Assessing the re-introduction of *Aedes aegypti* in Europe. Will the Yellow Fever Mosquito colonize the Old World?

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25th May 2020

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Highlights

- European coasts present favourable conditions for *Aedes aegypti* establishment

- We assess the species introduction and establishment using a process-based model

- We selected five ports: Algeciras, Barcelona, Venice , Genoa and Rotterdam

- Algeciras and Barcelona were the most suitable areas for the species establishment

- Climate change could make more suitable the northernmost study areas

Abstract

Aedes aegypti are feared invasive mosquitoes as they transmit pathogens which cause debilitating diseases in humans. Although mainland Europe has not yet witnessed re-establishment and diffusion of *Ae. aegypti* populations, several urban areas along coastlines represent suitable habitats for the species. In addition, European coastal areas are characterized by a high exotic species propagule pressure, due to the dense international ship traffic.

Here, we applied a process-based population dynamical model to simulate both the life cycle and dispersal of *Ae. aegypti* at the local scale after its introduction through ship traffic. We selected five European ports along a gradient of latitude by considering both environmental conditions and the economical importance of ports: Algeciras and Barcelona in Spain; Venice and Genoa in Italy and Rotterdam in the Netherlands. The model was informed using parameters relevant for *Ae. aegypti* biology, fine-scale temperature time-series, urban structures and road networks.

According to model results, the introduction of small quantities of *Ae. aegypti* eggs, from 10 to 1000, has the potential to cause species establishment, high local densities and slow initial dispersal in the two southernmost study areas, Algeciras and Barcelona, whereas Genoa may be considered only close to suitability. Barcelona had the highest simulated mosquito densities (584 females/ha), whereas Algeciras densities were never more than 32 females/ha, but remained higher during winter. The spatial spread of the species varied between a few hundred meters to 2 km/year and was affected by the structure of the road network, topography and urban sprawl along the coast in the surrounding of the port of introduction. The study areas of Genoa, Venice and Rotterdam were found not suitable for establishment of this mosquito species, however climate change could create conditions for *Ae. aegypti* invasion in

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these regions in the next decades.

It is commonly accepted that targeted monitoring and early control actions are the most effective methods to hinder the establishment of invasive species in new areas. Our findings and model framework may support surveillance initiatives for those European coastal urban areas which have a known high propagule pressure and a high modelled probability of *Ae. aegypti* establishment.

Keywords: Invasion ecology; process-based models; mosquitoes; biological invasions; spatial epidemiology; vector-borne pathogens.

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1   Introduction

Mosquitoes in the genus *Aedes* (Culicidae: Diptera) are amongst the most feared invasive species due to their competence for transmitting debilitating pathogens. Among them, *Aedes aegypti* is a global health concern due to its capacity to thrive in urban areas and because it is an unrivalled vector for viruses (Leta et al., 2018). This species evolved in Sub-Saharan Africa and was progressively brought outside its original geographical range by human trades. First, the slave trade between the 15th and 19th centuries moved the mosquito from West Africa to Europe and the Americas. Afterwards, intensified trade with Asia during the 18th and 19th centuries and troops movements during World War II caused further expansion of its geographical distribution (Powell et al., 2013).

To date, mainland Europe is the only continent, except Antarctica, where *Ae. aegypti* has no established populations, despite having been present from Portugal to the Black Sea until the 20th century (Cardamatis, 1929; Schaffner and Mathis, 2014). Improvements both in hygiene and in the management of water systems as well as the widespread application of insecticides to decrease the malaria burden are thought to have led the species to extinction in this continent. However, in recent years *Ae. aegypti* has been detected multiple times in mainland Europe or in areas geographically or politically connected with Europe. Multiple introductions of *Ae. aegypti* were detected in the Netherlands in 2010 and again in 2016 (Brown et al., 2011; Ibañez-Justicia et al., 2017). Established populations of *Ae. aegypti* were found along the coast of the Black Sea in 2008 (in Turkey, Georgia and Russian Federation; ECDC et al. 2019), in the Portuguese Island of Madeira, located about 900 km South-West of mainland Portugal, in 2004 and, more recently, from nearby Egypt (Abozeid et al., 2018).

