM, in logistic regression framework, previously developed in Cianci et al., (2013). So, we used model (3) in which we estimated the survival rate of wild mosquito males (λ in the model 3) and through this estimate, N can be estimated from the intercept of the model (3),

$$\alpha = -\log(N); \quad (5)$$

$$\hat{N} = \exp(-\hat{\alpha}). \quad (6)$$

The approximate 95% confidence interval has been calculated by:

$$\exp[-\alpha - zSE(\hat{\alpha})] < N < \exp[-\alpha + zSE(\hat{\alpha})], \quad (7)$$

Where z is a standard normal distribution.

As shown in Table 5 and Figure 6 in Caputo et al., (2021) the Fisher-Ford's equation estimate lower mosquitoes males/ha rather than the GLM model, either considering the sampling area (200 m and 50 m from the release point) and the trap type (ie BG-traps and HLC collections). This may be due to the fact that in the Fisher-Ford's equation we used a survival rate from the literature (and did not estimate it from MRR data) and providing a distort estimate of population size. However, it is important to stress that both methods should be improved in order to obtain more accurate population size estimate. For instance, our estimate did not take into account any spatial correlation into the traps. The spatial correlation could potentially influence the estimate of population size since the

introduction of a parameter that takes into account the space (ie the location of traps) may influence the estimate of the dependent variable (in our case the population size).

2.3 Dispersal

In Caputo et al., (2021) we estimated the dispersal of adult mosquitoes males as mean distance travelled a parameter which is not inherently biased for trap location or size of study area (Lillie et al., 1985; Morris et al., 1991; White and Morris, 1985). The MDT equation depends on the number of annuli in the study area and on the number of recaptures that would be expected if trap density was constant within annulus (ER, expected recapture). ER follows the equation

$$ER = \frac{N^{\circ} \text{ of recaptured in each annulus}}{N^{\circ} \text{ traps in each annulus}} * CF \quad (8),$$

CF is a correction factor to account for differences in trap densities among annuli

$$CF = \frac{\text{area of the annulus}}{\text{trapping area}} * \text{Number of traps in the study areas} \quad (9)$$

The estimate of MDT for marked males for BG and HLC data was similar, showing that the MDT is not influenced by sampling methods. As discussed in Caputo et al.,(2021) the marked males showed a short-range dispersal <60 mt daily distance, and our estimates are lower than the estimates for *Ae. albopictus* females in Italy (Marini et al., 2019). It is important to note that the equation of MDT does not consider any information about the time, space and other abiotic factors that could have an influence on the estimate of mosquito dispersal.

In Virgillito et al., (2021) we tried to bridge this gap, providing a more realistic representation of the dispersal process using an advanced mathematical equation, based on partial differential equations (PDEs) in a stochastic framework. The big advantage in the use of PDEs is the possibility of including time, space, and daily mortality in only one mathematical equation, thus, the equation used in Virgillito et al., (2021) had this form

$$\frac{\partial M}{\partial t} = D \left(\frac{\partial^2 M}{\partial x^2} + \frac{\partial^2 M}{\partial y^2} \right) - M(\mu + \beta) \quad (10)$$

where μ is the mortality rate, β is the capture rate, (x, y) represent location coordinates as distance (in meters) along the x and y spatial axis from a given origin (x0, y0) respectively, t is the time (i.e., days or hours), M(x,y,t) is the density of the mosquitoes of interest (individuals/meters²) at location (x, y) at time t. The term $\left(\frac{\partial^2 M}{\partial x^2} + \frac{\partial^2 M}{\partial y^2} \right)$ indicates the diffusion in space and D is the diffusion coefficient that measures dispersal rate (distance²/time). The dispersal of release mosquitoes was also quantified as Flight range (FR), that measures the area travelled/covered by released mosquitoes in a unit of time. Classical methods, such as the regression model, estimate the FR with the cumulative number of expected recaptures at the end of the MRR experiment. FR50 and FR95 values were calculated from the equation of regression line as 50% and 95% of the largest ER value. If the classical statistical methods have been used the cumulative number of expected recaptures at the end of the MRR to calculate FR, while the PDE method used the daily recapture. The FR equation by PDE method is

$$FR = Norm((0; 0), \sqrt{2Dt}) M_0 e^{-\mu t} \quad (11)$$

Where M_0 is the initial number of females marked release.

As discussed in Virgillito et al., (2021) the FR95 resulting from the PDE approach is significantly higher than the one estimated using regression analysis (Marini et al., 2019). This is maybe due to the fact that since the standard regression approaches for the computation of the flight range do not take into account time dependency, but they model cumulative captures, over the entire study period, as a function only of the distance.

In Virgillito et al., (2021) we tried to obtain more realistic dispersion estimates by improving existing methods. Recently other studies have tried to improve estimates of both population size and dispersion. Villela et al., (2015) developed a hierarchical Bayesian model in order to estimate the population size and spatial distribution of wild mosquitoes. One big advance of hierarchical model is that it involved more complex mathematical calculations, and this allows to take into account some factors that could influence the estimation of population size, such as mosquito ecology (i.e. survival rate), technical data (trap probability to capture mosquito as function of distance from release point) and dispersal of mosquitoes. In Marini et al., (2019) a GLM was used, with various probability distribution (i.e Bernoulli, Gamma) and Zero Altered Gamma model (hereafter ZAG) in order to provide estimates of dispersal of mosquitoes taking into account the time of release.

Aedes albopictus bionomics data collection by citizen participation on Procida Island, a promising Mediterranean site for the assessment of innovative and community based integrated pest management methods

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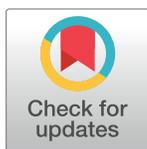
RESEARCH ARTICLE

Aedes albopictus bionomics data collection by citizen participation on Procida Island, a promising Mediterranean site for the assessment of innovative and community-based integrated pest management methods

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Abstract

In the last decades, the colonization of Mediterranean Europe and of other temperate regions by *Aedes albopictus* created an unprecedented nuisance problem in highly infested areas and new public health threats due to the vector competence of the species. The Sterile Insect Technique (SIT) and the Incompatible Insect Technique (IIT) are insecticide-free mosquito-control methods, relying on mass release of irradiated/manipulated males, able to complement existing and only partially effective control tools. The validation of these approaches in the field requires appropriate experimental settings, possibly isolated to avoid mosquito immigration from other infested areas, and preliminary ecological and entomological data. We carried out a 4-year study in the island of Procida (Gulf of Naples, Italy) in strict collaboration with local administrators and citizens to estimate the temporal dynamics, spatial distribution, and population size of *Ae. albopictus* and the dispersal and survival of irradiated males. We applied ovitrap monitoring, geo-spatial analyses, mark-release-recapture technique, and a citizen-science approach. Results allow to predict the seasonal (from April to October, with peaks of 928–9,757 males/ha) and spatial distribution of the species, highlighting the capacity of *Ae. albopictus* population of Procida to colonize and maintain high frequencies in urban as well as in sylvatic inhabited environments. Irradiated males shown limited ability to disperse (mean daily distance travelled <60m) and daily survival estimates ranging between 0.80 and 0.95. Overall, the ecological characteristics of the island, the acquired knowledge on *Ae. albopictus* spatial and temporal distribution, the high human

Data Availability Statement: All relevant data are within the manuscript and its [Supporting Information](#) files.

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and *Ae. albopictus* densities and the positive attitude of the resident population in being active parts in innovative mosquito control projects provide the ground for evidence-based planning of the interventions and for the assessment of their effectiveness. In addition, the results highlight the value of creating synergies between research groups, local administrators, and citizens for affordable monitoring (and, in the future, control) of mosquito populations.

Author summary

Mosquitoes represent a nuisance as well as public health risk due to their ability to transmit pathogens to humans. The Asian tiger mosquito *Aedes albopictus* is an invasive species nowadays established in all Mediterranean countries, reaching highest abundance in Italy. Innovative control approaches have been proposed to complement conventional ones and to increase the success of the fight against this aggressive day-biting species, but still need to be validated in the field. Small islands are ideal places for these validation experiments as they are naturally protected by the spill over of mosquitoes from neighbouring untreated areas. The island of Procida, in the gulf of Naples (Italy), has the right ecological characteristics (e.g., homogeneous landscape and high densities of human and *Ae. albopictus* populations) to represent an ideal experimental site. In collaboration with the Municipality and the residents we obtained relevant data on the mosquito distribution and seasonality on the island and performed preliminary experiments, creating an instrumental baseline information which will facilitate the effective testing of novel control schemes.

Introduction

The Asian tiger mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae) is an invasive species that in the last few decades has greatly expanded its range from southeast Asia to all other continents except Antarctica, mostly by the passive transportation of its drought-resistant eggs via used tire trade [1]. Since the first identification in Albania in 1979 [2], this exotic species invaded several European countries thanks to its ability to adapt to seasonal variations and to use man-made water containers for oviposition. Italy is one of the most heavily infested countries in Europe. *Ae. albopictus* was first reported in 1990 in Genova (northwest Liguria region) [3] and quickly spread over the whole territory, in particular in the northeast area and central and southern coastal areas, including major and minor islands [4,5]. *Ae. albopictus* represents a relevant public health risk due to its vector competence for pathogens such as arboviruses and filarial worms [6]. In European Mediterranean regions it has been involved in the last decade in various autochthonous transmission events of chikungunya virus (CHKV) (France [7]), dengue virus (DENV) (Spain [8], Croatia [9] and France [10]) and, more recently, of Zika virus (ZIKV) (France [11]). In Italy in 2007, *Ae. albopictus* was the primary vector during the first European chikungunya outbreak, with >200 human cases in northeast Italy (Emilia Romagna) [12,13]. Ten years later, a second chikungunya outbreak occurred in central and south Italy (Lazio and Calabria), with >500 cases, including cases in the metropolitan city of Rome [14,15] and in a coastal village of south Italy (Calabria) [14].

Mosquito spreading is contrasted with several different methods including microbial larvicides or chemical pesticides. The negative impact of the overuse of chemical compounds on the environment and on human health, the problem of growing insecticide resistance in

mosquito populations including *Ae. albopictus* [16–18], and the absence of available vaccines against most arboviruses, render the development of eco-sustainable alternative mosquito-control methods an urgent need, as recently underlined by WHO [19].

A promising experimental complementary methods for mosquito control are represented by the Sterile Insect Technique (SIT) [20] and the Incompatible Insect Technique (IIT) [21]. SIT relies on mass rearing and mass release of radiation-sterilized males into target areas to suppress local infesting populations. SIT technique has been successfully applied in the frame of area-wide integrated pest management (AW-IPM) programs [22], against several insect species including agricultural pests (*Ceratitis capitata*, *Cydia pomonella*), livestock pests (*Cochliomyia hominivorax*) and vector species such as the tsetse fly *Glossina austeni* [23–26]. SIT potential against mosquitoes has been experimentally demonstrated for the first time in Italy with a three-year long study based on the release of 2 million sterile males of *Ae. albopictus* [27]. By releasing 896–1,590 sterile males/ha/week in small villages it was possible to induce egg sterility in the range 18.72–68.46% causing a significant decline in the egg density [28,29]. From these encouraging pilot studies, the development of experimental SIT tests against mosquitoes has rapidly advanced in recent years thanks to the research and coordinating efforts of the Joint Food and Agriculture Organization of the United Nations (FAO)/International Atomic Energy Agency (IAEA) Insect Pest Control Subprogramme and their collaborators, involved in the development of mass rearing devices, sexing systems and protocols for field evaluation for both *Anopheles* and *Aedes* species [30,31]. IIT relies on mass rearing and mass release of males depleted of their natural *Wolbachia* endosymbiont and harbouring a *Wolbachia* strain different from that present in the wild target mosquito populations. *Wolbachia* is a gram-negative bacterium, a common symbiont of insects, including mosquitoes [32], capable of inducing cytoplasmic incompatibility, i.e., mating between *Wolbachia*-infected males and wild females, without or with a different *Wolbachia* strain, results in embryonic lethality [33]. Pilot studies carried out in Kentucky (USA) and Italy support IIT as a valuable approach to suppress *Ae. albopictus* populations [34,35]. Combining SIT and IIT and releasing millions of factory-reared *Ae. albopictus* adults over a two-year period on two small islands in Chinese city of Guangzhou, Zheng and colleagues have recently demonstrated that the near elimination of field populations of a mosquito specie is achievable [36].

More than thirty SIT or SIT/IIT pilot trials are ongoing worldwide, mainly against *Aedes* mosquitoes [37]. Recently, IAEA and WHO released a guidance framework for the assessment of the feasibility of SIT as a mosquito-control tool against *Aedes*-borne diseases [38] and a phased conditional approach guideline for mosquito management using SIT [37]. According to these guidelines, testing and implementation of Integrated Pest Management (IPM) techniques for mosquito control, including a SIT and/or IIT components, require, as essential premises, i) the selection of a proper and, possibly geographically isolated, site, ii) the baseline data on the bionomics of the local infesting mosquito populations and iii) the local community engagements [37]. In particular, a strong support and commitment of the local community and the early engagement of stakeholders, including residents in study sites and policy makers, are considered essential for the success of field trials [38].

The island of Procida, in the Gulf of Naples (Italy), offers a unique combination of features ideal for the testing of innovative IPM approaches against *Ae. albopictus*. First, the ecological and demographic features: i) a small size (only 4.1 km²), ii) a completely urbanized and accessible territory, iii) a high abundance of small water deposits (e. g., containers in private gardens), iv) a high human population density (27.83 inhabitants/ha—ISTAT 31/12/2020), and v) a history of infestation by *Ae. albopictus*, in the last ten years. Second, the willingness of citizens to participate to the project. Anecdotal evidence collected on the island show that many Procida citizens are familiar with SIT and its effectiveness in insect pest control programs.

This is mainly due to field performance tests of sterilized males of the Mediterranean fruit fly *Ceratitis capitata*, performed in the island during 1970's and 1980's, in a cooperative programme between the Italian National Committee for Research and Development of Nuclear Energy (ENEA) and the IAEA [39,40]. About 20 million sterile Mediterranean fruit fly males were released on the island from April to July 1986 leading to population suppression and protection of citrus fruits and therefore to a very positive perception of SIT by the residents thereafter.

In this paper, we tested a community-based approach to collect baseline data about *Ae. albopictus* bionomics on Procida. Here, we present the results of a four-year project, successfully implemented in strict collaboration with local administrators and citizens. We were able to collect baseline data on the temporal dynamics and the spatial distribution of *Ae. albopictus* on the island; to estimate the *Ae. albopictus* wild population size; and to estimate the dispersal and survival rates of irradiated *Ae. albopictus* males. These data provide insights on the bionomics of the Asian tiger mosquito in southern Europe and draw attention to Procida Island as an ideal site for testing innovative and community-based control programs against *Ae. Albopictus*. The island can be a study model to promote future implementation in other Mediterranean and temperate areas.

Methods

Ethics statement

This research was approved by the University of Naples Federico II CSV Ethics Board (Protocol # PG/2020/0090230) and by the Municipality of Procida with municipal resolution n° 52 of 07 July 2016. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was taken from all the participants involved in the study. Authors of the present manuscript and Procida volunteers gave their written consent to their participation to the study and to be photographed and to have their images published under a creative commons license. All authors declare no competing interests.

Study site

Procida is a 3.7 km² flat volcanic island (on average 27 m above sea level) in the gulf of Naples (Italy) with a 16 km-long rough coastline. The Procida territory is uniform in its organization, highly urbanized and completely accessible (S1 Fig). It is organized in hundreds of small residential buildings surrounded by private green areas including ornamental flower and vegetable gardens, orchards with citrus plants and family-type chicken and rabbit shelters. This landscape is associated with an extensive presence of anthropic water sources, representing ideal breeding sites for *Ae. albopictus* larvae. The island has a population density of 27.83 inhabitants/ha (ISTAT 31/12/2020) which approximately doubles during the touristic summer season. A 0.4 km² satellite island, Vivara, a natural uninhabited reserve, completes the Procida territory. The climate is temperate with an average annual temperature of 16.2°C and an average annual precipitation of 797 mm (<https://en.climate-data.org/>) and it is classified as Csa by Köppen and Geiger (Csa = Hot-summer Mediterranean climate) [41].

Citizen science and community engagement strategy

All experimental field activities presented in this work were performed in strict collaboration with local administrators and citizens of Procida, following the workflow graphically described

in the S2 Fig. The first step was to establish a relationship of mutual trust with the local administration by presenting the *Ae. albopictus* monitoring project, clarifying the logistical support and authorizations needed and drawing a memorandum of understanding between the Municipality and the University of Naples, which was approved with a public deed in July 2016. In parallel to these actions, in September 2015, we assessed the propensity of the island's citizens to volunteer in contributing to our project by administering a questionnaire to about 200 randomly selected Procida residents. The questionnaire included one question to assess the knowledge about the public health relevance of the Asian tiger mosquito, six questions on the preventive measures implemented by people to protect themselves from mosquitoes, and five questions about people possible commitment in control programs against the Asian tiger mosquito. The same questionnaire was administered again in September 2019 to a similar number of randomly selected persons on the island.

Local administrators assisted us in selecting twelve volunteers interested in participating in the *Ae. albopictus* temporal dynamic monitoring effort using ovitraps (S3 Fig). A one week-long workshop, led by expert operators, was organised on site to train the volunteers on how to manage ovitraps and collect germination paper strips to be delivered weekly for egg counting. In April 2016, four teams, composed each of an expert operator, a municipality member and three volunteers, visited the island territory to select twenty-six sites for the ovitrap positioning (S4 Fig). Volunteers managed 13 out of 26 ovitraps from April 2016 to December 2016, while the remaining ovitraps were managed by expert technicians. Then, with a second round of visits to households, we selected 79 families who allowed the access to their private properties to place the ovitraps required for the spatial analysis. 83 households were visited over four days by three teams organized as described previously. Each visit took about 15–20 minute. During the visit, the team members talked with the residents to explain the aim of the study, its relevance for a future control program on the island based on the release of sterile mosquito males and a pamphlet with general information about mosquito biology and control measures was hand-delivered. With a similar approach and supported by a local “facilitator” recommended by Procida administrators, we involved in September 2018 the local community of “La Chiaiolella” area for the mark-release-recapture experiments. Twenty families, two local merchant associations and a parish, which agreed in hosting in their private properties the recapture stations (see below) and two field laboratories for the managing of the sampling instruments and the egg/adult quantification (S3 Fig). The launch of the project in 2016 and the participation of volunteers as citizen scientists during the whole research project period, were covered by various press releases on local and national newspapers, in collaboration with the media office of the Procida municipality (S2 Fig).

During the study period (September 2015 –September 2019), several additional public activities were performed to further promote public awareness and participation of the Procida community to our experiments (S2 Fig). These include: 1) two informative campaigns on the island (October 2017 and September 2018) with hand-distribution of pamphlets about the project progresses and general information about mosquito biology and control measures. 2) one educational activity (May 2019) with fifty students at the secondary school about mosquito biology, monitoring and control. The activity included stereomicroscope observations of life-stages of *Ae. albopictus* in the classroom, in collaboration with science teachers, and homework observations, to be performed under the supervision of the student's parents, of mosquito larvae provided in Falcon tubes to each student. Students were asked to observe and to take photos and drawings of the larvae during its development and to identify the species and the sex of the hatched adults, reporting the results in a brief relation. 3) two public outreach events, held during the European Researchers' Night Week (September 2018 and 2019) as part of the Marie Skłodowska-Curie Actions. Laboratory activities were organized to let citizens to

perform egg counting, sterile males' collections using electric aspirators and observations of mosquitoes marked with fluorescent powder under the stereomicroscope.

Ovitrap collections for temporal and spatial population dynamics

Cylindrical black plastic jar, 15 cm high, 12 cm in diameter, with an overflow hole at 8 cm from the base, were utilized as ovitraps. Ovitrap were filled with about 600 ml of tap water and walls were lined with heavy-weight seed germination paper strips, 30 x 9 cm in size, (Anchor Paper Co. USA) as oviposition substrate. The strips were lined to the internal wall of the ovitraps to overcome the overflow hole and to prevent possible egg loss due to water overflow. Ovitrap were located at ground level, in shaded areas near the vegetation. For the temporal analysis, 26 ovitraps were distributed all over the island and monitored weekly from 2016-04-14 to 2016-12-31 (S4 Fig and S1 Table). For the spatial analysis on Procida Island, 75 additional ovitraps were deployed across the island and monitored weekly, together with the previous 26, for two weeks in July 2016 (weeks 29–30) and two weeks in September 2016 (weeks 37–38). For the spatial analysis on Vivara Island, 31 ovitraps were located along the two main paths of island and monitored in September 2018 (week 38) and in July 2019 (week 28) (S1 Table and S5 Fig). Collected germination paper strips were brought to the laboratory and eggs counted under a stereomicroscope.

Production of sterile males

Experimental sterile *Ae. albopictus* male mosquitoes were produced from eggs collected in June 2018 by ovitraps within the study area. Eggs were transported to the laboratory of Sanitary Entomology and Zoology at the Centro Agricoltura Ambiente “Giorgio Nicoli” in Crevalcore (CAA—Bologna, Italy) and utilized to establish a Procida *Ae. albopictus* strain named PRO1. PRO1 strain was reared as previously described [28] in a climate-controlled insectary ($28 \pm 1^\circ\text{C}$, $80 \pm 5\%$ RH, 14:10 h L:D photoperiod). CAA is certified with ISO9001, ISO14001 and ISO45001. Larvae obtained after standardized hatching procedures [27] were reared at a fixed larval density (2 larvae/ml) and fed with a standard diet of brewer yeast (IAEA-BY) liquid diet (5.0% w/v) at a mean daily dose of 0.5 mg/larvae [42] for the first four days of development. Pupae were harvested once per cycle at about 24 h from the beginning of pupation and males were separated using a 1,400-micron sieve (Giuliani Tecnologie S.r.l., Via Centallo, 62, 10156 Torino, Italy). With this method, males can be separated out with 99.0% accuracy [43]. However, for this trial particular attention was paid to reduce the female contamination in the released males. Quality control tests carried out on samples of approximately 300 pupae, resulted in 0% of females. Collected male pupae were aged 24 h before being subjected to the irradiation treatments at the Medical Physics Department of St. Anna Hospital (Ferrara, Italy) using a gamma irradiator (IBL 437C, CIS Bio International, Bagnols sur Ceze, France; 65.564 TBq ^{137}Cs linear source) at a dose of 35 Gy and a dose rate of 2.1 Gy/min ($\pm 3.5\%$). Following the irradiation procedure, no loss of pupae was observed. Irradiated pupae were then placed in petri dishes (12 cm diameter) filled with water and placed in cardboard boxes (12 x 12 x h 18 cm), supplemented with additional separators to increase the resting areas, closed at the top with mosquito net, for emergence and shipment. Cotton pads soaked with 10% sugar solution were provided and secured at the top of each box. Each box contained 1,500–2,000 adults and provided a vertical resting surface area of 1.3–1.0 cm²/adult. Mosquito boxes were maintained at about 21°C for two days. After adult emergence the cardboard boxes were transferred inside larger polystyrene container with adequate quantity of gel packs to maintain a constant temperature of 10–15°C during shipment. About 20,000 adult sterile males were sent by express courier, via ground transportation, in two different expeditions.

Mark-release-recapture (MRR) experiments

Sterile male releases were performed on 14 (MRR1) and 21 (MRR2) September 2018, at 15:00 PM (40°45'04.6"N, 14°00'27.5"E). Temperature and relative humidity at the release sites were 28°C and 59% and 27°C and 61% during MRR1 and MRR2, respectively. Immediately before release, males were marked, at room temperature, with a coloured fluorescent powder (PRO-CHIMA s.r.l., Calcinelli di Saltara (PU), Italy, product code PG-661). A fix dose of fluorescent powder (0.3gr/1000 males) was used per each cardboard box and applied, using a manual insufflator (Hygienic vaginal douche, mod. IntimWash, PicSolution, Italy), to disperse the powder uniformly on mosquitoes, following the FAO/IAEA guidelines for Mark-Release-Recapture procedures of *Aedes* mosquitoes [44]. This marking procedure should ensure a successful capture of marked individuals up to 17 days from the release as shown also in Marini et al., 2010 [45]. Fluorescent dust coverage on male body parts was evaluated on samples of about 100 mosquitoes randomly collected from cardboard boxes upon each release. The use of an UV light source was employed to facilitate the identification of dust on the collected male mosquitoes. A purple and green dye was used in MRR1 and MRR2, respectively, to differentiate males of the two releases. Dusted 5 days-old sterile males were released by placing and opening the cardboard boxes in a sunny area without vegetation to favour dispersal. The cages were gently shaken for about 30 min, to induce the males to exit. The males that remained in the cage after 30 min were counted and deducted from the total. Recaptures began approximately 24 hours after each release and performed daily for 13 consecutive days in MRR1 and for 6 consecutive days in MRR2. Recaptures were performed in 39 sampling stations distributed in four concentric annuli (50 meter-distance from each other) around the release point, located in the touristic district of "La Chiaiolella" (~3.1 stations/hectare), in the southern part of the island (S1 Table). At each sampling station, recaptures were performed by BG-Sentinel traps (operating continuously) and by Human Landing Catches (HLC), during the late afternoon peak of *Ae. albopictus* activity (indicatively from 4:30 to 7:30 PM). BG-Sentinel traps baited with BG-Lure were placed at ground level in shaded locations close to domestic areas. HLC has just been utilized for male *Ae. albopictus* captures in previous works, since males are known to be nearby the human host, attracted by the females [46–49]. HLC were carried out by targeting exclusively adult male and female mosquitoes flying around the operator legs and using locally made battery-powered hand-held electric aspirators for 15 minutes at each station. We did not perform any male mosquito collections within the vegetation. Field-collected mosquitoes were identified using ECDC morphological keys [50] and marked males were detected under stereomicroscope and UV lamp.

Eco-climatic parameters

A spatial dataset to analyse land cover, geomorphological and climatic variables on Procida Island has been generated using open-source Geographic Information System, specifically GRASS GIS [51] for data processing and spatial analysis and Quantum GIS [52] for spatial analysis and layout generation. Land cover variables were retrieved from supervised classification of digital multispectral aerial imagery collected on 16 June 2016 and 07 May 2011 at 0.5 m spatial resolution (Source: Italian National Geoportal, b), using the methodology described in Manica et al. (2016) [53]. Mapped land cover classes were 'trees', 'grasslands', 'roads/concrete', 'buildings', 'bare soil', 'water bodies', 'seawater'. Two main classes are derived from the land cover classified map: artificial surfaces' (including 'roads/concrete' and 'buildings') and 'natural cover' (including 'wood', 'grassland', 'bare soil'). Topography of Procida Island is described in the Digital Terrain Model (DTM) at 2 m spatial resolution, generated from LiDAR acquisitions (Source: Italian National Geoportal, <http://www.pcn.minambiente.it/GN/>). The

following additional geomorphological descriptors have been computed from DTM data using GDAL library (GDAL/OGR contributors, 2020): slope, aspect, terrain roughness, Topographic Position Index (TPI) and Terrain Ruggedness Index (TRI). In the context of climatic variables, daily spatial maps at 30 m spatial resolution of air temperature climate variable on Procida has been computed combining *in-situ* measured meteorological data and satellite estimated Land Surface Temperature (LST). LST data represent the estimation of skin temperature detected at earth surface by remote sensing sensor. Meteorological data were downloaded from the 'Ciraccio—INAPROCI2' weather station using the Weather Underground database (<https://www.wunderground.com/>). LST has been estimated from satellite images acquired by OLI and TIRS sensors aboard LANDSAT-8 satellite due to unavailability of LST estimates from MODIS. LST maps at 30 m spatial resolution were computed using the Plank equation, after estimating brightness temperature and emissivity from LANDSAT-8 satellite spectral bands. A total of 16 cloud free satellite images acquired throughout solar year 2016 have been used to estimate LST, using the Land Surface Temperature Estimation QGIS Plugin [54]. A regression analysis has been performed to transform LST estimates, describing the skin temperature of earth surface objects, to the located above air temperature, measured by the weather station at the same satellite acquisition time. The analysis allowed the creation of a regional regression model, that has been trained only accounting for LST estimates in the pixel corresponding to meteorological station location. Finally, the regression model has been used to estimate spatial maps of daily air temperature in Celsius degrees for year 2016 from *in-situ* meteorological measurements, accounting for the spatial variability described in LST maps.

Statistical analysis

Temporal analysis. A generalized linear additive mixed model (GAMM) was used to assess the relationship between the number of *Ae. albopictus* eggs collected in the 26 ovitraps monitored weekly from April to December 2016 and meteorological variables. GAMM was applied on the series of collected eggs with the following equation:

$$Eggs_{ij} = NB(\mu_{ij}, \theta), \quad (1)$$

$$E(Eggs_{ij}) = \mu_{ij}; \text{Var}(N_{ij}^h) = \mu_{ij} + \frac{\mu_{ij}^2}{\theta}, \quad (2)$$

$$\log(\mu_{ij}) = \beta_0 + \beta_1 Rain_{ij} + \beta_2 Wind_{ij} + f(Temperature_{ij}) + \varepsilon_i, \quad (3)$$

$$\varepsilon_i \sim N(0, If_{trap}^2), \quad (4)$$

$Eggs_{ij}$ is the total number of collected eggs at collection week j in ovitrap i and was assumed to follow a Negative Binomial distribution of mean μ_{ij} and dispersion parameter θ (Eqs (1) and (2)). A log link function was considered to model μ_{ij} as a function of independent variables (Eq (3)). $Rain$ is the cumulative precipitation during the week of collection, $Wind$ is the average wind speed during the week of collection, $f(Temperature)$ is the temperature trend modelled by a first order random walk model and $Temperature$ is the average temperature during the week of collection. Given that multiple observations were collected from each ovitrap, ovitrap was considered as random effect (ε , Eq (4)). Penalized complexity priors ($U = 0.05$; $\alpha = 0.05$) were used for the random walk model of the temperature trend, a log-gamma distribution was used for the priors of the log-transformed precision of the random effect trap, while normal distributions of mean 0 and precision 0.001 were used for the priors of β parameters.

Finally, all quantitative independent variables were standardized (subtracted their mean value and divided by their standard deviation) [55]. GAMM was fitted in a Bayesian framework using INLA [56] and R version 3.5.1 [57]. Assessment of the statistical assumptions in the model was carried out by autocorrelation function, variogram and graphical inspection of the residuals. Model fit was evaluated by computing the Bayesian p-value for each observation and the conditional predictive ordinate (CPO) using 'leave one out' cross validation. 2016 and 2017 meteorological data was used to predict the mean number of eggs in ovitrap for weekly collection and estimate the start and end of the breeding season. The start and end of the season were defined as the week when the cumulative number of eggs collected exceed the 5% and 95% quantile, respectively.

Spatial analysis. The geo-referenced field data from the 101 ovitraps monitored in July and September 2016 were identified on the projection system UTM Zone 33N with datum WGS84 (EPSG code 32633), relating to the Italian cartographic system. The vector and raster maps were prepared and visualized on the Open-Source Quantum GIS version 2.18.2 Las Palmas [52] software and the spatial statistical analysis was undertaken using interpolation by the kriging method on the Open Source R v3.3.2 [57], gstat package [58]. In geo-statistics, the random field (RF) $Z(u)$ is assumed to be intrinsic second order stationary if the first two moments (i.e., the mean or trend component m and the semi-variance $\gamma(h)$) of the two point RF increments exist and are invariant under translation and rotation within a bounded area D [59,60]:

$$m = E\{Z(u)\}, \quad (5)$$

$$\gamma(h) = \frac{1}{2}E\{[Z(u) - Z(u+h)]^2\}, \quad (6)$$

with theoretically infinite points locations $u(x) \in D$, and random variables (RV) $Z(u)$ and $Z(u+h)$ separated by the distance vector $h(x)$, where x represents the spatial coordinates $(x_1, x_2) \in \mathbb{R}^2$ in our study domain. In ordinary kriging the mean is deemed stationary in the local neighbourhood of locations u and unknown, which brings to the following kriging system in matrix notation:

$$Kl(u) = k, \quad (7)$$

Geostatistical interpolation offers the possibility to use the spatial dependency of the variable under investigation to get on the basis of field observations, the values of that target variable at any unsampled or unknown location over the whole study area—in our case in the area of the Procida and Vivara islands. Kriging weights:

$$l(u) = K^{-1}k, \quad (8)$$

are calculated after the fitting of an experimental variogram through an allowed model variogram, which allows in turn to derive the vector of data to unknown covariance:

$$k = \begin{bmatrix} C(u_1 - u) \\ \vdots \\ C(u_n - u) \end{bmatrix}, \quad (9)$$

The vector of weights:

$$l(u) = \begin{bmatrix} l_1(u) \\ \vdots \\ l_n(u) \end{bmatrix}, \tag{10}$$

is calculated by solving the kriging system in equation [8], where:

$$K^{-1} = (K^T K)^{-1} K^T, \tag{11}$$

The map of interpolation by ordinary kriging (OK) is calculated for each unknown location u of the grid by iterative linear combination of kriging weights with measurements $z(u_a)$ at sampling locations [59]:

$$z(u) = \sum_{a=1}^{n(u)} l_a(u) z(u_a), \tag{12}$$

Mean distance travelled, population survival and size. Mean distance travelled (MDT) was computed to estimate the dispersal of marked *Ae. albopictus* males from MRR experiments, taking into account the unequal trap densities within each annulus [61,62]. MDT is independent of the position of the traps or size of study area [63–65], and is defined by the following equation:

$$MDT = \frac{\sum_{i=1}^a ER_i \gamma_i}{\sum_{i=1}^a ER_i}, \tag{13}$$

where, a is the number of annuli ($a = 4$), is the median distance of each annulus, ER is the number of recaptures that would be expected if trap density were constant within annulus. See [S1 Text](#) for further details.

Based on MDT results, which provide an estimate of the area to consider in the estimation of the population size, a Generalized Linear Model (GLM) with Binomial distribution was used to estimate the population survival rate of marked *Ae. albopictus* males and the population size of the wild population [66]. GLM was applied on the series of collected marked males out of the total number of collected male mosquitoes with the following equation:

$$\log\left(\frac{\pi}{1 - \pi}\right) = -\log(N) - \lambda t + \log(M), \tag{14}$$

Where N is the population size, M is the number of mosquitoes released, λ is the rate of survival function and t is the time (days) between release and recapture of mark mosquitoes as in Cianci et al. (2013). See [S2 Text](#) for further details.

Moreover, the Fisher-Ford’s method modified for low recapture rate [66] was also applied to provide a second estimate of population size. The Fisher-Ford’s equation is the following:

$$N = \frac{\phi^t (n + 1)(M + 1)}{m + 1}, \tag{15}$$

where N is the population size, ϕ is the marked male survival function, t is the time between release and capture, n is the number of both marked and unmarked mosquitoes captured, m is the number of marked mosquitoes recaptured and M is the number of marked mosquitoes released. The confidence intervals related to the estimates are calculated with the method of percentile bootstrap [67] based on 1000 bootstrap replicates at 95% level.

Results

Community-engagement assessment

Over four years of activities on Procida Island, ~300 residents were involved as citizen scientists or collaborators. Twelve persons, including the Procida mayor himself and two municipal counsellors, contributed as volunteers to the ovitrap monitoring. Ten out of twelve volunteers participated in the ovitrap monitoring for a complete *Ae. albopictus* season, from April 2016 to December 2016. The two remaining volunteers dropped out after three months of activity, because of holidays abroad or loss of interest in the project. About 300 persons, belonging to 99 household families, contributed to the spatial analysis of *Ae. albopictus* distribution on the island and to the mark-release-recapture experiments. In addition, we estimated that ~2,000 people were directly or indirectly contacted during the four years of activity on the island (direct participation as volunteers, household families, students and their families, people receiving informative pamphlets, people participating in surveys and to the public outreach activities). In September 2015 we evaluated, using a questionnaire, the knowledge of Procida citizens about *Ae. albopictus*-associated public health risks, the kind of practices they utilized to protect themselves by mosquitoes on the island and their attitude in supporting and participating in mosquito research and control campaigns (Table 1).

Among the 200 randomly selected people interviewed (~2% of the resident population), 77% resulted aware of the capacity of mosquitoes to transmit diseases, 85% declared to use electric diffusers, mosquito nets or chemical repellents to protect themselves from bites, only 11% removed water containers from their properties and only 3% used larvicide products in standing water. The large majority of interviewed people was in favour of a mosquito control programme on Procida, but a minority of them agreed to contribute economically (33%) or to participate as volunteers in the control programme (25%) [68]. In September 2019, we administered again the same questionnaire to 191 randomly selected residents to record possible changes in feedback after our research activities on the island. While no significant changes were observed in the knowledge about the Asian tiger mosquito as vector and about preventive/protective measures taken (with the only exception of an apparent increase in the use of mosquito nets), we observed an increased interest in mosquito control program and an increased availability in supporting or participating to monitoring and control actions on the island.

Table 1. Results of public surveys carried out among Procida Island residents in 2015 (N = 200) and 2019 (N = 191) using a questionnaire.

Questionnaire question	Responses 2015 (%)		Responses 2019 (%)	
	YES	NO	YES	NO
Do you know that the Asian tiger mosquito can transmit viral diseases to humans?	77	23	73	27
Do you use protective measures against mosquitoes?	85	15	91	9
Do you use electric diffusers?	58	42	37	63
Do you use mosquito nets?	54	46	71	29
Do you use insect repellents?	45	55	47	53
Do you use larvicides?	3	97	2	98
Do you remove standing water?	11	89	11	89
Would you welcome a regional/municipal mosquito control programme?	88	12	96	4
Would you agree to the installation in your property of traps for the capture and monitoring of mosquitoes?	44	56	78	22
Would you agree to contribute personally to the financing of a mosquito control project?	33	67	54	46
Are you interested in participating, as a volunteer, to a mosquito monitoring and control programme in Procida?	25	75	33	67

<https://doi.org/10.1371/journal.pntd.0009698.t001>

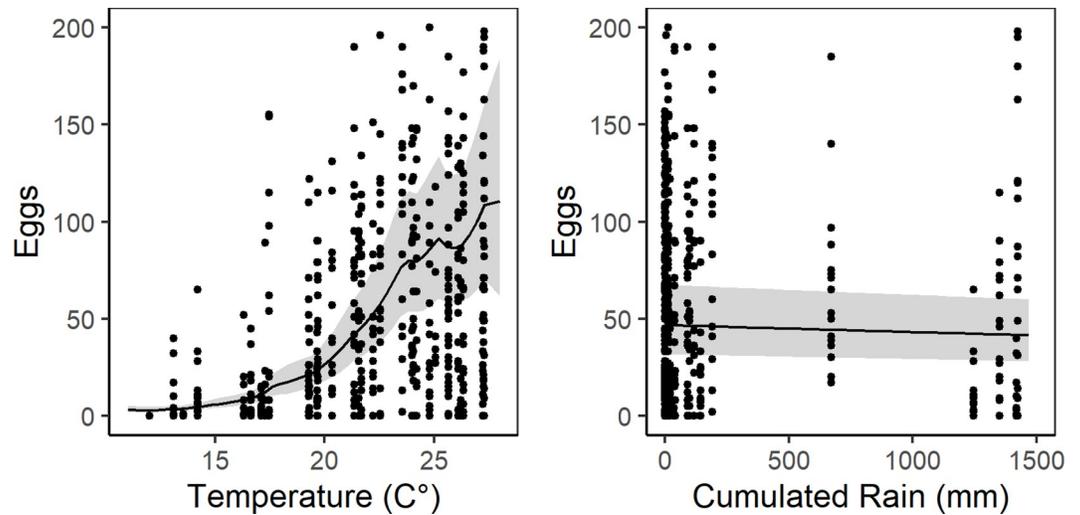


Fig 1. GMM posterior predictive values of *Ae. albopictus* eggs/ovitraps/week on Procida Island. Left panel = temperature dependent mean value of eggs/ovitraps/week. Right panel = rain dependent mean value of eggs/ovitraps/week. Solid lines = GMM posterior mean value of eggs/ovitraps/week. Grey areas = 95% credible interval. Black dots = observed values of eggs/ovitraps/week. y-axis = number of eggs/ovitraps/week. x-axis (left panel) = weekly averaged temperature. x-axis (right panel) = weekly accumulated precipitation.

<https://doi.org/10.1371/journal.pntd.0009698.g001>

Temporal analysis

A total of 44,244 *Ae. albopictus* eggs were collected by 26 ovitraps located across Procida and Vivara Islands in 2016 and two peaks of egg abundance were registered, one in July and one in September, in most ovitraps (see [Methods](#), S4 Fig and [S1 Table](#)). Results of a generalized additive mixed model (GAMM) carried out to model the relationship between meteorological variables and *Ae. albopictus* egg abundance, show evidence of a strong effect of temperature on the number of weekly collected eggs and low or not significant effects of cumulated rain and average wind speed, respectively ([Fig 1](#) and [Table 2](#)).

Model assessment shows no-specific violation of statistical assumptions (homogeneity, independence, autocorrelation, spatial correlation) ([S6 Fig](#)). However, GAMM shows some under-dispersion (dispersion statistic = 0.71) under the assumption of a Negative Binomial distribution and a strong over-dispersion under a Poisson distribution (dispersion statistic = 39.3). Moreover, the model predicts a lower number of zeros compared to the observed ones ([S7 Fig](#)). Model fit estimated by Conditional Predictive Ordinate (hereafter CPO, see

Table 2. Results for the GAMM. Dependent variable is the count of eggs in ovitraps, independent variables are wind, rain considered as fixed effect and temperature as spline function, while the position of ovitraps as randomised effect. The posterior mean values and 95% credible intervals for both parameters and hyperparameters are provided. When the 95% credible interval includes zero there is no statistical support of a correlation between the independent and the dependent variable.