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57   Mainland Europe has not yet witnessed the establishment and diffusion of *Ae. ae-*

58   *gypti* populations. However, urban areas distributed along coastlines represent suitable

59   habitats for the species (Kraemer et al., 2015). European coastlines are also character-

60   ized by extremely high exotic species propagule pressure (Lockwood et al., 2005; Dunn

61   and Hatcher, 2015; Blackburn et al., 2020), due to the dense international maritime trade

62   which nowadays accounts for 90% of the global exchange of goods in Europe (IMO,

63   2019). The Mediterranean basin plays a relevant role in global trades and it is expected to

64   become even more important in the next decades since massive investments are planned

65   in coastal and harbour infrastructures (e.g. see for instance the Chinese government's Belt

66   and Roads initiative; Ekman, 2018). Moreover, the Near and the Far East as well as North

67   America, where *Ae. aegypti* populations are widespread, represent the major sources of

68   goods shipped to Europe. Therefore, in the European context, coastal urban areas and the

69   Mediterranean basin should be considered as focal points when evaluating the potential in-

70   troduction of invasive mosquitoes. The precedent provided by *Ae. albopictus*, an invasive

71   species related to *Ae. aegypti* could serve as a cautionary tale. This species was detected

72   for the first time in the port of Durazzo, Albania, likely arrived from China (Adhami and

73   Reiter, 1998), and nowadays is distributed along most of south and central Europe. Its

74   introduction, establishment and dispersion in the European continent appear to have been

75   supported by maritime transportation of tires and other goods (Eritja et al., 2005).

76   Outbreaks caused by *Aedes*-borne pathogens such as chikungunya and dengue have

77   been recorded multiple times in Europe in the last two decades and, more recently, the

78   first cases of Zika infection in humans have been reported in Southern France (Rezza

79   et al., 2007; Venturi et al., 2017; Brady and Hay, 2019). Until now, these outbreaks have

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80 been limited in time and space and sustained by *Ae. albopictus*, which is an abundant
81 but more opportunistic feeder and, thus, less competent vector than *Ae. aegypti* (Richards
82 et al., 2006). The establishment of *Ae. aegypti* in Europe would dramatically change
83 the epidemiological landscape by increasing the risk of pathogen transmission among the
84 human population by this species, mimicking the public-health emergency which followed
85 its recent introduction in the West Coast of the US (Metzger et al., 2017).

86 Surveillance enabling early warning and rapid control actions is the best tool we have
87 to prevent species invasions, eradicate invading species or manage their population at low
88 levels (Simberloff, 2014). Here, to support surveillance and early detection of *Ae. aegypti*
89 in Europe, we applied a process-based population model which simulates both life cycle
90 and dispersal of *Ae. aegypti* after its introduction, for example through ship traffic in Eu-
91 ropean ports. To develop the model, we followed the theoretical model structure proposed
92 by (Otero et al., 2008) and extended in Montecino et al. (2016). We chose to simulate
93 introductions of *Ae. aegypti* in ports located along a latitudinal gradient to capture the
94 variability of climatic condition along European coastlines. We selected five of the busiest
95 European container ports (Eurostat, 2018) which were also located in areas predicted to be
96 suitable for *Ae. aegypti* (Kraemer et al., 2015, 2019) or where *Ae. aegypti* was detected in
97 the past, namely: Algeciras and Barcelona, Spain; Venice and Genoa, Italy and Rotterdam,
98 the Netherlands.

99 The main questions we asked in this study were:

100 • Could *Ae. aegypti* eggs introduced via ship traffic via the major European harbours
101   establish viable populations?

102 • If so, what is the likelihood that an introduction event brings about established inva-

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103   sive populations?

104   • At what latitude is an introduction more likely to happen?

105   • What is the minimum propagule pressure required for a successful introduction?

106   • What is the space-time spatial distribution trend which *Ae. aegypti* populations will

107   follow once established?

2   Methods

2.1   Overview of model structure

110   We applied a process-based and time-discrete model to simulate population dynamics of

111   *Ae. aegypti* after introduction in five study areas. *Ae aegypti* life cycle presents four main

112   developmental stages: egg, larva, pupa and adult stage. We simplified the life cycle of *Ae.*

113   *aegypti* by merging the larval and pupal stages in a unique compartment which we called

114   "immature". Thus, the model considers three main compartments: egg, immature and

115   adult. Temperature and space can be considered the main environmental drivers of inva-

116   sive mosquito ecology, thus events in the mosquito life cycle were treated as stochastic pro-

117   cesses with probabilities derived from temperature-dependent (development) or distance-

118   dependent (movement) functions (Fig. 1). Each event in the simulated life cycle occurred

119   once per day always in the same order and each study area was divided in a grid composed

120   of 250 m cells, which represented the fundamental spatial units into which*Ae. aegypti* life

121   cycle took place and among which adult mosquitoes dispersed. This simplified space-time

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122   arena allowed us to reduce dramatically the structure of *Ae. aegypti* life cycle, and there-

123   fore the computing burden of the model, while retaining temporal, spatial and "biological"

124   resolutions relevant for our questions (Pascoe et al., 2019). Model output consisted in a

125   numerical matrix containing the daily number of individuals in each life stage for each 250

126   m cell of each study area, for each iteration.

2.2   Study areas and road network

128   We considered five 100x100 km study areas placed along a gradient of latitude (Fig. 2)

129   and centered on the ports of Algeciras (lat=36.12, long=-5.43; Spain), Barcelona (41.34,

130   2.16; Spain), Genoa (44.40, 8.92; Italy), Venice (45.44, 12.25; Italy), and Rotterdam

131   (51.90, 4.50; the Netherlands). These study areas have very different climatic conditions:

132   Algeciras has a hot-summer Mediterranean climate with average annual temperature of

133   18.6°C and cumulative precipitation of 768 mm; in Algeciras summers are generally hot

134   and dry, whereas winters are mild and wet. Barcelona and Genoa have a hot-summer

135   Mediterranean climate but are surrounded by subtropical areas, reporting average temper-

136   atures of 21.2 and 16.7°C and cumulative precipitation of 640 and 1082 mm. Venice is

137   characterized by a humid subtropical climate, with average annual temperature and cu-

138   mulative precipitation of 14.5°C and 1101 mm, respectively. Winters in Venice can be

139   cold, with temperatures commonly below 0°C during December and January. Finally,

140   Rotterdam has a temperate oceanic climate, with average temperature and precipitation

141   of 10.9°C and 867 mm, respectively. Rotterdam summers are generally cool and win-

142   ter months rarely record temperature below 0°C (Fig. 3; Smith et al. (2011); Beck et al.

143   (2018)). In addition to variability in the temperature regime, the five study areas show dif-

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144   ferent environmental and topographic characteristics which represent natural barriers for

145   species active dispersal. Such barriers can be overcome by invasive mosquitoes, neverthe-

146   less, through human-aided dispersal, with movements facilitated by human transportation

147   (Service, 1997). The road network connecting European cities is amongst the most devel-

148   oped in the world, ensuring fast and easy movements of humans, goods and, unintention-

149   ally, introduced mosquito populations (Díaz-Nieto et al., 2016; Nelson et al., 2019). Here,

150   in order to inform passive dispersal of *Ae. aegypti*, we considered only the network of

151   primary roads in each 100 km^2 study area (Center for International Earth Science Infor-

152   mation Network - CIESIN - Columbia University and Information Technology Outreach

153   Services - ITOS - University of Georgia, 2013). Moreover, to consider the eusynantropic

154   ecology of *Ae. aegypti* (ECDC et al., 2019), we constrained both its life cycle and move-

155   ments within urban areas, whose boundaries were defined using a spatial GIS layer derived

156   from Schneider et al. 2009.

2.3   Temperature data

158   Daily temperature estimates at 2 m above-ground for the period 2016-2019 were obtained

159   using the R package *microclima* (Kearney et al., 2020) for each study area, considering

160   a region spanning 100 km^2 from the center of each port. Microclima makes use of 6-

161   hour climate and radiation data downloaded from the National Center for Environmen-

162   tal Prediction Reanalysis 2 database at a resolution of 1.8-2.5° (Kanamitsu et al., 2002).