Parameters	Mean (95% credible interval)
Intercept (β_0)	2.942 (2.633; 3.248)
Rain (β_1)	-0.310 (-0.450; -0.162)
Wind (β_2)	-0.101 (-0.218; 0.019)
Hyperparameters	
Negative binomial size parameter ($1/\theta$)	0.536 (0.474; 0.605)
Precision for Temperature random walk model	6.533 (3.615; 10.831)
Precision for ovitraps random effect	2.447 (1.199; 4.372)

<https://doi.org/10.1371/journal.pntd.0009698.t002>

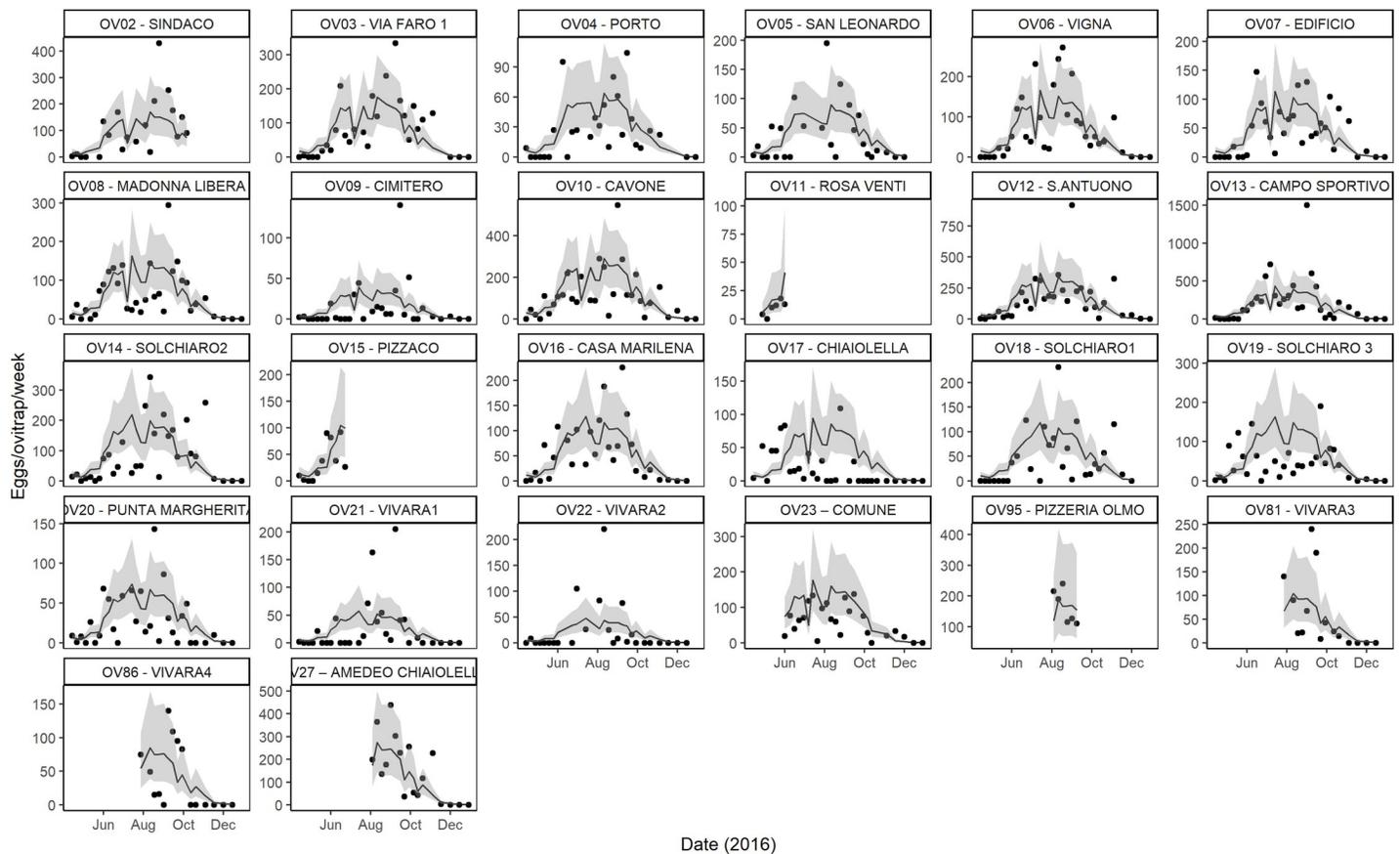


Fig 2. Observed and expected values *Ae. albopictus* eggs/ovitraps/week on Procida Island in 2016 estimated by GMM model. Each panel represents a single ovitrap. Black dots = observed values of eggs/ovitraps/week; solid line = GMM posterior mean value of eggs/ovitraps/week; grey area = 95% credible interval; x-axis = months of collections in 2016; y-axis = eggs/ovitraps/week; the scale differs per panel to help visualization.

<https://doi.org/10.1371/journal.pntd.0009698.g002>

Methods, section Temporal analysis) and Bayesian p-value is poor (S7 Fig), probably due to the large variation detected in ovitrap capture (range: 0–1500) coupled with a considerable proportion of zero captures (~25.7%) (Fig 2). However, fitted values positively correlate with observed values (Pearson's correlation: 0.697, df = 660, p-value <0.001) and the predicted oviposition temporal pattern is overall consistent with the observed one (Fig 2). In particular, the model well-captures the decreasing oviposition activity at the end of the season, but it is challenged by the high variability observed at trap level and during the season (see as an example OV09 or OV12).

Spatial analysis

A total of 40,811 *Ae. albopictus* eggs were collected by weekly monitoring of 101 ovitraps distributed across Procida in July (weeks 29–30) and September (weeks 37–38) 2016. By means of the experimental variogram, we detected the presence of a spatial structure in the data sampled (eggs/ovitraps/week) (Fig 3). A preliminary analysis of the cloud variograms (S8 Fig), showed a good spatial structure in the total number of eggs/ovitraps/week with a relatively high sill-nugget ratio (S9 Fig). This suggests that an important part of the sample variance can be explained by the spatial covariance and can be used, by means of kriging weights, to make spatial interpolation over a regular grid to get a continuous map of eggs deposited in the ovitraps.

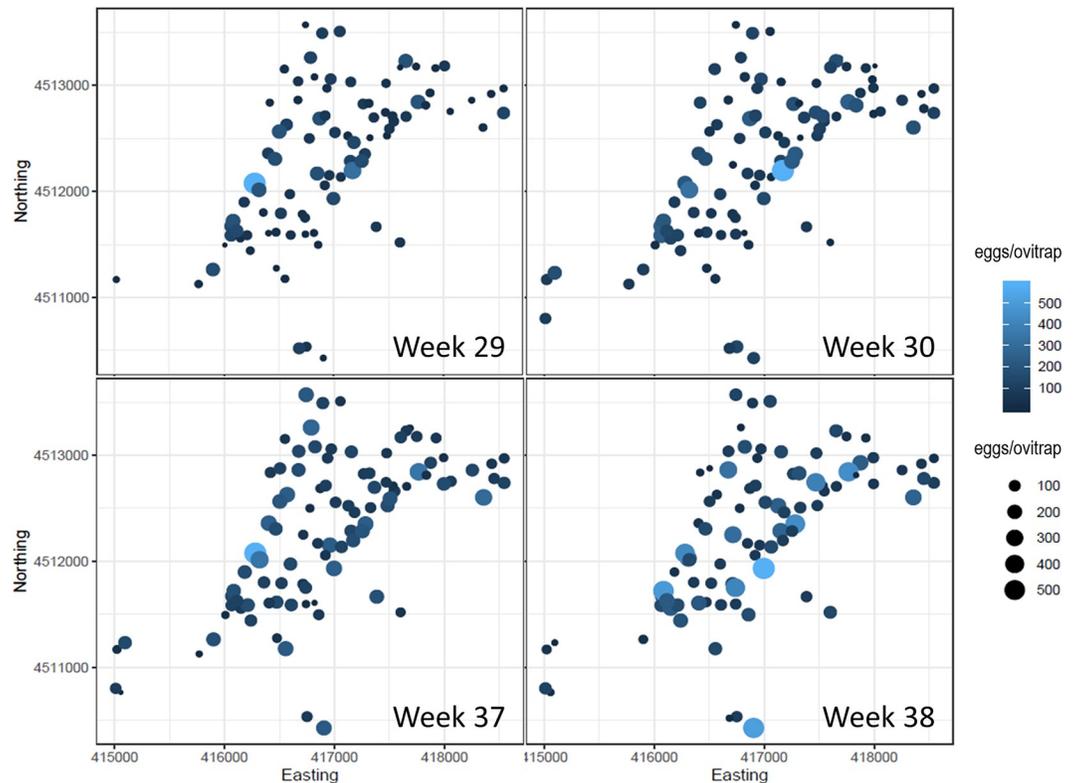


Fig 3. Number of *Ae. albopictus* eggs/ovitraps/week on Procida Island in July and September 2016. Spatial coordinates Northing and Easting are expressed in meters using the coordinate reference system with EPSG code 32633 (<https://epsg.io/32633>). Circles represent the positions of the 101 ovi-traps utilized for the spatial analysis. Circle size and colour scales are proportional to the magnitude of collected eggs/ovitraps. The double scale for the same parameter enables the resolution of the overlapping circles in the plot.

<https://doi.org/10.1371/journal.pntd.0009698.g003>

The ordinary kriging maps highlights a uniform presence of *Ae. albopictus* on the island, with some “hot spots” of oviposition activity (Fig 4). We calculated a set of geospatial covariates that could aid the spatial analysis of target measurements (ovipositions) in a multivariate context. Different data sources were used, such as a digital elevation model, a map of land use and land cover and a map of distance from the sea. Four rasters of growing degree days were calculated, one for each week, as a proxy of the spatial distribution of *Ae. albopictus* ovipositing females. All the tested covariates poorly contribute to the analysis of spatial variance of oviposition, as highlighted by very low Pearson correlation values, ranging from -0.19 to 0.28, and no significant correlations were observed (S2 Table).

Ovi-trap monitoring of *Ae. albopictus* in Vivara reserve

Temporal analysis in 2016 found *Ae. albopictus* also in Vivara, an inhabited natural reserve, where potential hosts are represented by rodents, birds and reptiles, only. To analyse the mosquito spatial distribution in the reserve area, we monitored the oviposition rate by 31 ovi-traps in 2018 and 2019 (see Methods, S1 Table and S5 Fig). All ovi-traps were found positive for *Ae. albopictus* eggs: a total of 18,891 and 6,608 eggs were captured in September 2018 (611 eggs/trap/week) and in July 2019 (213 eggs/trap/week), respectively. In addition, we performed adult collections by four BG-sentinel traps baited with BG-lure (S5 Fig). We collected 7 males and 31 females by BG-trap n°2 in September 2018 and 19 males and 27 females by BG-traps n°

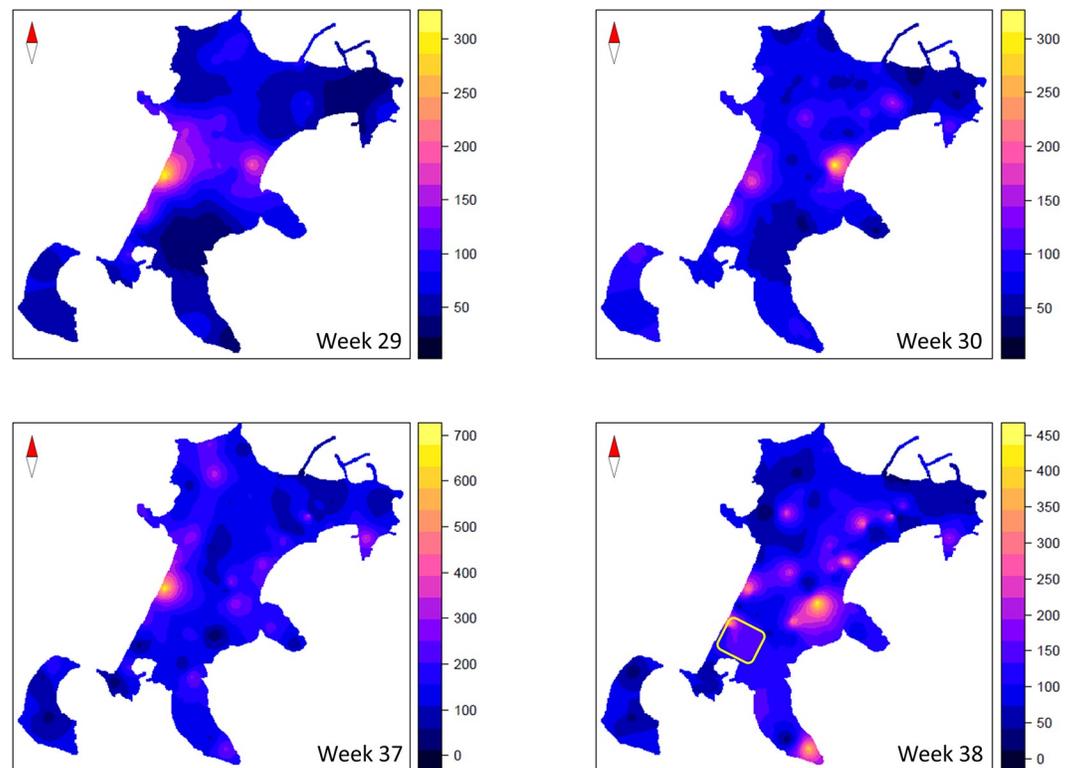


Fig 4. Ordinary Kriging maps of *A. albopictus* oviposition on Procida and Vivara islands. The figure shows the estimated ordinary kriging of *Ae. albopictus* oviposition on Procida Island in 4 weeks from July to September 2016. The colour gradient corresponds to the variation range of the estimated egg numbers. The yellow rectangle represents the area selected for the MRR experiments. The base layer of the geographic background map has been sourced from an open maps access (<https://glovis.usgs.gov/app>).

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2 and n³ in July 2019 (S1 Table). Both positive BG-traps were in proximity of two breeding sites present on the Vivara reserve (S5 Fig).

Mark-release-recapture experiments

We performed two mark-release-recapture (MRR) experiments in September 2018 (i.e., in the seasonal high-density peak according to results of 2016 spatial analysis) to estimate *Ae. albopictus* male density per hectare and to evaluate the dispersal and survival capacity of males sterilized by radiation (Fig 5).

In total, 7,836 (MRR1) and 9,680 (MRR2) marked sterile *Ae. albopictus* males (PRO1 strain) were released in September 2018. In MRR1, 2,009 wild males and 169 marked males were recaptured by HLC (N = 79) and by BG (N = 90) during 14 days of collection, with an overall recapture rate of 1.8% (N = 143) in the first 6 days. During this time interval, 97.8% of the males were recaptured within 50 m from the release site and none at ≥ 150 m from it (Table 2). In MRR2, 2,319 wild males and 165 marked males were recaptured by HLC (N = 111) and BG (N = 54) during 6 days of collection, with a total recapture rate of 1.7%. Among marked males, 87.9% and 6.7% were recaptured within 50 m and at ≥ 150 m distance from the release site, respectively (Table 3). Overall, only 4% and 9% of recaptured males were collected by BG sentinel and HLC beyond 50 m from the release point, respectively (Table 3).

Estimates of cumulative MDTs (1–6 days) was 51 m in MRR1 and 61 m in MRR2 based on BG-trap collections, and 52 m in MRR1 and 57 m in MRR2 based on HLCs (Table 4).

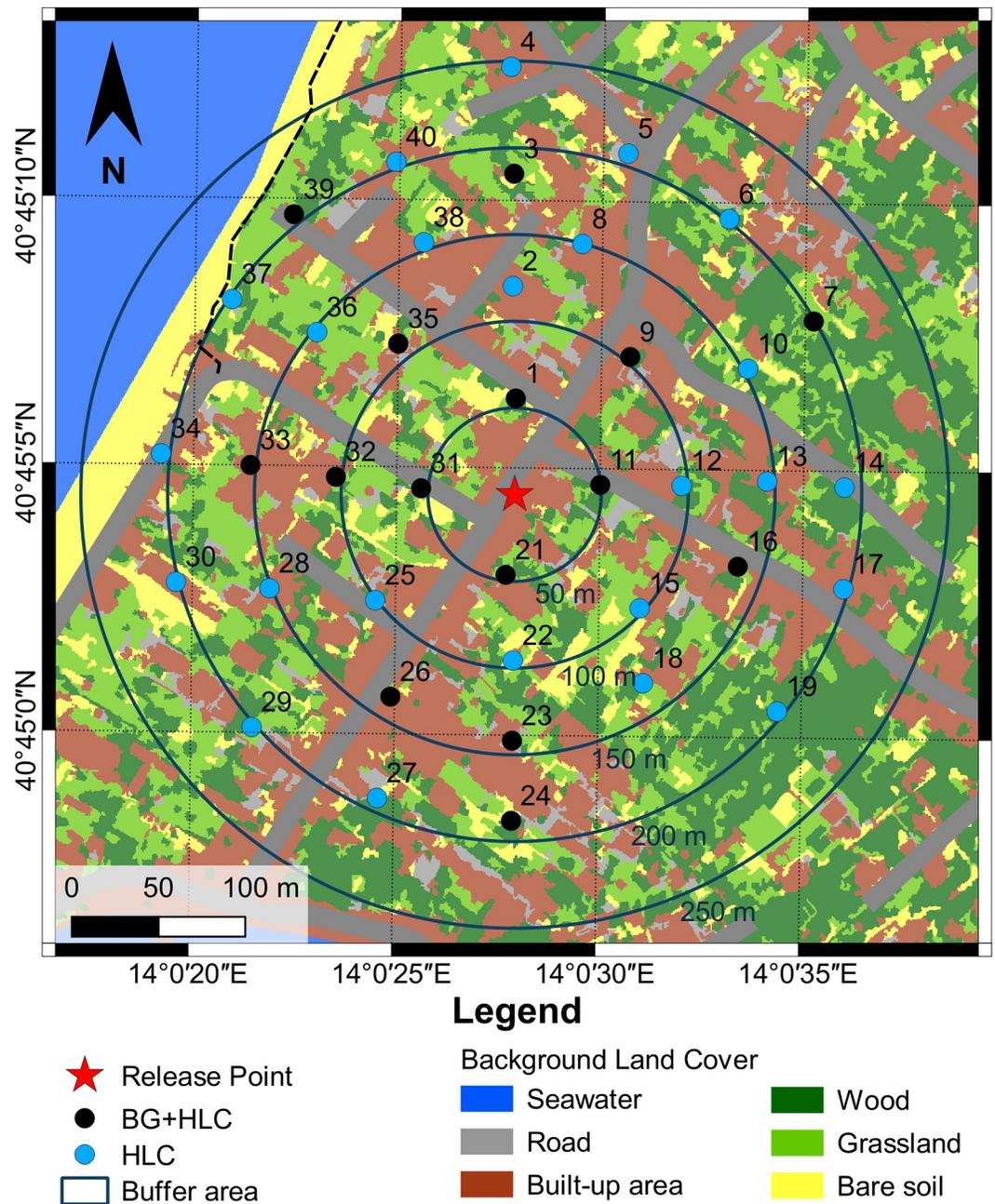


Fig 5. Distribution of *Aedes albopictus* sampling stations in the mark-release-recapture study site in “La Chiaiolella” (Procida). Red star = release site. Black circles = HLC + BG-traps recapture stations. Light blue circles = HLC recapture stations. Circles = 50 m-annuli around release site. The base layer of the geographic background map has been sourced from an open maps access (<https://glovis.usgs.gov/app>).

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Population size of wild *Ae. albopictus* males was estimated over areas of either 50 m (estimated flight range) or 200 m (whole sampling area) radius (Table 4 and Fig 6): results predict that the number of males/ha is higher within 50 m-radius area than in the 200 m-radius area. This effect is probably due to heterogeneities in mosquito abundance (as estimated by field collection), which increases at a lower rate than the size of the area (which increases

Table 3. Number of marked (and unmarked) *Ae. albopictus* males recaptured in two Mark-Release-Recapture (MRR) experiments on Procida Island. BG = BG-sentinel traps (number of BG within each annulus); HLC = Human Landing Catches (number of HLC sites within each annulus).

	Annuli (mt)	Trap/ sampling (number)	Days after release							
			1d	2d	3d	4d	5d	6d	7d	8-14d
MRR1	0–50	BG (4)	6 (35)	31 (88)	18 (72)	16 (51)	6 (19)	4 (36)	3 (32)	5 (377)
		HLC (4)	5 (24)	3 (21)	17 (68)	20 (75)	11 (45)	6 (44)	//	14(426)
	50–100	BG (3)	0 (28)	0 (13)	0 (28)	0 (40)	0 (23)	0 (86)	0 (36)	1 (345)
		HLC (8)	0 (13)	1 (44)	0 (51)	0 (32)	0 (43)	1 (38)	//	0 (225)
100–150	BG (5)	0 (16)	0 (14)	0 (20)	0 (22)	0 (17)	0 (10)	0 (17)	0 (124)	
	HLC (11)	0 (37)	0 (48)	0 (39)	1 (68)	0 (74)	0 (85)	//	0 (272)	
150–200	BG (3)	0 (12)	0 (9)	0 (2)	0 (9)	0 (5)	0 (4)	0 (2)	0 (46)	
	HLC (16)	0 (58)	0 (61)	0 (78)	0 (119)	0 (121)	0 (76)	//	0 (369)	
MRR2	0–50	BG (4)	2 (63)	27 (90)	10 (81)	5 (70)	2 (18)	3 (73)	//	//
		HLC (4)	11 (104)	33 (114)	38 (91)	10 (56)	3 (24)	1 (37)	//	//
	50–100	BG (3)	2 (43)	0 (46)	1 (57)	0 (118)	1 (62)	0 (19)	//	//
		HLC (8)	1 (43)	2 (31)	0 (52)	0 (44)	0 (25)	0 (60)	//	//
100–150	BG (5)	0 (26)	0 (30)	0 (34)	0 (8)	1 (10)	0 (16)	//	//	
	HLC (11)	0 (64)	0 (57)	1 (42)	0 (60)	0 (24)	0 (25)	//	//	
150–200	BG (3)	0 (12)	0 (13)	0 (6)	0 (8)	0 (5)	0 (2)	//	//	
	HLC (16)	0 (93)	0 (104)	10 (68)	1 (40)	0 (16)	0 (48)	//	//	

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quadratically). Estimates of mosquitoes/ha based on recaptures in the 50 m-radius area largely differ depending between GLM and Fisher Ford methods (Table 5 and Fig 6). The estimated survival parameter of marked mosquitoes ranges from 0.8 to 0.95, according to the sampling area and the trap type considered in the estimation.

Discussion

The application of sustainable innovations in mosquito control programmes requires strong partnerships with local communities [69,70]. The achievement level of such sustainable control programmes greatly depends on the degree to which citizens are effectively engaged into the work of entomological research and mosquito control, as reminded by WHO’s 2017–2030 Global Vector Control Response [71]. At the same time, bionomics data collection for the vector species of interest and the selection of pilot field test sites are considered essential premises for the successful implementation of sustainable vector control programmes, in particular for *Aedes* species [72]. Here, we present bionomics data of *Ae. albopictus* population on the island of Procida, an interesting site for field testing of innovative control methods. The data were

Table 4. Mean distance travelled (in meters) of recaptured *Aedes albopictus* sterile males in two mark-release-recapture experiments on Procida Island.

	MRR1 (BG)	MRR2 (BG)	MRR1 (HLC)	MRR2 (HLC)
day after release				
1	51	94	51	55
2	51	51	63	57
3	51	61	51	61
4	51	51	53	55
5	51	89	51	51
6	51	51	58	51
1/6 days	51	61	52	57

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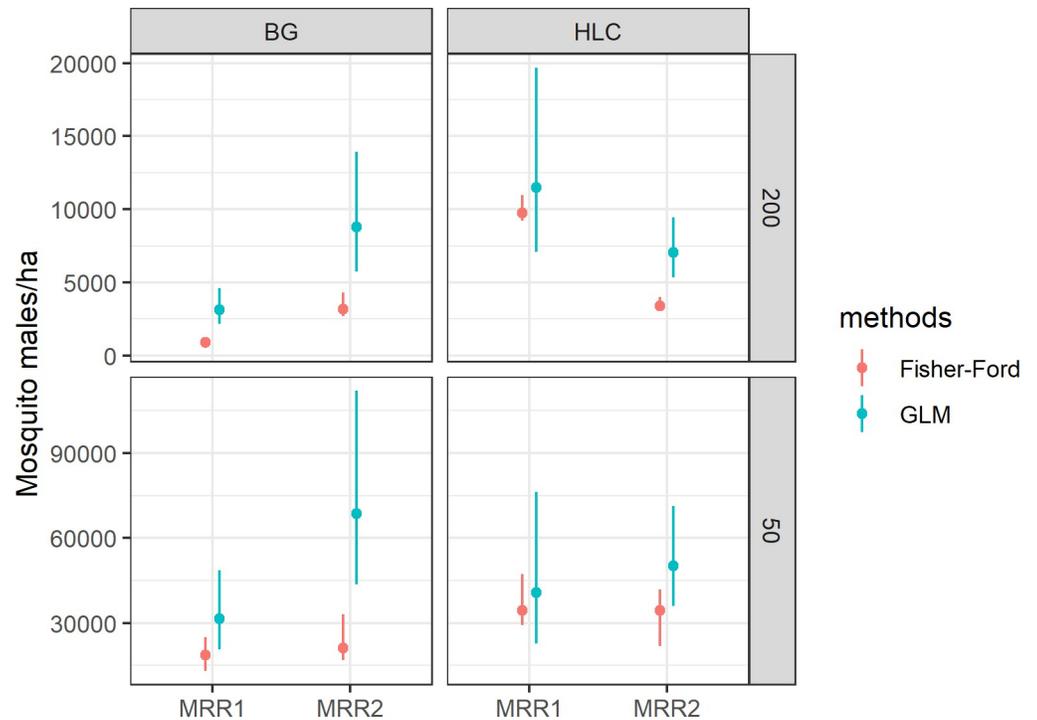


Fig 6. Wild *Aedes albopictus* population size on Procida Island estimated based on mark-release-recapture (MRR) experiments within either a 200m- and a 50m-areas around release site. Blue lines = GLM-based estimates; red lines = Fisher-Ford based estimates; dots = mean value of mosquito males/hectare; vertical segments = 95% confidence intervals using percentile bootstrap (red), using equation [5, S1 Text] (blue).

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Table 5. Wild *Aedes albopictus* population size on Procida Island and survival of sterile males estimated based on mark-release-recapture (MRR) experiments within either a 50m- and a 200m-areas around release site. Density/ha = number of males/ha; N = estimate of population size/day; λ = survival rate estimated by logistic regression; confidence intervals based on 1000 bootstrap replicates with the method of percentile bootstrap at 95% level; m = number of marked mosquitoes recaptured; n = total number of mosquitoes captured (marked+wild).

MRR	Methods	Type of traps	Annuli (m)	N	Density per ha	n	m	λ	95% CI per density per ha
1	Regression Model	BG	50	24692	31437	301	81	0.92	20753–48490
			200	39361	3132	659	81	0.8	2173–4618
	HLC	50	32013	40761	277	62	0.87	22786–76221	
		200	144447	11494	1350	65	0.95	7075–19673	
	Fisher-Ford(a)	BG	50	14727	18750	301	81	\\	13048–24835
		200	11659	928	659	81	\\	485–963	
2	Regression Model	HLC	50	27058	34450	277	62	\\	29223–47132
			200	122611	9757	1350	65	\\	9301–10989
		BG	50	53928	68663	395	48	0.81	43566–112028
			200	110256	8774	910	53	0.8	5753–13914
	HLC	50	39481	50267	426	96	0.94	36021–71275	
			200	88389	7034	1322	114	0.86	5333–9425
		BG	50	16618	21158	395	48	\\	16810–31257
			200	339776	3165	910	53	\\	2700–4276
	Fisher-Ford(a)	HLC	50	27024	34408	426	96	\\	21814–41779
			200	42421	3375	1322	114	\\	2526–3367

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collected using a community-based approach, which includes a citizen science component and several community-engagement activities, designed to involve the Procida community from the early stages of our research activities on the island.

Community-based approach to mosquito monitoring on Procida Island

Citizens have recently been included in citizen science projects for mosquito surveillance and control in several countries all around the globe, such as Germany [73], Spain [74] and USA [75]. In these studies citizens were asked to contribute by reporting the mosquito fauna by submitting mosquitoes collected in their private surroundings [73], by reporting tiger mosquito presence through a mobile app [74], or by reporting about potential mosquito habitat such as larval breeding sites [75]. Recently, Schoener and colleagues reported the involvement of citizens in Austria to monitor *Aedes* species by using ovitraps during summer months, from July to October 2017 [76].

We actively involved Procida citizens in monitoring ovipositing mosquitoes on the island over an entire season, from April to December 2016. To the best of our knowledge, this is the first citizen science project successfully involving volunteers in ovitrap management for such a long period of time. We observed a drop out of only two volunteers out of twelve during the nine months of ovitrap monitoring. The active participation of the mayor and of the two municipal councillors as volunteers in the project was an example for the other volunteers and certainly contributed to motivate citizens to participate in the monitoring until the project conclusion. This also represents a novelty in the community-engagement strategy for mosquito control programs that could be further explored in the future.

We also obtained a high success rate in the family involvement in the analysis of spatial distribution of *Ae. albopictus* on the island: 99 out of 106 (93.4%) contacted families gave access to their private properties to place the ovitraps or to set the re-capture stations for the MRR experiments. This provided us with capillary access to the whole island territory, enabling an easy and appropriate spatial distribution of the sampling stations.

Our results clearly show that the effort of the research groups in working in synergy with public administration and citizens of Procida was instrumental to implement high quality monitoring and experimental procedures despite the limited budget available. According to the surveys carried out in 2015 and 2019, Procida citizen involvement in mosquito monitoring and control can be further increased. The limited commitment of the residents in eliminating standing water and in larvicide usage highlights the need to increase knowledge on best practices to be implemented at the individual level to reduce mosquito reproduction (e.g., by specific educational projects in schools and/or door-to-door activities on mosquito biology and control) as a relevant step to lower mosquito densities and creating a condition more suitable for the success of pilot SIT/IIT studies and public mosquito control activities.

Spatial-temporal population dynamics on Procida Island

Results from ovitrap monitoring allowed to characterize the temporal dynamics and the spatial distribution of the *Ae. albopictus* on the island of Procida and to develop predictive models useful for optimization of conventional mosquito control interventions, as well as for the assessment of the effectiveness of control tools, such as SIT and/or IIT which still need to be validated in the field.

From the seasonal perspective, modelling of 2016 ovitrap and meteorological data allowed to predict the start of *Ae. albopictus* seasonal activity on Procida in April/early May and its end in October/early November (S10 Fig). Egg abundance dynamics is shown to be dependent on temperatures (Table 1 and Fig 1) and is characterized by two peaks in July and September. These

predictions are consistent with a >6 month active season in central Italy [53,77], while the species seasonality is known to be shorter in northern regions [78]. It is important to note that the results obtained by the temporal analysis had a limitation due to the monitoring of the population dynamic for just one year and to the use of the ovitraps, known to have large variation in collections. However, our model results are useful for evidence-based identification of the most appropriate period to start implementing mosquito control interventions on Procida (i.e., not later than April), as well as for assessing the effectiveness control approaches by comparing the predicted natural seasonal dynamics of the species with that observed after interventions. Further field data collection and analyses will allow the validation of the GAMM model, which could represent a trustable predictive model exploiting locally collected meteorological data to define more precisely the starting period of control interventions on Procida Island and in other Mediterranean sites.

To analyse the spatial distribution of the *Ae. albopictus* on Procida Island we applied a geostatistical approach. Geostatistical approaches, with particular emphasis on ordinary kriging, have been previously applied to study spatial distribution of insect vectors, e.g., to study either the association between vector distribution and pathogen transmission [79] and the critical low temperature for the survival of *Aedes aegypti* in Taiwan [80], or to relate entomological indicators to the incidence of dengue [81] and to analyse seasonal and spatial distribution of *Ae. aegypti* and *Ae. albopictus* in San Paulo municipality (Brazil) [82]. Our results highlight an overall uniform and massive presence of the *Ae. albopictus* mosquito consistent with the nature of the island territory, completely urbanized and fragmented in hundreds of private properties with residential buildings surrounded by gardens. Indeed, it was very easy to identify several anthropic water sources ideal for the species larval breeding in all sites selected for ovitrap positioning. In the hypothesis of the release of males within SIT and/or IIT plans, this finding points to the need of an island-wide capillary release of males (e.g., by drones [83] and/or by a large network of volunteers involved in ground releases) in order to achieve an effective and long lasting suppression trial. Furthermore, the uniformity of the Procida territory and the observed uniform and massive presence of the *Ae. albopictus* mosquito make easy the identification of island's areas to be utilized as test and reference field sites for a robust testing of SIT and/or IIT, as recently suggested by Oliva and colleagues [72].

As for the main island, a high *Ae. albopictus* densities have been also observed in the natural reserve of Vivara. This finding confirms that also in the Mediterranean area, as shown by several recent studies in invaded countries of the New-World [84–86], the species maintains the capacity to colonize sylvatic environments. In addition, this observation highlights the importance of also considering these kinds of habitats for the proper planning of future mosquito control interventions on Procida.

Procida *Ae. albopictus* population size estimates

We estimated *Ae. albopictus* population size at the end of the reproductive season (September) by MRR experiments. Estimates based on 200m-dispersal (928–9,757 males/ha with Fisher-Ford and 3,132–11,494 males/ha with regression) are comparable with the values estimated in the north of La Reunion island (i.e., 639 males/ha in September 2016 to 6,000 males/ha in December 2015) [87] and higher than estimates based on MRR experiments conducted in Rome in September 2009 (i.e., 135 mosquito/ha with regression and 294 m/ha with Fisher-Ford) [66]. Higher population size values (18,750–68,663 male/ha) are estimated by both statistical methods applied (despite GLM provided greater values than Fisher-Ford equation), under the assumption of a 50m dispersal. Although population size estimates are to be taken with caution as they are affected by the assumptions made (e.g., on dispersal) and by the methodological approaches applied (e.g., releases of males or females, recapture method, statistical

method), the very high number of *Ae. albopictus* individuals estimated on Procida confirms the capability of this species to reach very high population densities with detrimental effect on the quality of life of residents and tourists and, hence, the overall tourism-centred economy of the island.

Dispersal, mean distance travelled and survival rates of sterile *Ae. albopictus* males

By applying the MRR technique, we observed a short-range dispersal of irradiated males, likely favoured by the uniform landscape of Procida with plenty of resting and swarming sites for adult males. However, the <60 m mean daily distance travelled by the males is in the range of values estimated for non-irradiated *Ae. albopictus* males in Switzerland [88] and La Reunion Island [87], and lower than values estimated for *Ae. albopictus* females in Italy [61,89] and Switzerland [88]. This suggests that the flying capacity of the released males is not affected by manipulations, irradiation and transportation and highlights the need of planning multiple short-distance ground releases and/or aerial uniform releases within a future SIT intervention program.

We observed a daily survival estimates of irradiated males ranging between 0.80 and 0.95, which are in the range of estimates for not-irradiated males in Missouri (USA) (survival rate = 0.77) [90]; Reunion Island (survival rate = 0.90 [87] and 0.94–0.95 [91]) and Switzerland = 0.88 [88]. These survival rates lessen the concern of excessive field mortality being a critical bottleneck for the success and sustainability of a SIT intervention [92]. In addition, this suggests that not only the dispersal capacity, but also the longevity of the released males is not affected by manipulations, irradiation and transportation, supporting the potential effectiveness of SIT interventions based on male production and sterilization in centralized facilities, even if distant from intervention area.

Conclusions

The multifaceted approach described in this paper represents a guide for the identification of areas to test the cost-effectiveness and the challenges of mosquito control programs, integrating conventional (e.g., larval source removal and larvicide treatments) and innovative (e.g., SIT and/or IIT) control methods in Mediterranean Europe and in other temperate regions. Our approach could be used as a reference to design similar studies to evaluate conventional/innovative mosquito control methods and to improve community-engagement strategies for mosquito control.

In addition, results obtained represent an excellent endorsement of Procida Island as a site for future experimental trials. The ecological characteristics of the island, the high human and *Ae. albopictus* densities, the positive attitude of the resident population in being active parts in innovative mosquito control projects and the acquired knowledge on *Ae. albopictus* spatial and temporal distribution provide the ground for evidence-based planning of the interventions and for the assessment of their effectiveness. Finally, our results highlight the value of creating synergies between research groups, local administrators, and citizens for affordable monitoring (and, in the future, control) of mosquito populations.

Supporting information

S1 Fig. Procida island. A) Procida Island map. Yellow box shows the extent of map showed in Fig 5, red box shows the extent of map showed in S5 Fig. The base layer of the geographic background map has been sourced from an open maps access (<https://glovis.usgs.gov/app>). B) Procida island satellite picture (<https://eol.jsc.nasa.gov/SearchPhotos/>). In both

representations of the island, a clear uniformity in the territory organization is visible. Most of the Procida territory is organized in private properties with residential buildings surrounded by green areas, including gardens with ornamental flowers, vegetable cultivations and/or orchards with citrus plants and family-type farming of chickens and rabbits. The only exception is represented by the Vivara natural reserve, which is inhabited by humans and connected with the main island by a concrete bridge (150m long).

(TIF)

S2 Fig. Workflow of the community engagement approach utilized during the 4 years of activities on Procida island. (*) MoU = Memorandum of Understanding. (**) ZanzaMapp is a mobile app for mosquito monitoring (<https://www.zanzamapp.it/>) [93] that was tested on Procida island during September 2016. (***) ERN = European Research Night. The public activities were organized on Procida island in the frame of the MEETmeTONIGHT project (<http://www.meetmetonight.it/>) funded by EU.

(TIF)

S3 Fig. Volunteers involved during the three phases of the research program on Procida and Vivara Islands. All the people (co-authors of the present manuscript and Procida volunteers) present in this figure gave their written consent to be photographed and to have their images published under a creative commons license.

(TIF)

S4 Fig. Position of the ovitraps on Procida Island. The position of the 101 ovitraps utilized for temporal (red) and spatial (red and green) analyses are reported. The base layer of the geographic background map has been sourced from an open maps access (<https://glovis.usgs.gov/app>).

(TIF)

S5 Fig. Positions of ovitraps and BG-traps on Vivara Island. Purple circles represent the position of the ovitraps. Black circles represent the position of BG-sentinel traps. Red circles indicate the position of two identified breeding sites, in both cases represented by ancient cisterns for the collection of rainwater. The base layer of the geographic background map has been sourced from an open maps access (<https://glovis.usgs.gov/app>).

(TIF)

S6 Fig. GAMM model validation. Upper right panel: Pearson's residuals versus fitted values. Upper left panel: Pearson's residuals versus temperature. Middle right panel: Histogram of % of zeroes obtained by simulating 10000 databases, the red dot represents the observed % of zeroes. Middle left panel: Pearson's residuals versus fitted date of collection. Lower right: Pearson's residuals versus ovitraps. Lower left: Variogram of Pearson's residuals. Autocorrelation function of each ovitrap time series did not show serious violation of independence.

(TIF)

S7 Fig. Assessment of GAMM model fit. Left panel: On the x axis the conditional predictive ordinate (CPO) which represents the posterior probability of observing that observation when the model is fit using all data except that one. On the y-axis the observation. Right panel: the frequency distribution of the probability of a new value to be lower than the actual observed value (Bayesian p-value).

(TIF)

S8 Fig. Example of cloud variogram for week 29. Each point on the plot represents a couple of two locations separated by a distance vector in 2D spatial domain (x axis) and having a

semivariance value reported on y axis.

(TIF)

S9 Fig. Experimental semivariograms of total eggs/ovitrap/week on Procida. Each point represents the average value of semivariance of couple of locations belonging to the same lag. This is called semivariogram and it is the main tool in geostatistics to discover the existence of spatial structure in the data. It is used to inform the interpolation by kriging.

(TIF)

S10 Fig. Pattern of *Ae. albopictus* eggs/ovitrap/week and temperature on Procida Island in 2016 (upper panel) and 2017 (lower panel). On the x-axis the date. The black solid lines represent the GAMM posterior predictive mean value of eggs in ovitrap (left y-axis), the shaded areas represent their 95% credible intervals. The red solid line represents temperatures (right y-axis). In the upper panel the black solid line (the posterior mean) is estimated from observed data (2016).

(TIF)

S1 Text. Equations for mean distance travelled computation.

(DOCX)

S2 Text. Equations for expected recapture rate calculation.

(DOCX)

S1 Table. Data and coordinates for the temporal, spatial and MRR analyses.

(XLS)

S2 Table. Pearson correlation coefficients between ovitrap egg numbers in spatial analysis, terrain parameters derived from digital terrain model (dtm) and sea distance.

(XLS)

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Modelling arthropod active dispersal using Partial differential equations: the case of the mosquito *Aedes albopictus*

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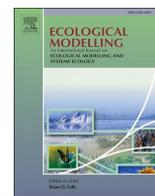
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Modelling arthropod active dispersal using Partial differential equations: the case of the mosquito *Aedes albopictus*

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ABSTRACT

Dispersal is an important driver for animal population dynamics. Insect dispersal is conventionally assessed by Mark-Release-Recapture (MRR) experiments, whose results are usually analyzed by regression or Bayesian approaches which do not incorporate relevant parameters affecting this behavior, such as time dependence and mortality. Here we present an advanced mathematical-statistical method based on partial differential equations (PDEs) to predict dispersal based on MRR data, taking into consideration time, space, and daily mortality. As a case study, the model is applied to estimate the dispersal of the mosquito vector *Aedes albopictus* using data from three field MRR experiments. We used a two-dimensional PDE heat equation, a normal bivariate distribution, where we incorporated the survival and capture processes. We developed a stochastic model by specifying a likelihood function, with Poisson distribution, to calibrate the model free parameters, including the diffusion coefficient. We then computed quantities of interest as function of space and time, such as the area travelled in unit time. Results show that the PDE approach allowed to compute time dependent measurement of dispersal. In the case study, the model well reproduces the observed recapture process as 86%, 78% and 84% of the experimental observations lie within the 95% CI of the model predictions in the three releases, respectively. The estimated mean values diffusion coefficient are 1,800 (95% CI: 1,704–1 896), 960 (95% CI: 912- 1 128), 552 (95% CI 432–1 080) m²/day for MRR1, MRR2 and MRR3, respectively. The incorporation of time, space, and daily mortality in a single equation provides a more realistic representation of the dispersal process than conventional Bayesian methods and can be easily adapted to estimate the dispersal of insect species of public health and economic relevance. A more realistic prediction of vector species movement will improve the modelling of diseases spread and the effectiveness of control strategies against vectors and pests.

1. Introduction

Animal dispersal refers to movements away from the place of birth towards another location for reproduction. The main drivers of dispersal are related to the avoidance of kin competition and inbreeding and escaping deteriorating environmental conditions (Bowler and Benton, 2005). In the case of insects, assessing the active dispersal range might be of crucial importance particularly in the case of species which damage agricultural productions, unsettle ecosystems, and threat human health. Among more than 6 million species of insects known, only less

than 100 are either important pests for major crops or relevant vectors of human and/or animal diseases. The deep knowledge of these species dispersal is instrumental to develop proper integrated pest management plans which maximize cost-effectiveness of interventions and protect the environment in a sustainable way.

Although many theoretical models are available, empirical studies are generally lacking due to the difficulties of linking observations to the quantification of dispersion (Tesson and Edelaar, 2013). Advanced satellite radio telemetry and acoustic are giving new opportunities to study dispersion of big size animals such as large mammals or sharks

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(Cagnacci et al., 2010; Spaet et al., 2020), but are less useful to quantify the dispersal of small animals. In the case of insects, Mark-release-recapture (MRR) is the most widely used technique for quantifying dispersal: specimens are first collected/reared and marked, then released from a single site and subsequently recaptured through traps placed at different distances in a given study area (Pollock et al., 1990).

The most common statistical approaches used to estimate dispersal from MRR data are regression techniques which aim to estimate the mean distance travelled (MDT) and the flight range (FR_x) of a specified fraction \underline{x} of the population, rather than the diffusion process of individual marked specimens. Other methods to estimate insect dispersal follow a Bayesian framework which explicitly models the diffusion process (Villela et al., 2015). For instance, the hierarchical Bayesian model proposed by Villela et al. (2015) for the mosquito *Aedes aegypti* takes advantage from the flexibility of the Bayesian approach and expands the frequentist approach by including three components: two probabilistic models, describing the spatial distribution of specimens and the daily survival of marked and native individuals, and an observation model describing the sampling process.

Partial Differential Equations (PDEs) represent a standard mathematical method to model diffusion processes, such as the gas dynamics and heat distribution (Borthwick et al., 2016). In ecology, PDEs have been applied to study spatial-temporal dispersal of animal populations in a continuous domain (Bassett et al., 2017; Kareiva et al., 1990), such as the home-range dynamics of meerkats (*Suricata suricatta*) (Bateman et al., 2015) and the dispersal of butterflies (Ovaskainen, 2004).

The aim of this work is to provide a PDE-based analytical method to estimate insect dispersion based on MRR field data. This method, compared to previously quoted ones, allows to estimate the dispersion taking into account the daily mortality of marked release insects and the days after release (time) in a single mathematical equation. In particular, we applied the proposed modeling framework to estimate the dispersal of blood-fed females of the tiger mosquito, *Aedes albopictus*, during the egg laying phase. This species represents a significant public health burden due to its capacity to transmit exotic arboviruses, such as dengue (DENV) and chikungunya (CHIKV), capable of induce serious diseases in humans (Zeller et al., 2016). The species was the primary responsible of the thousands of DENV cases recorded in the southwest of the Indian Ocean in 2015–2018 (Vincent et al., 2019) and of the first autochthonous cases of both viruses in Europe (Marrama Rakotoarivony and Schaffner, 2012), where it caused two large CHIKV outbreaks with hundreds of human cases (Italy 2007 and 2017; Caputo et al., 2020).

We expect that the proposed approach can be applied to quantify dispersal, and hence improve control of diseases transmitted by *Ae. albopictus* and by other mosquito species of global relevance (such as the major arbovirus vector, *Ae. aegypti*, and malaria vector species), as well as of agricultural pest species.

2. Materials and methods

2.1. Modelling

Our model expands the one proposed in (Lutambi et al., 2013). Precisely, we included in the main equation the mortality and capture processes of the species of interest. Thus, the equation assumes the following form:

$$\frac{\partial M}{\partial t} = D \left(\frac{\partial^2 M}{\partial x^2} + \frac{\partial^2 M}{\partial y^2} \right) - M(\mu + \beta) \quad (1)$$

Where μ is the mortality rate, β is capture rate, (x, y) represent location coordinates as distance (in meters) along the x and y spatial axis from a given origin (x_0, y_0) respectively, t is the time (i.e., days or hours), $M(x, y, t)$ is the density of the population of interest (individuals/m²) at location (x, y) at time t . In the equation [1] we assume that the initial number of

individuals are $M(x, y, 0) = M_0 \delta(x, y)$ where $\delta(x, y)$ is the Dirac delta function in two dimensions so $\delta(x, y) = 0$ for $x^2 + y^2 \neq 0$ and $\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \delta(x, y) dx dy = 1$ and therefore, $\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} M(x, y, 0) dx dy = M_0$. The number of specimens in a general rectangular $S = [0, a] \times [0, b]$, is given by $\int_S M(x, y, t) ds$.

The term $\left(\frac{\partial^2 M}{\partial x^2} + \frac{\partial^2 M}{\partial y^2} \right)$ indicates the diffusion in space and D is the diffusion coefficient that measures dispersal rate (distance²/time). In addition, we assumed Neumann boundary conditions i.e., $\frac{\partial M}{\partial x}(0, y) = \frac{\partial M}{\partial x}(L, y) = 0$ and $\frac{\partial M}{\partial x}(x, 0) = \frac{\partial M}{\partial x}(x, L) = 0$. This implies that we assumed neither immigration (which is reasonable considering that we are modelling only the dispersal of released mosquitoes) nor emigration of mosquitoes from the study area (such assumption should be accounted for in the study design when selecting the sampling area). The analytical solution of [1], as shown in (Lutambi et al., 2013), is:

$$M(x, y, t) \sim Norm((R_x; R_y), \Sigma) * M_0 e^{-(\mu+\beta)t} \quad (2)$$

Where *Norm* is bivariate normal distribution, $(R_x; R_y)$ is the distance on the x and y axis from the initial point (x_0, y_0) , and Σ is the following 2×2 covariance matrix:

$$\Sigma = \begin{pmatrix} \sigma_{xx}^2 & \sigma_{xy}^2 \\ \sigma_{yx}^2 & \sigma_{yy}^2 \end{pmatrix} \quad (3)$$

Here, we suppose that x and y are independent, meaning that the diffusion process is equal with respect to x and y , thus: $\sigma_{xy}^2 = \sigma_{yx}^2 = 0$; $\sigma_{xx}^2 = \sigma_{yy}^2 = 2Dt$. In other words, we assume that the movement of the species of interest follows a Brownian motion (Lutambi et al., 2013), meaning the diffusion coefficient D is equal for both x and y directions. In our case, the bivariate normal distribution models the probability of finding specimens of the species of interest in a point (x, y) at time t .

While our model was developed to estimate the diffusion coefficient of a selected species, it could also have other unknown parameters such as the mortality or the capture rate. We denote the set of free parameters with θ . Different techniques can be used to estimate θ , for instance, Markov chain Monte Carlo (MCMC), maximum likelihood, and particle filtering (Gelman et al., 2013).

2.2. Case study

We apply the proposed model to estimate the dispersal of blood-fed *Ae. albopictus* females using MRR field data. The entomological data were collected through three MRR experiments carried out on August 3rd (MRR1, N released females = 1049), August 24th (MRR2; $N = 1600$) and September 9th (MRR3; $N = 1200$) 2009 in the municipality of Piove di Sacco (Padua province, Veneto, northeastern Italy) (see Marini et al., 2019), for a detailed description of the experiments). In brief, blood-fed females were released from a single site and recaptures were carried out by 96 sticky-traps (STs, Facchinelli et al., 2007) distributed within 10 concentric 50m-radius annuli around the release site (see Table S1 in the Appendix). Data used in the model refer to daily recaptures of marked mosquitoes carried out in the first five consecutive days after release, i. e., 76 during MMR1, 183 during MMR2 and 29 during MMR3 (see Table S2 in the Appendix).

Mosquito mortality value used in the model is based on results of an assessment carried out on marked mosquitoes under semi-field conditions in parallel with the MRR experiments (see Table S3 in the Appendix). Empirical data were fitted with several functions: (i) $M_0 e^{-\sigma t}$ exponential, (ii) $e^{-\frac{A}{B}(1-e^{Bt})}$ Gompertz, (iii) $e^{-\frac{c}{d}t^d}$ Weibull, (iv) $\frac{e^{a+bt}}{1+e^{a+bt}}$ GLM; where t is day of experiment and M_0, σ, A, B, c, d are free-parameters estimated through the nlsLM (Non-Linear Square Levenberg-Marquardt) method (see Table S4 in the Appendix) and a (Intercept), b estimated through the Generalized Mixed Model using Binomial distribution (see Table S5 in the Appendix). We computed the likelihood of

daily mosquito mortality, which is assumed to follow a binomial distribution, for each tested function. In addition, we evaluated the Root Mean Square Error (RMSE) for each function to quantify differences between predicted and observed values. Finally, we selected the best function for mortality considering both measures. So, in this case study we remark that the mortality rate μ is a function of time t .

The capture rate β is defined as $\beta = \alpha * \frac{N \bullet A}{A_s} * \zeta$, where α is the average daily capture rate of a single sticky-trap. Here, α (estimated value = $1.24 \cdot 10^{-4}$) has been computed using field data from three different MRR experiments carried out in Rome (Italy) in 2009, in which the same sticky-traps were used to recapture *Ae. albopictus* females (Marini et al., 2010). The parameter α is defined as the number of recaptured marked mosquitoes divided by the product between the total number of mosquitoes released, the total number of sticky-traps deployed and the total number of days of MRR experiment. Such rate (α) was rescaled by the factor $N \bullet A / A_s$ to take into account the different density of active traps within each annulus, where N is the number of operational traps, A is the area of the inner (and smallest) annulus where a single trap was present, and A_s is the area of the annulus s . Finally, we multiplied the rescaled rate for a correction factor ζ , to be will estimated (so it is a free parameter belonging to set θ).

Thus, in our study case, the set of free parameters is $\theta = \{D_i, \zeta\}$ and their posterior distribution was estimated following a MCMC approach. We initially explored the parameter space by sampling 1000 values of each free parameter from a uniform distribution (range: 0–2). The set of parameters that best fitted the data was then used as a starting point for the MCMC algorithm.

We simulated mosquito dispersal sampling and the mosquito location at each time step (12h) from equation [2]. We assumed mosquito survival to follow a binomial process of parameters $1-\mu(t)$ and $M(t)$ where $\mu(t)$ is the computed mortality at each time step t , and $M(t)$ are the surviving mosquitoes define as $\int_S M(x, y, t) ds$ from time step t . Thus, we verify if every mosquito is alive with a binomial extraction with probability $1-\mu(t)$ given by the selected mortality function. We assumed that the number of captured adult female mosquitoes at given day t and annuli s follows a Poisson distribution with mean $\lambda_{\{t,s\}}$; therefore, the likelihood of the observed data given a parameter set θ was:

$$L \prod_{i=1}^{\varphi} \prod_{t=1}^{\Psi} \prod_{s=1}^{\Delta} \frac{e^{-\lambda_{\{t,s\}}(\theta_i)} \lambda_{\{t,s\}}(\theta_i)^{n_{\{i,t,s\}}}}{n_{\{i,t,s\}}!} \quad (4)$$

where $i \in \varphi$ are the number of replicas of the mark release experiments, $t \in \Psi$ are the consecutive days after release of MRR, $s \in \Delta$ are the number of annuli, $\lambda_{\{t,s\}}(\theta_i)$ is the expected number of captures at day t and annuli s defined as $\lambda_{\{t,s\}}(\theta_i) = \beta_i \int_S M(x, y, t) ds$ simulated by the model

with parameter set $\theta_i = (\mu_i, \beta_i, D_i, \zeta)$. Finally, $n_{\{i,t,s\}}$ is the observed number of trapped marked mosquitoes at day t ($t = 1, \dots, \Psi$) and annulus s ($s = 1, \dots, \Delta$) during experiment i ($i = 1, \dots, \Phi$).

Here we used aggregated captures from each annuli s and day t and applied a sensitivity analysis study to assess the robustness of our stochastic model. Afterwards, we considered two additional scenarios for the parameter set θ : $\theta_i = (\mu_i, D_i, \zeta)$ and $\theta_i = (\mu_i, D_i)$ that were compared using the Akaike Information Criterion (AIC) (see Table S7, Figs. S3 and S4 in the Appendix). In addition, we assumed a different likelihood function under the hypothesis that the capture process followed a Negative Binomial distribution rather than a Poisson distribution (see Eq. (1) in Additional results section in the Appendix).

A total of 15,000 MCMC iterations with a burn-in of 5000 were performed and the convergence was checked by visual inspection on the trace plots of chains. Finally, we simulated 1000 releases, using the estimated parameters (D, ζ) discarding the first 5000 MCMC iterations, to compare our model predictions with the observed data. All statistical analysis and model simulations were performed using the statistical software R version 3.6.1 (R Foundation for Statistical Computing., 2018) and packages tidyverse (Wickham, 2017), mvtnorm (Ekström, 2010).

The R code and the data are available at <https://github.com/Chia1992/Partial-Differential-Equation>.

As mentioned in the introduction, a standard method to quantify the dispersal is measuring the flight range (FR). The FR measures the area travelled/covered by marked mosquitoes in a unit of time. Classical methods, such as the regression model, estimate the FR with the cumulative number of expected recaptures at the end of the MRR experiment (Lillie et al., 1985; Morris et al., 1991; White and Morris, 1985), while the PDE method used the daily recapture. We used the parameters obtained by MCMC approach and the equation [2] to calculate the FR, thus the equation is:

$$FR = Norm\left((R_x; R_y), \sqrt{2Dt}\right) M_0 e^{-\mu} \quad (5)$$

FR₅₀ (area travelled by 50% of marked mosquitoes) and FR₉₅ (area travelled by 95% of marked mosquitoes), were calculate from the equation [5] considering 50% and 95% of marked mosquitoes that are found each day t in the annulus s .

3. Results

In this section we present: (i) the estimated values for the diffusion coefficients (D_1, D_2, D_3 for MRR1, MRR2 and MRR3 respectively), the correction factor (ζ) and the daily mortality rate (μ_1, μ_2, μ_3 for the first, second and third semi-field experiments); (ii) the simulation of the dispersal process of the marked mosquitoes during five consecutive days after release; (iii) a validation of the mathematical-statistical model proposed here using the analytical solution of equation [2].

3.1. Diffusion coefficients and correction factor (D, ζ)

The diffusion coefficients estimated by the stochastic model vary among the three MRR experiments. The mean values are 1800 (95% CI: 1716–1907), 983 (95% CI: 912–1143), 564 (95% CI 442–1103) m^2/day for the MRR1, MRR2 and MRR3 experiment respectively (see Table S6 and Fig.S1 in the Appendix), which can be interpreted as the area covered in a day by the dispersion process of the mosquitoes, i.e., the area travelled by the mosquitoes. The mean value of the correction factor (ζ) for the capture rate of sticky-traps is 81.35 (95% CI: 72.47–88.44) (see Table S6 and Fig.S1 in the Appendix Our model well reproduces the marked mosquito recapture observed dynamics, with 86%, 78%, 84% of the experimental observations lying within 95% of the model predictions in the first, second and third releases, respectively (Fig. 1).

The estimated mean diffusion coefficient allows to evaluate the flight ranges over time. In MRR1, the predicted FR₉₅ is on average 163 m (95% CI: 155–171 m) at day 1 and increases up to 361 m (95% CI: 342–382 m) at day 5 after release, whereas in MRR2 and MRR3 the FR₉₅ start from 119 m (95% CI: 115–124 m) and 91 m (95% CI: 87–96 m) and increase up to 267 m (95% CI: 256–280 m) and 204 m (95% CI: 194–213 m) in the first and fifth day after release, respectively (Fig. 2).

To validate the stochastic model, we compared the predicted density of marked *Ae. albopictus* females with the analytical solution of the PDE (equation [2]). As shown in Fig. 3, there is a very good agreement between the analytical solution and model simulations: 68%, 86%, 100% of the analytical results are within 95% of the simulated model data in MRR1, MRR2 and MRR3, respectively.

3.2. Mortality rate estimate

The comparison of the considered mortality functions shows that the exponential and the Weibull functions provide the best fit (higher likelihood) of the mortality data of marked mosquito in the semi-field experiment (see Table 1 and Fig.S2 in the Appendix).

The functions with the highest values of likelihood (Exponential and Weibull) were compared by means of a likelihood ratio test. Since no

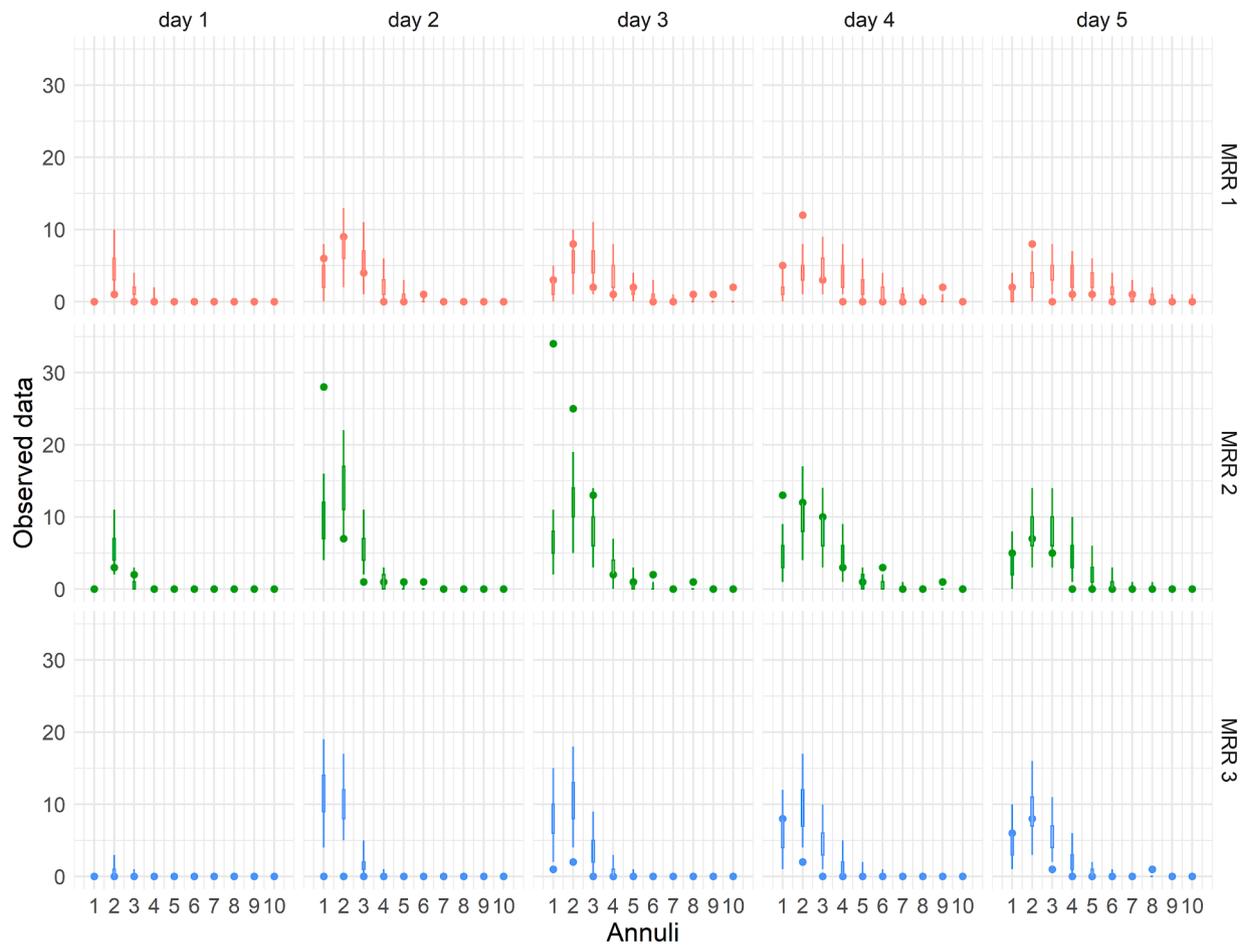


Fig. 1. Model fit. Boxplot (2.5%, 25%, 75% and 97.5% quantiles) of the number of recaptured marked mosquitoes predicted per annulus (each annulus 50 mt) by the model (boxplots representing the 2.5%, 25%, 75% and 97.5% quantiles) for each MRR experiments (rows: MRR1 red, MRR2 green, MRR3 blue) in the first five days after release (columns). Dots represent the number of marked mosquitoes recaptured during the MRR experiments.

statistical differences were observed between Exponential and Weibull likelihood value (p -value = 0.21, $df = 1$), the exponential function was chosen, given its simpler formulation compared to Weibull. The estimated daily mortality rates using the exponential function were 2.1%, 1.8%, 1.3% in MRR1, MRR2 and MRR3, respectively.

4. Discussion

Here we presented a PDE-based stochastic framework to estimate insect dispersal based on MRR data. We tested it in a specific case-study to overcome the limitations of the commonly used analytical approaches, i.e. regression analysis (Marini et al., 2019) and hierarchical Bayesian models (Vilella et al., 2015). Indeed, the big advantage in the use of PDEs is the possibility of including time, space, and daily mortality in only one mathematical equation, thus providing a more realistic representation of the dispersal process, which could improve the planning of control activities of human vectors and agricultural pests, as well as the study of their collective behavior.

We applied the PDE method to study the dispersal of *Ae. albopictus*, which represents a major source of nuisance and a public health threat also in temperate regions, in order to highlight the potential of PDE approach in studies aimed at predicting the transmission dynamics of vector-borne pathogens and at planning vector control interventions. We exploited data from three MRR experiments, during which daily mortality rates of marked mosquitoes were experimentally estimated under semi-filled conditions. A good agreement between the stochastic model and observed data was observed. Moreover, given the analytical solution, the calculation of quantities of interest such as FR is

straightforward. It is important to stress the implication that such result may have on control strategies, as it may allow to tailor the spatial radius deemed necessary to target the population of interest (Marini et al., 2019; Yamashita et al., 2018).

In the case study, the FR_{95} resulting from the PDE approach is significantly higher than the one estimated using regression analysis (Marini et al., 2019), i.e. 361 m, (95% CI: 342–382 m) vs 250 m in MRR1, 267 m (95% CI: 256–280 m) vs 209 m in MRR2, and 204 m (95% CI: 194–213 m) vs 177 m in MRR3. This is due to the inclusion in the analysis of daily mortality and time (day after release) and is thus expected to be more realistic. The spatio-temporal patterns of *Ae. albopictus* blood-fed females modelled through the PDE approach suggest that the dispersal increases over time, a conclusion which could not be reached using a conventional regression approach (Marini et al., 2019).

The latter result suggests that in case of arbovirus transmission, the size of the area covered by vector control interventions should change over time to target the same fraction of the mosquito population potentially involved in the transmission event. For instance, in Italy, it is recommended that in case of autochthonous arbovirus transmission, vector control intervention (i.e. insecticide sprayings, larvicide application, door-to-door activity) are carried out in a 100 m buffer around the residence of a suspected or confirmed case (Ministero della Salute et al., 2019). Our model estimates that the probability of a mosquito being within this buffer is equal to 0.88 and 0.5 after 24 h and 3 days from the potentially infectious blood meal, respectively.

Indeed, the flight range estimated for all MRR experiments suggested a very rapid dispersal, as in the case of MRR1 when 95% of mosquitoes travelled beyond 163 m (95% CI: 155–171 m) 1 day after release and

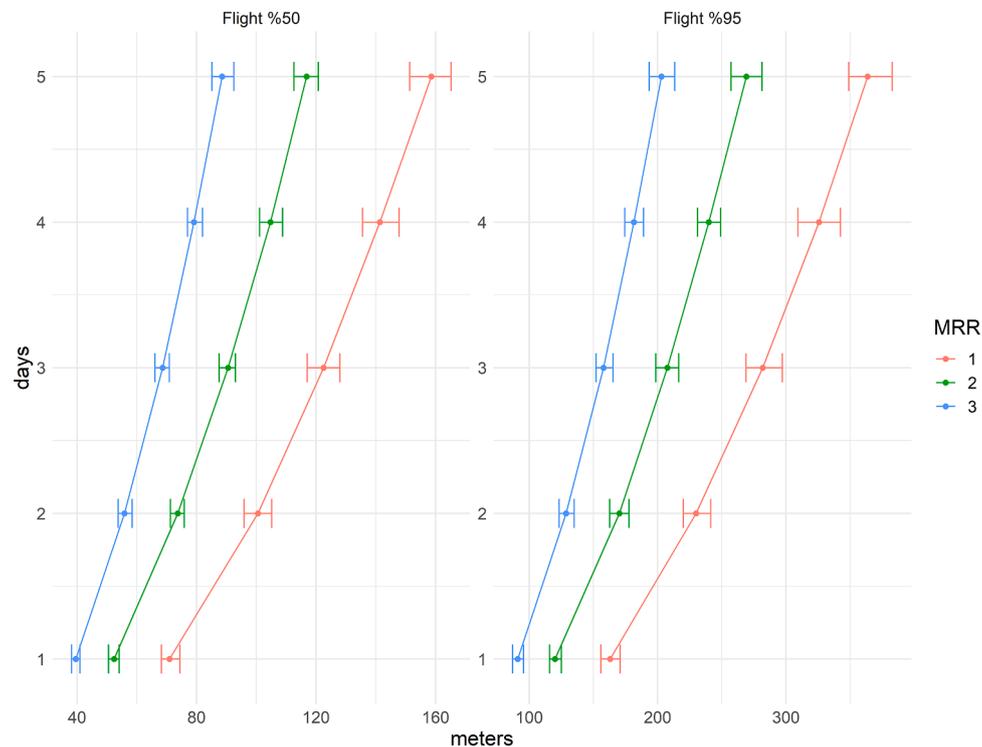


Fig. 2. Flight range of marked blood-fed females of *Aedes albopictus* estimated by the model (meters/day traveled by 50% and 95% of mosquitoes). Dots represent the average predicted distance (in meters) from the release point covered by 50% (left panel, i.e., FR50) and 95% (right panel, i.e., FR95) of marked mosquitoes for MRR1 (red), MRR2 (green), and MRR3 (blue). The segments represent the 2.5- 97.5% quantiles. x-axis: Meters, y-axis: days after release.

over 361 m (95% CI: 342–382 m) 5 days after release.

Standard regression approaches for the computation of the flight range do not take into account time dependency but model cumulative captures, over the entire study period, as a function only of the distance. It should be noted that, given the short time span of the recapture data in our MRR dataset (5 days) and the low observed mortality, we did not need to make any assumption on the spatio-temporal variation of diffusion coefficient. However, in the PDE we assumed that mosquito dispersal is homogeneous in space (with respect to x and y coordinates) and temporally constant. It would be interesting in the future to address the spatio-temporal dependency of the diffusion parameter D (i.e., individuals of the species will not disperse indefinitely, nor randomly). In addition, we included in the model estimates of capture and daily mortality rates, which may be difficult to be estimated under different experimental conditions. In our study we calibrated the capture rate, evaluated by [Marini et al., \(2019\)](#), to our data by estimating the correction factor (ζ). It is important to remark that if the mortality and capture rate are not available, they can be considered as free parameters and thus can be estimated by the stochastic model. Adding these free parameters into the model would increase the complexity of the model.

Finally, our proposed framework could be improved by including other parameters affecting insect dispersal, such as wind ([Bowen et al., 1991](#); [Cummins et al., 2012](#); [Knols and Meijerink, 1997](#); [Raffy and Tran, 2005](#); [Smallegange et al., 2005](#)), provided that they are appropriately estimated during the MRR experiments. Indeed, differences either in wind direction and force, or in other meteorological features, might account, at least partially, for the different estimates in the diffusion coefficients between the three MRR experiments, as hypothesized in [Marini et al., \(2019\)](#).

It is critical to highlight that estimate obtained from our analytical approach are unavoidably affected by the experimental design (e.g., the physiological stage of marked insect, the ecology of the site of release and recapture, the size of study areas, the recapture methods). FR_{90} estimates for *Ae. albopictus* are higher in a study conducted in Switzerland ([Vavassori et al., 2019](#)) compared to ([Marini et al., 2019](#)).

However, the experimental design of the two studies largely differ: i) freshly emerged adults instead of blood-fed females were released in Switzerland; ii) BG-Sentinel trap instead of blood-fed ones were released in Switzerland to focus on dispersal triggered by host-seeking, rather than by egg-laying; iii) the study area was larger in Switzerland (1 km radius instead of 500 m). Application of PDE method to this dataset could allow a more realistic comparison of the two datasets.

Epidemiological models are used to evaluate the risk of outbreaks occurrence and, more generally, to study the circulation, possibly spatial and temporal, of a given mosquito-borne pathogen ([Li et al., 2021](#)). Most of these models include into the equations various parameters that can influence the transmission of pathogens such as the vector mortality and oviposition rates and the length of gonotrophic cycle (e.g. [Poletti et al., 2011](#); [Otero et al., 2006](#); [G. Marini et al., 2019](#)). The dispersal of mosquitoes can be incorporated into these models by introducing either the dispersion term or the value of the diffusion coefficient (D) estimated in this work. In fact, during an outbreak, mosquito dispersion might be an important factor. Seventy percent of focal infections during a large chikungunya outbreak mediated by *Ae. albopictus* in the Lazio region (Italy) in 2017 were transmitted within a distance of 200 m, demonstrating the key importance of the dispersal of infected mosquitoes in the spatial spread of mosquito borne diseases ([Guzzetta et al., 2020](#)). Finally, our results on the dispersion of mosquitoes could be useful both for models aimed at predicting mosquito abundance ([Zheng et al., 2018](#)) and for models carried out to evaluate demographic effects of SIT techniques ([Haramboure et al., 2020](#)).

5. Conclusions

The results here obtained are useful for the definition of the optimal buffer on which to focus emergency mosquito-borne virus control interventions (i.e. deployment of adulticides insecticides aimed at eliminating potentially infected mosquitoes in the area surrounding the residence of an arbovirus infected person). This information is crucial for public authorities, as it has already been shown that enlarging the

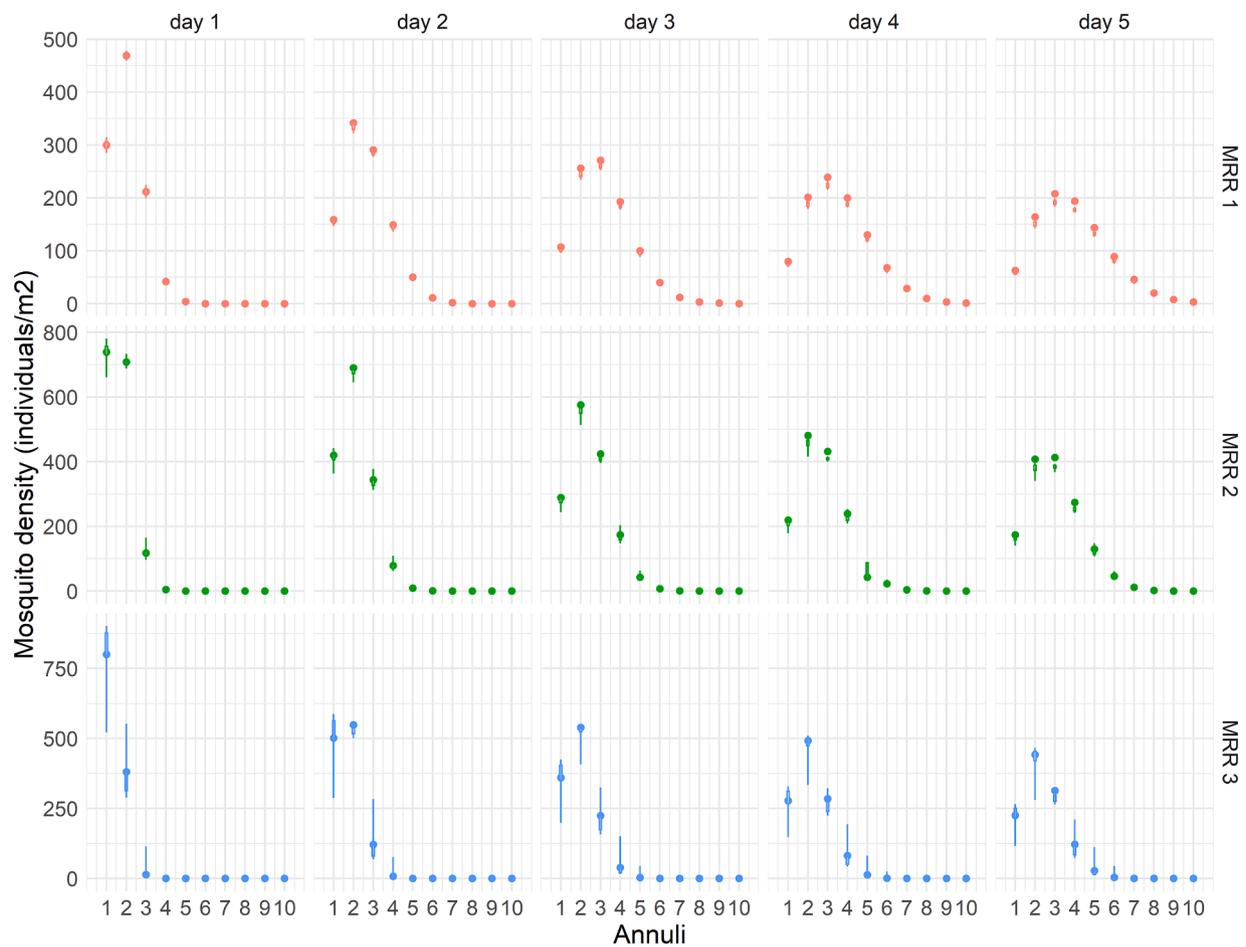


Fig. 3. Comparison between mosquito density (mosquitoes/m²) predicted by the analytical solution and the model. Number of mosquitoes predicted per annulus by the analytical solution (dots) and by the stochastic model (boxplots representing the 2.5%, 25%, 75% and 97.5% quantiles) for each day (in columns) and MRR experiment (in rows, MMR1: red, MRR2: green, MRR3: blue).

Table 1

Summary of RMSE and likelihood for the four mortality functions of marked mosquitoes in semi-field condition. The values represent the sum of three MRR experiments (RMSE = Root Mean Square Error).

	Exponential	Gompertz	Weibull	GLM
RMSE	0.062	0.057	0.053	2.58
Likelihood	-65.69	-67.79	-64.93	-72.2

size of the area to be treated and reducing the time interval between infective case identification and the control implementation might substantially increase the effectiveness of the intervention during an arbovirus outbreak (G. Marini et al., 2019).

The mathematical/statistical framework developed here can be easily adapted to estimate the dispersal of other insect species of public health and economic relevance. Indeed, MRR data are available for major arbovirus (i.e., *Ae. aegypti*, (e.g. Villela et al., 2015)) and Afro-tropical malaria vectors (e.g. Epopa et al., 2017), as well as for agricultural pest species, such as *Drosophila suzukii* which damages the fruit during the ripening stage (Asplen et al., 2015), or the beetle *Platypus koryoensis*, vector of the fungus *Raffaelea quercus-mongolicae*, which is fatal for oaks (Lee et al., 2019).

6. Data availability statement

The R code and the data are available at <https://github.com/Chi1992/Partial-Differential-Equation>

CRediT Author Statement

Chiara Virgillito: Methodology, Data curation, Writing. **Mattia Manica:** Methodology, Data curation, Writing. **Giovanni Marini:** Methodology, Data curation, Writing. **Beniamino Caputo:** Collecting data, Writing. **Alessandra della Torre:** Collecting data, Writing-Reviewing. **Roberto Rosà:** Methodology, Data curation, Writing-Reviewing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ecolmodel.2021.109658](https://doi.org/10.1016/j.ecolmodel.2021.109658).

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Chapter 3: Estimation of the effectiveness of conventional and innovative mosquito control tools in Italy by Bayesian statistical methods.

The aim of mosquito vector control in Europe is to reduce either the nuisance and/or the potential for transmission of diseases, such as Dengue, Chikungunya (Bellini et al., 2020) and West Nile. While in endemic areas the effectiveness of control against major malaria and arbovirus vectors can be measured against clinical parameters (e.g. number of hospitalizations or of deaths), the assessment the effectiveness of mosquito control in reducing the risk of transmission in epidemic area is less straightforward. Similarly, the effectiveness of interventions aimed to reducing mosquito nuisance are difficult to evaluate as the desired outcome as the level of annoyance tolerated varied greatly among individuals. Therefore, the most common experiment design for testing the effectiveness of conventional and innovative vector control is to quantify the reduction of mosquito population in the treated area under assumption that the lower the mosquito the lower the nuisance and risk. This assessment is generally carried out by comparison of counts of adult mosquitoes or one of their aquatic stages in a treated versus an untreated area at the same timeframe (Reiter, 2010). Methods for the analysis of such data consist in non-parametric statistical tests (ie. Mann–Whitney U, Wilcoxon), basic mathematical equation (Mulla's equation), and statistical inference approaches such as regression models. In statistical inference both frequentist and Bayesian approaches may be applied. The Bayesian approach is more flexible, allowing the use of a more extensive range of statistical distributions as well as more complex models and dependency structures. Bayesian models assume that the parameters to be estimated are randomly distributed. As such, they can be understood in terms of probability distributions and interpreted more intuitively (Zuur et al., 2013). Technically, the Bayesian inference determines the posterior distribution of the parameters starting from their prior distributions and maximizing the likelihood of fitting observed data. Often the analytical calculation of the posterior distribution might not be possible and computation methods may be required. Markov Chain Monte Carlo (MCMC) algorithms are the standard methods to compute parameter posterior distributions in a Bayesian setting.

The present chapter reports the results of the application of Bayesian approaches implementing MCMC algorithms to the assessment of the effectiveness of: 1- conventional larvicide treatments against *Cx pipiens*, accounting for temporal dynamics (Virgillito et al., 2022), and 2- an innovative control approach to suppress *Ae. albopictus* populations in Italy (manuscript in preparation).

In Virgillito et al., (2022), we evaluated the combined effect of *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus* against *Cx pipiens* larvae in highly vegetated ditches in north-east Italy by applying a family of Generalized Additive Mixed Models (GAMM) in a Bayesian framework and we advocated for such models to be routinely applied to account for the non-linear fluctuation in mosquito populations.

The conventional formula applied to assess the effectiveness of larval control is the Mulla's equation (Mulla et al., 1971). Mulla's equation quantifies the effectiveness of larvicide treatments by measuring the relative reduction of the vector abundance compared to an untreated site. Specifically, the percentage of reduction is calculated as follows:

$$\text{Reduction (\%)} = 100 - \left(\frac{C_1}{T_1} - \frac{T_2}{C_2} \right) 100,$$

where C_1 and T_1 are the average of vector abundance pre-treatment in the control and treated site respectively, and C_2 and T_2 are the average vector abundance post-treatment in the control and treated site, respectively. It is important to point out that this formula is based on several assumptions. First, changes in vector populations are assumed to take place at the same level and same rate in both control and treated site. Second, treated and control sites are assumed to be uniform with reference to environmental and climatic factors contributing to vector productivity. In addition, the equation does not consider the efficacy of vector control over the time but provide an estimate over a predetermined period or endpoint. In general, Mulla's formula neglects the spatio-temporal structure of mosquito populations as well as the external factors and the impact on the prediction of the multiple field observations

Alternatively, to Mulla's equation, regression models could be used to assess treatment effectiveness. Regression models allow to adjust estimates accounting for covariates of interests and to model the estimated effectiveness in time. However, to fully exploit these features, advanced regression modelling techniques ought to be applied. These richly parametrized models often require well-designed experiments with appropriate sampling size. Another drawback is that the numerical/computational complexity may be intractable in a frequentist framework, whereas the Bayesian one, although with his own limitation, provides more flexibility.

To overcome these limitations in the analysis of *Bacillus thuringiensis subsp. israelensis* and *Bacillus sphaericus* against *Cx pipiens* larvae, we applied a family of Generalized Additive Mixed Models (GAMM) in Bayesian framework that consider the non-linear fluctuation of the response variable and compare the dynamics of mosquitoes in the control and treated sites. We also included random effect in the model to account for repeated samplings in multiple locations. We included O'Sullivan splines to account for the temporal pattern of larvae and in addition we compared those splines (by exploiting the Bayesian approach) simply by subtracting one from the other and obtaining a posterior distribution for the different temporal pattern in the two site. Then, it is straightforward to compute averages and credible intervals (Zuur et al. 2009).

In the second study we applied a Bayesian method to evaluate the effectiveness of an innovative method to suppress *Ae. albopictus* population by releasing males transinfected with a *Wolbachia* strain of *Cx pipiens* which are supposed to sterilize wild females harbouring a different *Wolbachia* strain upon mating. Results from similar studies conducting in the US (Atyame et al., 2016; Mains et al., 2016) assessed the sterilizing effect of the releases by comparing eggs hatch rates in the treated and control sites by non-parametric statistical test (ie. Mann-Whitney U, Wilcoxon-) or Generalized Linear Models (GLM), which do not account for the spatio-temporal structure of mosquito populations and the external factors and the impact of the multiple observations. We applied a hierarchical model in order to employ the advantage of Bayesian approach. The hierarchical models are flexible tools and allow a more objective approach to the statistical inference by estimating the parameters of prior distributions from data rather than using prior subjective information (Efron et al., 1975; James et al., 1961). Specifically, we developed a hierarchical model to estimate the fertility rate of single wild females collected in the treated area - where *Ae. albopictus* males manipulated

with a *Cx. pipiens* Wolbachia strain (hereafter ARwP) were released - and compared it with the fertility rate of single females collected in the untreated area. In addition, in order to assess whether the females have or have not mated with ARwP males, we modelled the mating event of a single female with a ARwP males as Bernoulli process, with parameter θ , and including the posterior distribution of θ on the binomial model build to estimate the fertility rate of wild female.

In both studies presented we carried out the model validation suggested by Zuur et al., (2013) includes 3 steps:

- I) assess the mixing of MCMC chains.
- II) check for overdispersion in the response variable.
- III) check models' statistical assumptions (homogeneity, independence, autocorrelation).

I) to assess the mixing of MCMC chains, we checked whether the chains are auto-correlated, by creating auto-correlation functions for each chain and for each parameter. If autocorrelation is present, it is necessary increase the thinning rate, simplify the model or use a different method for sampling new iterations in the MCMC process. In Virgillito et al., (2022) a minimal autocorrelation in betas parameters was found.

II) To check for overdispersion (only in the case that the likelihood of observed data follows a Poisson or Negative Binomial distribution) is needed to calculate the Pearson residuals for the observed data and simulated data and, to avoid overdispersion on average the sums of squares of the Pearson residuals for the observed data will be similar to those of the simulated data (adding in the MCMC code the discrepancy measures).

III) to check the model' statistical assumption is needed to calculation the Person Residual ($E = \frac{y - E(y)}{\sqrt{var(y)}}$) and plot them against fitted values and versus each covariate in the model as well as versus each covariate not in the model. In moth studies the models built were not violated the statistical assumption.

Evaluation of *Bacillus Thuringiensis subsp. Israelensis* and *Bacillus sphaericus* combination against *Culex pipiens* in highly vegetated ditches

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EVALUATION OF *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* AND *BACILLUS SPHAERICUS* COMBINATION AGAINST *CULEX PIFIPIENS* IN HIGHLY VEGETATED DITCHES

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ABSTRACT. Among the few mosquito larvicides available in the market, *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) and *B. sphaericus* (*Bs*) represent the most environmentally safe alternatives. The combination of the 2 products is known to overcome their specific limitations by producing a synergistic effect. The aim of the study was to assess the effect and persistence of a single treatment with a granular *Bti* + *Bs* formulation on highly vegetated ditches in northeastern Italy that represents the primary rural larval sites for *Culex pipiens*, the primary vector of the West Nile virus in Europe. The analysis takes into account the nonlinear temporal effects on the population dynamics of larvae and pupae. The results showed a dramatic reduction in mosquito larval abundance 24 h posttreatment (93%) and was effective against larvae up to 22 days (100%). The residual effect after 28 days was 99.5%, and a limited residual effect was observed after 39 days (31.2%). A reduction in pupal density was observed after 4 days (70%) and was >98% from days 14 to 28 posttreatment, persisting for up to 39 days (84% after 39 days). The results demonstrate the effective use of the *Bti* + *Bs* formulation against *Cx. pipiens* in vegetated ditches in rural areas. Our modeling framework provides a flexible statistical approach to predict the residual effect of the product over time, in order to plan a seasonal intervention scheme.

KEY WORDS *Bacillus*, control, *Culex pipiens*, larval reduction, larvicide, mosquito

INTRODUCTION

Larval control is a key component of integrated mosquito control management, particularly in temperate regions (Marrama and Schaffner 2012). However, few larvicide products are currently available. The commercial introduction of new products is hindered by the time and financial investment required for basic research and product development, registration, and commercialization. In Europe, insect growth regulators (e.g., S-methoprene, pyriproxyfen, diflubenzuron; hereafter IGRs) are the only chemical commercial products available for larval mosquito control (EU 2012; regulation 528/12). Insect growth regulators are widely applied due to their persistence (with a maximum persistence for 3–4 weeks with diflubenzuron and a minimum for 1–2 weeks with s-methoprene.), which reduces implementation costs where multivoltine or sequentially appearing target species are present. However, there

are increased reports of resistance to IGRs (e.g., diflubenzuron) in Mediterranean populations of *Culex pipiens* (Linnaeus): France (Fotakis et al. 2020), Turkey (Guz et al. 2020), and Italy (Porretta et al. 2019), with particular reference to Italy where resistance has been phenotypically observed (Grigoraki et al. 2017). Physical products, such as monomolecular films that induce the drowning of eggs and suffocation of larvae and pupae (Mbare et al. 2014), are increasingly used. However, they may affect nontarget species and are only effective on larval sites with limited vegetation that breaks their monomolecular surface (Garrett and White 1977, Nayar and Ali 2003).

Bacterial larvicides (e.g., *Bacillus thuringiensis* subsp. *israelensis* de Barjac, *B. sphaericus* Neide, and their combinations), which produce toxins lethal to the larvae of Diptera (e.g., mosquitoes, fungus gnats, and black flies), are very specific, resulting in lower environmental impact, and thus are preferred in natural habitats (Boisvert and Boisvert 2000, Lacey 2007). The United States Environmental Protection Agency and the European Union have published guidelines for the assessment of risks associated with the use of microbial pesticides (EPA 2000, EU 2012).

Bacillus thuringiensis israelensis (hereafter *Bti*) is largely used against mosquitoes in rural areas, particularly in nonpolluted water (e.g., gutters, ditches). It shows higher persistence than in water with high levels of organic matter which binds with toxins, leading to more rapid denaturation (Mulla 1990, Russell et al. 2009), thus increasing implementation costs due to the need for frequent applications. So far, no resistance to *Bti* formulations has been

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Fig. 1. Left: A typical rural ditch hosting mosquito larvae and pupae in Padua Province (Italy). Right: Collector sampling for mosquito immature stages using dipper.

documented in mosquito populations even after long and widespread field applications (Becker 1997, Regis and Nielsen-LeRoux 2000). In contrast, *B. sphaericus* (hereafter *Bs*) is utilized predominantly in organically rich habitats and has been shown to have longer residual activity and higher tolerance to organic pollution (Mulla et al. 1999) than *Bti* (Lacey 2007). However, *Bs* is effective against a narrower range of mosquito species than *Bti*, and a potential for resistance development has been observed in the *Cx. pipiens* complex (Rao et al. 1995, Yuan et al. 2000).

Combinations of *Bti* and *Bs* (hereafter *Bti + Bs*) present the desirable attributes of both bacterial species in a single formulation: they are effective in clear and highly organic waters and show a greater persistence than *Bti* alone (28 days compared with 7–14 days of *Bti*). Moreover, they were shown to act synergistically, enhancing the toxicity against some mosquito species, such as *Culex Quinquefasciatus* (Say) and *Aedes aegypti* (Linnaeus). Free-flowing granular formulations with a narrow particle size are available for direct application by hand or granule spreaders (WHO 2016, EPA 2018).

The *Bti + Bs* combination effectiveness and persistence have been tested in small- and large-scale field trials on a variety of mosquito species (WHO 2016) (e.g., *Ae. aegypti*, *Aedes Albopictus* (Skuse), *Cx. Pipiens*, *Cx. Quinquefasciatus*) depending on the type of treatment (e.g., manual, or aerial application), and habitats such as catch basins (Anderson et al. 2011, Guidi et al. 2013), water tanks (Cetin et al. 2015), and natural or artificial rainwater (Su 2008). However, very few data are available on vegetated larval sites. In polluted water ditches in India, *Cx. Quinquefasciatus* larval and pupal densities were significantly reduced for 8–15 days at dosages of both 500 and 1,000 mg/m² (WHO 2016); in Brazilian fishponds, *Anopheles darlingi* (Root) larval abundance was reduced for up to 7 days after an application of *Bti + Bs* (Fontoura et al. 2020). Notably, the shorter time of the residual effect reported in Brazilian fishponds may potentially be due to the overlapping of the rainy season in the

Amazon, when heavy rainfall raises the water level of larval habitats, diluting the product.

Traditionally, the experimental design to establish the efficacy of insecticide includes a comparison of a treated versus an untreated area. Most of the field and laboratory *Bti + Bs* experiments evaluate the reduction of larvae/pupae pre- and posttreatment, using Mulla's equation (Mulla et al. 1971). However, such an approach does not consider the temporal dynamics of the larvae/pupae population along with other abiotic factors that could have had an impact during the course of the experiment.

We carried out a case-control, longitudinal field experiment to assess the persistence of the lethal effect of the *Bti + Bs* combination in highly vegetated ditches along roads in a rural area in northeast Italy (Fig. 1). These ditches collect water from rainfall, civilian dwellings, and agricultural/industrial activities, and represent the main larval sites for *Cx. pipiens*, not only a high-nuisance species in the country, but also the primary vector of the West Nile virus (WNV), a pathogen responsible for hundreds of autochthonous human cases in Europe each year. The results are expressed as a function of larval/pupal abundance after treatment, including their nonlinear temporal dynamics, which allow a better assessment of the treatment's effectiveness and persistence under field conditions than conventional analytic approaches.

MATERIALS AND METHODS

Study area and experimental procedures

Twelve ditches of various depths between 0.4 and 1 m, hosting mosquito larvae, were selected in the rural area of the municipality of Brugine (45°17'47.731"N, 11°59'42.406"E; province of Padua, northeast Italy) (Fig. 1, left panel). The selected ditches had not been treated with larvicide for at least 1 year.

The experiment was carried out from June 6 to July 15, 2019. Five randomly selected ditches (treated sites) were treated by distributing 15 g/10 m² of a granular formulation of *Bti* (4.7% p/p) and *Bs*

(2.9% p/p) (Vectomax FG®; INDIA Industrie Chimiche srl, Padua, Italy) manually on the water surface. The remaining 7 ditches were left untreated.

Larval and pupal sampling was carried out using a 500-ml standard telescopic dipper in both treated and untreated sites at the beginning of the experiment and at 7 additional time points (i.e., 24 h and 4, 7, 14, 21, 28, and 39 days posttreatment) (Fig. 1, right panel). In each ditch, 2 dippings were carried out close to each bank and 1 dipping in the central part, waiting 30 sec between each dipping. The collected water was transferred into a white bucket, and mosquito larvae and pupae were counted. Morphological identification of Culicidae genera was carried out only for 3rd and 4th instars. A subsample of larvae was reared to adult stage and identified to species (Severini et al. 2009).

Statistical methods

The abundance of larvae and pupae in treated and untreated sites was assessed by a generalized additive mixed model (GAMM) in a Bayesian framework. The response variable (the total number of specimens collected in a ditch at a given time point) is assumed to follow a negative binomial distribution with mean μ , dispersion parameter θ , and logarithmic link function. Four smoothing functions modeling the temporal dynamic during the experiment (O'Sullivan spline with 3 internal knots) were considered in the GAMM framework: one for larvae at the treated site, one for pupae at the treated site, one for larvae at the untreated site, and one for pupae at the untreated site. Moreover, we included 4 dichotomous variables identifying which life stage (larva or pupa) and site (treated or untreated) the observed number of specimens belonged to. This model structure makes it possible to quantify independently the nonlinear temporal effect of the number of specimens at the 2 sites. The number of days of field experiment (40 days) was standardized (after subtracting its mean value and dividing by its standard deviation) to improve the numerical stability of the model (Schielzeth 2010). Finally, we modeled the individual variability of ditches (which represent the observational unit of our experiment and were resampled over 40 days) across sites as a random effect term in the GAMM, whose equations are the following:

$$Y_{i,j} \sim NB(\mu_{i,j}, \theta)$$

$$E(Y_{i,j}) = \mu_{i,j}; \text{Var}(Y_{i,j}) = \mu_{i,j} + \frac{\mu_{i,j}^2}{\theta}$$

$$\begin{aligned} \log(\mu_{i,j}) = & \beta_0 + \beta_1 X^{P,T} + \beta_2 X^{L,U} + \beta_3 X^{P,U} \\ & + f_{L,T}(\text{Day}_{i,j}) + f_{P,T}(\text{Day}_{i,j}) \\ & + f_{L,U}(\text{Day}_{i,j}) + f_{P,U}(\text{Day}_{i,j}) + \varepsilon_j \end{aligned}$$

$$\varepsilon_j \sim \text{Norm}(0, \sigma^2)$$

where $Y_{i,j}$ is the total number of specimens (either larvae or pupae) collected during sampling i ($i = 1, \dots, 8$) in the j th ditch ($j = 1, \dots, 11$); $\beta_0, \beta_1, \beta_2$, and β_3 are model parameters, with β_0 representing the average value of larvae in the treated site; X are dichotomous variables identifying if $Y_{i,j}$ are either pupae (P) or larvae (L) in the treated (T) or untreated (U) site; $f()$ is the smoothing function on the number of days on 1st sampling (Day); and ε_j is the ditch random effect with mean 0 and variance σ^2 . Diffuse normal priors were used for regression parameters, while Cauchy priors were used for standard deviation parameters (Gelman 2006). Finally, model assumptions were checked by graphical inspection of model residuals. To estimate the model parameters, 3 Markov chains were built running 50,000 iterations with a burn-in of 40,000 and a thinning rate of 10. The reduction in larvae and pupae density in the treated site was calculated by the following equation:

$$\text{Reduction (\%)} = \frac{e^{\mu_{i,j}} - e^{\mu_{i,j}}}{e^{\mu_{i,j}}}$$

where $\mu_{i,j}$ is the predicted mean value of either larvae or pupae only in the treated site, as obtained from the GAMM model. All analyses were carried out using the R and R2jags (Su and Yajima 2020) statistical software packages.

RESULTS

A total of 16,843 mosquito larvae and 5,774 pupae were collected during 7 sampling periods after the treatment of ditches with $Bti + Bs$ (i.e., 1,421 larvae and 1,319 pupae in the treated ditches and 16,843 larvae and 4,455 pupae in the untreated ditches). In the treated ditches, the number of larvae and pupae collected ranged from 0 to 360 and from 1 to 65, respectively, while for untreated sites these ranges were slightly higher (20–380 and 25–130, respectively). The temporal dynamics of larvae and pupae collected in treated and untreated ditches at each time interval is shown in Fig. S1. The morphological identification of a subsample of adults emerged from collected larvae suggests the exclusive presence of *Cx. pipiens* in the ditches included in the study. Precipitation and Temperature data were obtained by the Regional Environmental Agency (http://www.arpa.veneto.it/bollettini/meteo60gg/Staz_111.htm). The average daily temperatures ranged between 19.8°C and 33.6°C, and the rainfall was negligible (precipitation = 1.31 mm during the whole study period). The overall percentage of samplings with zero specimens after treatment is estimated to be 74.3% and 49.7% for larvae and pupae in the treated site and 7.9% and 11.9% in the untreated site, respectively. Figure S2 shows the percentage of samplings with zero specimens in the 7 collections after treatment.

The GAMM results show a comparable larval and pupal abundance in treated and untreated sites before

Table 1. Expected mean abundance (log-link scale) of mosquito larvae and pupae in ditches treated with a combination of *Bacillus thuringiensis* subsp. *israelensis* and *B. sphaericus* and untreated ditches, as estimated by a generalized additive mixed model based on a Bayesian framework, estimated mean values, and 95% credible confidence intervals of model parameters.

Parameter	Mean	SE	2.5%	97.5%
Treated—larvae (intercept)	0.730	0.265	0.186	1.251
Treated—pupae	0.039	0.130	-0.216	0.283
Untreated—larvae	3.069	0.338	2.364	3.703
Untreated—pupae	1.805	0.350	1.051	2.471
Negative binomial size parameter ($1/\theta$)	1.079	0.058	0.968	1.197
Random effect of ditches	0.586	0.154	0.359	0.943

treatment, and a significantly lower abundance in the treated site compared with the untreated site after treatment (Table 1). The estimated differences between smoothers in treated and untreated sites (Fig. S3) highlighted that the significantly lower abundance persistence is up to 28 and 32 days after the product application for larvae and pupae, respectively. Figure 2 shows the 4 estimated nonlinear smoothers representing the temporal dynamics of sampled larvae and pupae in the treated and untreated sites. In the treated sites, a significantly sharp decrease in larval abundance is observed immediately (24 h) after the larvicide application, whereas the decrease of pupae is delayed by 4 days. An increase in larval and pupal abundance is observed after 21 days in treated sites, and larvae reach levels comparable to those observed in untreated sites slightly before pupae (28 versus 32 days posttreatment).

The mean net reduction of larval abundance was evident in all treated ditches 24 h posttreatment (92.95%). No live larvae were sampled up to 22 days after treatment, and the reduction (99.5%) was significant for up to 28 days. A residual effect was observed for up to 39 days posttreatment (31.2% reduction). As expected, based on the need for the

toxins to be ingested in order to exhibit a lethal effect on pupal density was observed after 4 days (70.1%). Pupal reduction was >98% from 14 to 28 days posttreatment and persisted up to the end of the experiment (84% after 39 days) (Fig. S4 and Table 2). The estimated variance of the random effect variable (i.e., ditches) in GAMM is 0.586 (95% CI 0.359–0.943), indicating the presence of some heterogeneity among ditches.

DISCUSSION

We report the results of a field experiment conducted to test the persistence of a single application of *Bti* + *Bs* granular formulation on *Cx. pipiens* mosquitoes in highly vegetated rural ditches in Italy. The effectiveness of *Bti* + *Bs* has been rarely evaluated in this kind of habitat, despite their suitability as optimal breeding sites for this mosquito species, primary vector of the WNV. The result of the statistical analysis provided strong evidence of a reduction across time in the relative abundance of larvae and pupae in treated ditches.

A comparison of the results obtained with the existing literature is hampered by differences in the type of treated larval habitats, target species,

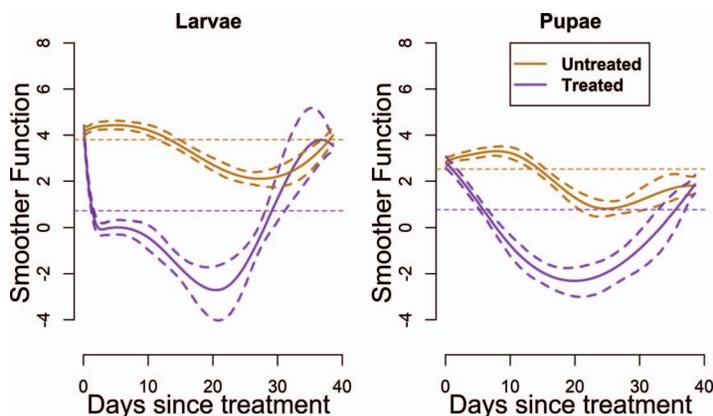


Fig. 2. Estimated nonlinear smoothed curves of the temporal effect on the number of mosquito larvae (left) and pupae (right) in ditches treated with a combination of *Bacillus thuringiensis* subsp. *israelensis* and *B. sphaericus* (*Bti* + *Bs*) (purple) and untreated ditches (orange). Solid lines = posterior mean in log-link scale estimated by generalized additive mixed model (GAMM) model. Dashed lines = 95% credible confidence interval. Horizontal dashed lines = estimated posterior mean for the smoother.

Table 2. Percent reduction in mosquito larvae and pupae abundance in ditches with abundant vegetation that were treated with a combination of *Bacillus thuringiensis* subsp. *israelensis* and *B. sphaericus*. Percentages are based on the results of a generalized additive mixed model. *N* = number of collected larvae and pupae.

Days posttreatment	Reduction, % (<i>N</i>)	
	Larvae	Pupae
24 h	92.95 (174)	21.3 (722)
4	98.46 (103)	70.14 (351)
7	98.44 (22)	89.1 (23)
14	99.5 (21)	98.71 (21)
21	100 (0)	99.3 (1)
28	99.5 (27)	98.97 (7)
39	31.77 (1,074)	83.96 (197)

experimental designs, and statistical approach. In fact, most of the available studies have assessed the impact of *Bti* + *Bs* on mosquito abundance only during the day of field sampling using the Mulla's equation (Mulla et al. 1971)

$$100 - \left(\frac{\text{Control}_{pre}}{\text{Treated}_{pre}} \times \frac{\text{Treated}_{post}}{\text{Control}_{post}} \times 100 \right)$$

which does not allow for a proper evaluation of effectiveness over time. In addition, we applied Mulla's formula (Table S1) to our data, obtaining similar results to those of our statistical model.

Our results are similar to those obtained in wetlands in the USA, where a single aerial application of *Bti* + *Bs* (8.9 kg/ha) was effective against *Cx. tarsalis* Coq. larvae and pupae for up to 28 days posttreatment (Dritz et al. 2011). We estimated a higher efficacy for *Bti* + *Bs* compared with a previous evaluation in Brazilian fishponds (Fontoura et al. 2020), where a single application (10 kg/ha) substantially reduced (>95%) anopheline larval densities for 7 days. However, Fontoura et al. (2020) reported residual effects for up to 21 days after reapplication of *Bti* + *Bs* (20 kg/ha). Our results, obtained from rural ditches, estimated *Bti* + *Bs* to be less effective with respect to other studies conducted in different settings, such as a septic tank in Turkey (Cetin et al. 2015) and urban catch basins in the USA (Anderson et al. 2011) and Switzerland (Guidi et al. 2013). The results obtained have a great operational value, by predicting the residual effect of *Bti* + *Bs* over time. This is particularly relevant in some areas of Europe (Italy, France, Spain, and Switzerland [EU regulation 528/12]) where only 8 *Bti* + *Bs* treatments are allowed during each season. According to model results, a treatment every 3 wk would prevent the emergence of *Cx. pipiens* adults for 5–6 consecutive months. However, treatments at 28-day intervals would reduce the emergence of 99.5% of adults in the treated sites. Notably, the model did not include abiotic variables (such as temperature and/or rainfall) or biotic ones (such as ecological differences among

the ditches tested), which were included as random effects accounting for unexplained variability by model predictors. Future studies could exploit the model to evaluate the interaction between larvicide and a wide range of different climatic conditions. Finally, our results support the effectiveness of *Bti* + *Bs* granular formulations for the treatment of vegetated ditches that represent the main rural breeding sites of *Cx. pipiens* in some regions of northeastern Italy where WNV is endemic.

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Innovative vector control method: *Wolbachia*

In preparation

Authors

list of authors still to be defined

Material and Methods

Assessment of the viability rate of *Ae. albopictus* eggs collected by ovitraps

The temporal dynamic of the viability rate (p), here defined as the ratio between the number of viable (i.e. hatched and embryonated) eggs (N) and the total number of collected eggs (N^{tot}), was investigated using a generalized additive mixed model (GAMM-1) in a Bayesian framework. Model covariates were site (qualitative: treated/control) and day of the year (quantitative). The day of the year was included as a smoothing function (O'Sullivan spline with five internal knots) to model the non-linear temporal effect of the viability rate in the two sites. The interaction between day of the year and site was also considered to allow different temporal patterns of viability rate between treated and control sites. Moreover, to improve the numerical stability of the model and help with interpretation of the results, day of the year was standardized (subtracted its mean value and divided by its standard deviation). The mixed-effects model approach with the random effect ovitrap was considered to take into account that observations were collected repeatedly from each ovitrap during the experiment. p was modelled using a logistic link function assuming that N follows a binomial distribution with parameters p and N^{tot} . Therefore, GAMM-1 equations are the following

$$\begin{aligned}
 N_{i,j} &= \text{Binomial}(p_{i,j}, N_{i,j}^{tot}) \\
 E(N_{i,j}) &= p_{i,j}N_{i,j}^{tot}; \text{Var}(N_{i,j}) = p_{i,j}(1 - p_{i,j})N_{i,j}^{tot} \\
 \text{logit}(p_{i,j}) &= \pi_{i,j} \leftrightarrow p_{i,j} = \frac{e^{\pi_{i,j}}}{1 + e^{\pi_{i,j}}} \\
 \pi_{i,j} &= \alpha + \beta \text{Site}_{i,j} + f_k(\text{Day}_{i,j}) + \varepsilon_j \\
 \varepsilon_j &\sim \text{Norm}(0, \sigma^2_1)
 \end{aligned}$$

Where $N_{i,j}$ is the total number of viable eggs observed at collection i ($i = 1, \dots, 16$) in the j th ovitrap ($j = 1, \dots, 60$), α and β are the model parameters, $f_k()$ is the smoothing function where the index k indicates a different smoother per site (treated, control), and ε_j is the ovitrap random effect with mean 0 and variance σ^2_1 . Missing values and ovitrap data with zero eggs collection were discarded. Diffuse normal priors [Norm (0,1000)] were used for the smother's parameters and for regression parameters β , informative prior [Norm (3,1)] was used for regression parameters α as high viability was expected in the control site, while Chauchy priors were used for the variance terms of both smoothers and random effect [$\sigma^2_1 \sim$ |Norm(0,25) / Norm(0,1)|].

Assessment of fertility rates of single ovidepositing females

The fertility rate of single females collected in the field was also investigated by analysing the viability of individual eggs batches using a generalized linear model (GLM-1). The percentage of viable eggs laid by single females was modelled using a logistic link function assuming that the number of collected eggs that were viable of the total number of laid eggs follows a binomial distribution. However, a female mosquito in the treated site may or may not have mated with ARwP males. Therefore, mosquito mating was modelled as a Bernoulli trial with parameter (θ). If the female was modelled as ARwP mated then the viability rate (p^{Wpos}) of its eggs would differ from that of a non-ARwP-mated female (p^{Wneg}), which is assumed to correspond to the eggs viability rate of single

females collected in the control sites. Beta priors were used for both viability rates [informative prior based on the assumption that mating between wild female and ARwP males results in sterility $p^{Wpos} \sim \text{Beta}(a = 1, b = 20)$; uninformative prior $p^{Wneg} \sim \text{Beta}(a = 1, b = 1)$] and the mosquito ARwP mating [$\vartheta \sim \text{Beta}(a = 1, b = 1)$]. This is a simplification that does not take into account the possibility of multiple matings. Therefore, GLM-1 equations are as follows:

For observations in control sites

$$N_{i,j}^{fertile} = \text{Binomial}(p^{Wneg}, N_{i,j}^{laid})$$

$$E(N_{i,j}^{fertile}) = p^{Wneg} N_{i,j}^{laid}; \text{Var}(N_{i,j}^{fertile}) = p^{Wneg} (1 - p^{Wneg}) N_{i,j}^{laid}$$

$$\text{logit}(p^{Wneg}) = \pi_{i,j}^c \leftrightarrow p^{Wneg} = \frac{e^{\pi_{i,j}^c}}{1 + e^{\pi_{i,j}^c}}$$

For observations in the treated site

$$\Theta_{i,j} \sim \text{Ber}(\vartheta_{i,j}) \text{ where } \vartheta_{i,j} \in \{0,1\}$$

$$p_{i,j}^T = \Theta_{i,j} p_{i,j}^{Wpos} + p_{i,j}^{Wpos} (1 - \Theta_{i,j})$$

$$N_{i,j}^{fertile} = \text{Binomial}(p_{i,j}^T, N_{i,j}^{laid})$$

$$E(N_{i,j}^{fertile}) = p_{i,j}^T N_{i,j}^{laid}; \text{Var}(N_{i,j}^{fertile}) = p_{i,j}^T (1 - p_{i,j}^T) N_{i,j}^{laid}$$

$$\text{logit}(p_{i,j}^T) = \pi_{i,j}^t \leftrightarrow p_{i,j}^T = \frac{e^{\pi_{i,j}^t}}{1 + e^{\pi_{i,j}^t}}$$

Where $N_{i,j}^{fertile}$ is the total number of viable eggs observed for female i captured in the j th collection, ϑ is the expected probability of mating with a ARwP male in the treated site. Finally, model assumptions were checked by graphical inspection of model residuals. To estimate parameters of both models, three chains were used running 50 000 iterations with a burn-in of 40 000 and a thinning rate of 10.

Results

In total, 32 721 *Ae. albopictus* ARwP males (corresponding to an average number of 4 674 males per week) were released during the 7-week experiment (Table 1).

Date of ARwP	Release Arwp male (N)	Time since last ARwP release (days)	Males recaptured	Males Tasted	ARwP frequency in release site (N)
21 June	4800	-	-	-	-
24 June	-	3	68	20	80(16)
27 June	-	6	15	15	53,33(8)
28 June	4800	7	-	-	-
1 July	-	3	34	20	45(9)
4 July	3850	6	12	12	41,67(5)
8 July	1420	2	30	20	60(12)
11 July	4435	3	39	20	55(11)
15 July	-	4	24	20	50(10)
18 July	-	7	18	18	38,89(7)
19 July	5461	8	-	-	-
22 July	-	3	58	20	50(10)
25 July	-	6	22	20	40(8)
29 July	-	10	17	17	58,82(10)
30 July	4395	11	-	-	-
1 August	-	2	39	20	90(18)
5 August	-	4	47	20	40(8)

Table 1. *Aedes albopictus* ARwP males released and recaptured in green area within urban Rome (Italy). Date of release, number of ARwP males released and ARwP frequency of the overall sampled males

Viability rates of eggs collected by ovitraps

A total of 17 143 *Ae. albopictus* eggs (5 008 in the treated site and 11 210 in the control sites) were collected in 718 ovitrap/collections (304 in the treated site and 414 in the control sites). Overall, the mean percentage of viable eggs calculated from day 1 after the first release to 8 days after the last release is estimated to be 67.7% (average absolute deviation 29.8%) and 99.1% (average absolute deviation 1.63%) for eggs collected in the treated and control sites, respectively. According to the results of the Bayesian model (GAMM, Table 2), egg viability rate in the treated site shows a strong temporal pattern (Fig. 1, Fig S1) and is on average significantly lower than in the control sites (Table 2), suggesting that released ARwP males had successfully sterilized a fraction of wild females. Before the first release, viability rate (calculated for 2 604 and 5 189 eggs collected in 252 ovitrap/collections in treated and control sites, respectively) is estimated to be >96% in both sites with no statistical difference, as shown by the overlapping credible intervals in Fig. S1. The estimated variance of the random effect variable (i.e. ovitrap) in GAMM is 0.736 (95% CI: 0.575–0.929), indicating the presence of some heterogeneity in viability rates among ovitraps.

Figure 1 shows the estimated temporal dynamic of the egg viability rates in the study sites based on GAMM. Overall, the estimated viability rate is >96% in the control sites, whereas in the treated site it starts decreasing following the first ARwP male release and reaches ~60% concurrently with the fifth release, when the highest difference between viability rates in the treated and control sites was

recorded (35% at day 22 from first release, i.e. day 195 in Fig. 1). Viability rate in the treated site remains <80% during the following three releases and increases 10 day from the last release (i.e day 200 in Fig. 1). It should be highlighted that viability rate was calculated based on eggs classified as hatched/embryonated of the total of hatched, embryonated and sterile eggs.

Variable	Mean	SE	95% CI
Intercept(Control)	4.223	0.164	3.922/4.566
Site (Treated)	-1.364	0.343	-2.228/-0.901

Table 2. Viability rate of *Aedes albopictus* eggs in treated and control sites in Rome (Italy) as estimated by GAMM. Estimated mean values and 95% credible intervals of model parameters

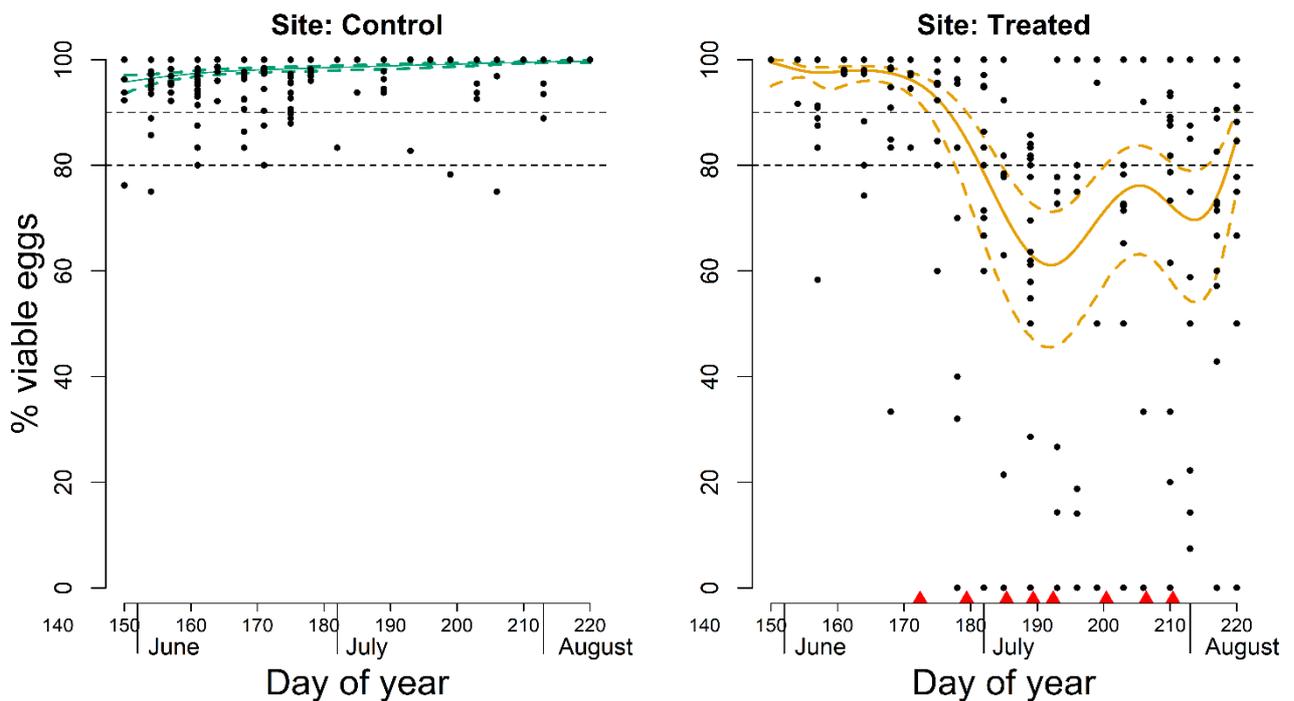


Figure 1. Temporal dynamics of the proportion of viable *Aedes albopictus* eggs in treated and control sites in Rome (Italy) as estimated by GAMM. Black solid line = estimated % of egg viability; dashed lines = 95% credible intervals; black dots = observed % of viable eggs/ovitrap; red triangles = ARWP male release dates.

Fertility rates of single ovipositing wild *Aedes albopictus* females

The fertility rate of single ovipositing females collected between day 3 from the first ARwP male release and day 7 after the last release was assessed on 4736 eggs laid by 122 females collected in the treated site and 2349 laid by 60 females collected in the control sites. Around one third (23%) of females collected in the release spots laid 100% infertile eggs; all were confirmed as inseminated by the presence of sperm in the spermathecae. Results of Bayesian analysis (GLM; Table 3) show a bimodal pattern of viability in eggs laid by females collected in the treated site, as opposed to a high viability rate in eggs laid by in females collected in the control sites (Fig. 2). Based on GLM estimates, the proportion of females classified as mated with ARwP males in the treated site is 41.78% (95% CI 33.18–50.80) (black dots in Fig. 2). The viability rate of eggs laid by these females (9.48; 95% CI 8.06-9.99) is on average lower than that of females classified as not-mated with ARwP males in both the treated and control sites (87.76; 95% CI 86.60–88.82) (vertical bars in Fig. 2).

Variable	Mean (%)	SE	95%CI
p^{Wpos}	9.48	0.522	8.06/9.99
p^{Wneg}	87.76	0.562	86.60/88.82
ϑ	41.78	4.580	33.18/50.80

Table 3. GLM estimates of fertility rate of *Aedes albopictus* females classified as mated (p^{Wpos}) and not mated (p^{Wneg}) with ARwP males and of the expected probability of mating with ARwP males in treated site (ϑ)

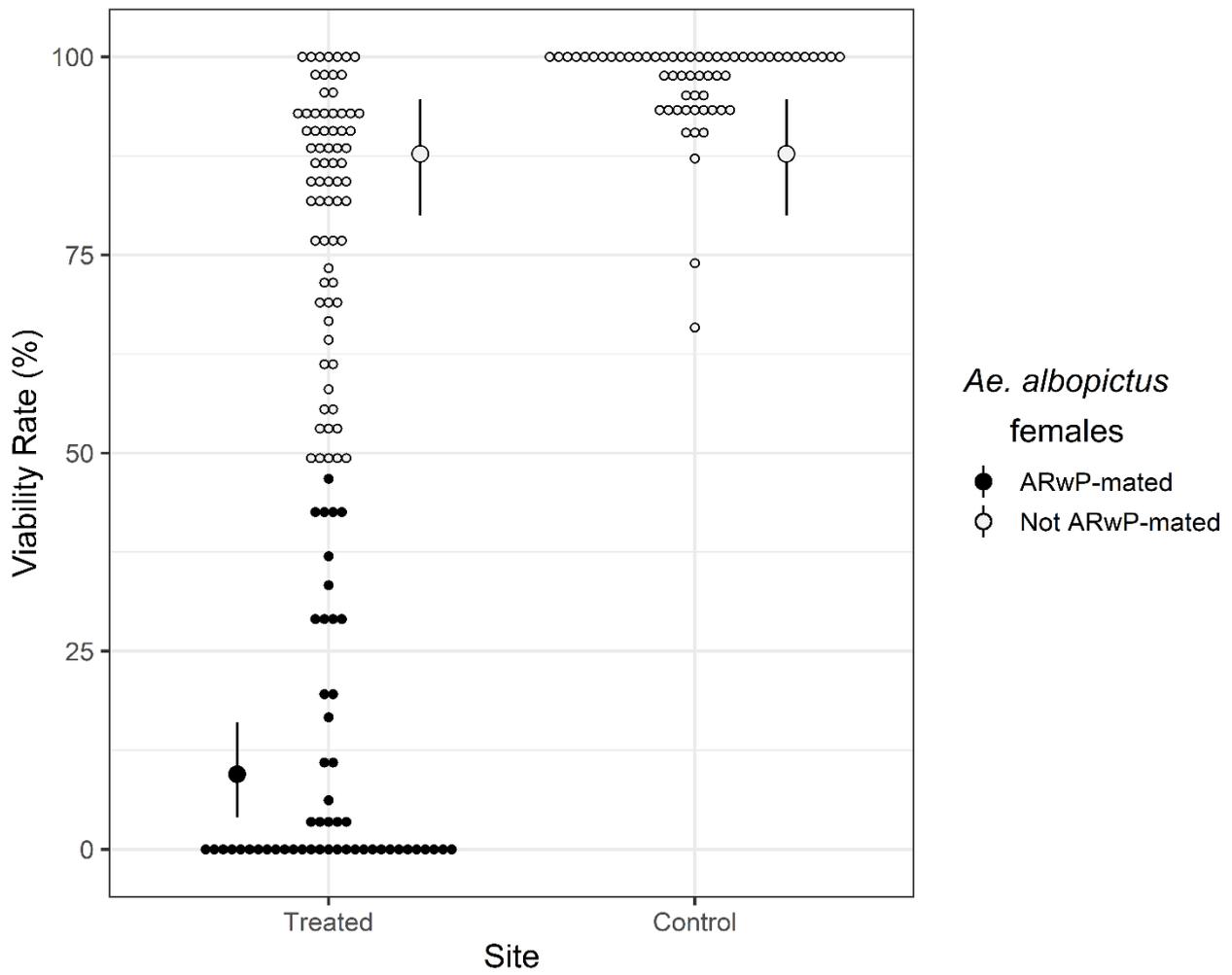


Figure 2. Egg viability in single ovipositing *Aedes albopictus* females in Treated and Control Sites. Egg viability rates were observed and estimated by GLM in Treated and Control Sites. Dots = observed fertility rates; bars = estimated mean viability rate of single ovipositing females classified as ARwP-mated (filled dots) or not mated (empty dots); vertical solid lines = 95% prediction intervals.

Ratio of ARwP to wild *Aedes albopictus* males

A total of 423 *Ae. albopictus* males was collected in the release spots within the treated site every 3–4 days from the first release until 6 days after the last release (Table 1). After the first male release (21 June), a frequency of 80% and 53.3% ARwP males was measured at day 3 and 6 respectively. ARwP male frequency decreased to 38.89% at 7 days after fifth release of male and increased to 90% at day 2 from last release. The mean frequency of ARwP males collected between 3 days from the first release and day 4 after the last release was 54.07% (95% CI 23.49-84.84.61), corresponding to a mean ARwP/wild-type ratio of 1.12:1

Supplementary Materials

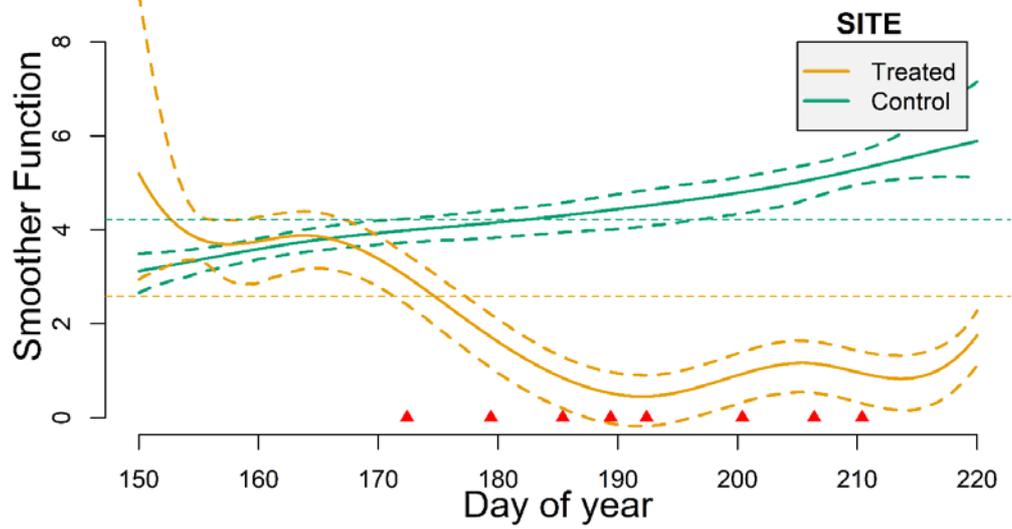


Figure S1. GAMM estimated smothers of *Aedes albopictus* egg fertility rate in Treated and Control Sites in Rome. Solid lines are the estimated smoother also considering the site parameter, the dashed lines are the 95% credible intervals. Dashed horizontal lines are smoother site average values (GAMM1: Control = 4.223; Treated = 2.859). Red triangles are the dates of the seven releases of ARwP males

Conclusions

My thesis work focuses on the development of quantitative methods in the field of medical entomology, with a specific emphasis on relevant epidemiological traits of mosquito-borne disease. During my PhD, I exploited my background in mathematical modelling and I learned and applied basic and advanced statistical techniques to model mosquito dynamics and mosquito-borne pathogen transmission. This has been possible thanks to the complementary expertise of my tutors during these 3 years. Through my tutors within the Medical Entomology group at the Dept. of Public Health and Infectious Diseases in Sapienza University I acquired a good knowledge of mosquito bionomics and mosquito-borne disease epidemiology and public health relevance and had access of data from experiments and surveys carried out in the field, on which I focused my analyses. Thanks from constant training and advice by my tutors at the Eco Health Research Unit at Fondazione Edmund Mach, I learned and exploited new approaches for field data analyses and applied my mathematical background to the estimation of epidemiologically relevant mosquito parameters. More in detail, during the first part of my PhD, I learned and applied inferential statistic techniques based on frequentist approach. Afterwards, I exploited my mathematical background to develop advanced mathematical tools based on Partial Differential Equations and to become familiar with advanced statistical techniques based on Bayesian approach and applied them to field data analysis.

With reference to statistical inference based on frequentist approach, I applied Generalized Linear Models and Generalized Additive Models to the analysis of field data from two studies on species of the *An. gambiae* complex from Afro-tropical malaria-endemic regions. These studies aimed at investigating the seasonal dynamic of main malaria vector species, as well as malaria transmission, in Senegal and Ivory Coast, respectively. I took advantage of using a multivariate approach to consider, within a single model, all the independent variables and their interactions that influenced the response variables (abundance of malaria and filariasis vector species). Interaction terms make model more complex, but often give useful insights to better understand how covariates (ie exophilic/endophilic behaviour, seasonal dynamic and host-seeking trap type) interact and permit to disentangle complex eco-epidemiological relationships and obtain insights of the underlying process. Such approach allows to not only quantify the average effect but more importantly to quantify the uncertainty characterizing the relationship tested. In addition, I emphasized the model validation part in terms of model goodness of fit and assessment of model assumption (e.g., residuals, R-squared, confidence intervals).

With reference to advanced mathematical approaches, I used both partial differential equations (PDE) and well-studied mathematical equation to estimate epidemiologically relevant parameters (population size, survival rate and dispersal) of *Ae. albopictus* in Italy from two Mark-Release-Recapture experiments. These parameters are instrumental to develop risk model of mosquito-borne diseases and to optimize interventions for mosquito control. In the first study, I applied basic mathematical equations to predict the population size of wild mosquitoes (Fisher-Fords equation) and the dispersal of *Ae. albopictus* males (Mean distance travel) sterilized by irradiation and released in Procida island, to assess the feasibility of this approach to suppress wild populations. In addition, I

applied a Generalized Linear Model within the frequentist framework, to infer the survival rate of released sterilized males and population size of the wild mosquitoes. Notably, standard approaches consider all entomological parameters as independent, and generally do not include the effect of either survival or population size on dispersal. To address this issue, in the second study, I applied a Partial Differential Equation to estimate the dispersal of wild *Ae. albopictus* females marked and released in a site in Padua Province as a function of time, space, and survival. To my knowledge this is the first application of Partial Differential Equation (which are a consolidated mathematical technique) to the field of mosquito entomology.

Finally, I enriched my knowledge on statistical inference by studying and also applying the Bayesian framework. The Bayesian approach assumes that the parameters to be estimated are realization of random variables with their on-characterizing probability distributions and is more flexible than the frequentist one, allowing the use of a more extensive range of statistical distributions, as well as more complex models and dependency structures. I then applied the Bayesian approach to evaluate the effectiveness of traditional and innovative mosquito control tools. In the first study, I evaluated the effectiveness of a bio-larvicide (i.e. a combination of *Bacillus thuringiensis* subsp. *israelensis* and *Bacillus sphaericus*) against *Cx pipiens* larvae in high vegetated ditches from Padua province by applying a family of Generalized Additive Mixed Models (GAMM) accounting for non-linear fluctuations in mosquito population. This approach overcomes the limitation of the Mulla's equation (the most used analytical approach for the evaluation of vector control effectiveness), which provides an estimate over a predetermined period of time (ie pre and post treatment) without taking into account the mosquito temporal dynamic. In the second study, I applied a family of Generalized Additive Mixed Models (GAMM) to estimate the eggs viability rate of *Ae. albopictus* over the time after the release of *Ae. albopictus* males transinfected with a *Wolbachia* strain from *Cx pipiens*. In this kind of studies, eggs viability rate is usually investigated using non-parametric statistical test (ie. Mann–Whitney U, Wilcoxon), which does not take into account the spatio-temporal structure of mosquito populations, nor the temporal dimension (mosquito seasonal dynamic), nor the impact of the multiple observations (repetitive collections from each ovitraps during the experiment). Moreover, I applied a hierarchical model, to estimate the viability rate of wild female may or may not mated with the male transinfected with *Wolbachia*. The hierarchical model allows to model in a single equation the mating event of a single female with males manipulated with a *Cx pipiens* *Wolbachia* strain and its viability rate.

In conclusion, my PhD training and research allowed me to extend and deepen my knowledge in the research field of vector ecology and vector-borne disease, which represents a serious public health concern. In all studies described, I was responsible to develop mathematical and/or statistical approach and to carry out the analyses in order to provide quantitative estimated of eco-epidemiological parameters. In doing this, I combined empirical observations with the application of mathematical and statistical models, highlighting the added value provided by quantitative methods in these research field. The results presented in the thesis have been already published or, in single case, are being finalised in a manuscript.

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