163   The four daily observations are then interpolated hourly with spline interpolation and cor-

164   rected considering topographic effect using a digital terrain model at 250 m resolution

165   downloaded from EUDEM (Copernicus project, 2019). The microclima package allows

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166 to define an habitat category as defined in MODIS Land use datasets (MODIS product

167 MCD12Q1v006) (M. Friedl, 2015), which is used to calibrate a Gaussian curve to es-

168 timate hourly vegetation features using a-priori established relationship between habitat

169 type and observed vegetation characteristics. We decided to consider the vegetation cover

170 across our study areas according to the MODIS habitat class closer to the ecology of *Ae.*

171 *aegypti*, which is "Urban and built-up" (MODIS category 14). Coastal effects on tempera-

172 tures were also considered as described in (Maclean et al., 2019). Finally, the temperature

173 times series for each study area derived with Microclima was further fine-tuned to local

174 weather conditions using data from the weather station from the NOAA network (Smith

175 et al., 2011) closest to the relevant port.

176 **2.4   Description of the population dynamical model**

177 In the proposed model arena, which we described by space i and time t, the number of

178 individuals in each developmental stage dying or surviving day t in cell i is an outcome

179 of binomial draws with probabilities derived from temperature-dependent functions pa-

180 rameterized with data from the appropriate scientific literature. All processes with their

181 associated probability distributions, parameters and references are described in Table 1.

182   The egg stage (E) is composed by four sub-compartments (ESC) which contain eggs

183 of different ages. Eggs enter ESC-1 when laid by ovipositing adult females; the first

184 three sub-compartments contain eggs one, two and three days old that are undergoing

185 embrionation (i.e., incubation time; Christophers (1960a)). The number of eggs (*E*) in

186 *sc*-1 during day *t-1* that die or move to *sc* in day *t* is defined by a binomial random draw

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187 based on mortality and survival probabilities $1 - p_{E_s}$ and p_{E_s}, respectively:

$$E_{i,t,sc} \sim Binomial(E_{i,t-1,sc-1}, p_{E_s}) \tag{1}$$

188 Four days-old eggs can hatch as immature to move to the "immature" (*I*) compartment.
189 The hatching event occurs with probability p_{Eh}, which is established by a random draw
190 from a Beta distribution. The two shape parameters of this Beta distribution were derived
191 using the average (0.076) and low 95% CI (0.023) proportion of *Ae. aegypti* eggs hatched
192 after being submerged in plastic containers filled with water in field conditions (Soares-
193 Pinheiro et al., 2016).

194 The immature compartment is composed by six sub-compartments (ISC) that contain
195 immatures of different age or stage. Every day immatures in ISC-1 to ISC-5 can die
196 or move to the next ISC based on temperature-dependent probability $1 - p_{I_s}$ and p_{I_s},
197 respectively.

$$I_{i,t,sc} \sim Binomial(I_{i,t-1,sc-1}, p_{I_s}) \tag{2}$$

198 When immatures reach ISC-6, they become ready to emerge as adults and therefore can
199 die, survive as other ISC-6, or emerge to move to the "adult" compartment with probability
200 p_{I_e}, which is the outcome of a temperature-dependent polynomial function as defined in
201 (Yang et al., 2009). The pool of immatures moving every day to the adult compartment is
202 subjected to an additional Binomial random draw in order to limit the adult compartment
203 to only female mosquitoes (assuming a 1:1 male/female ratio).

204 The adult compartment is composed by five sub-compartments that contain adult fe-

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males in different ages or physiological status. ASC-1 contains 1-day old adults which are developing gonads and therefore are not yet sexually mature (Christophers, 1960a). Adults in ASC-1 can die or move to ASC-2, which contains host-seeking adults, following a random draw with temperature-dependent probability p_{A_s} (Yang et al., 2009), which also drives the fate of ASC-2 to 5. ASC-2 can die or move to ASC-3 where they are assumed to have taken a blood meal and entered the gonotrophic cycle. ASC-3 is therefore composed of blood-fed adult females maturing eggs (i.e., completing the gonotrophic cycle). If adult females in ASC-3 survive day *t-1* they can complete the gonotrophic cycle and move in day *t* to ASC-4 where they become ovipositing females. The completion of the gonotrophic cycle by ASC-3 is driven by a binomial random draw based on temperature-dependent probability p_{A_g} (Yang et al., 2009). Females in ASC-4 in day t, first oviposit, then die or move to ASC-5 which contains females in the second day of oviposition. The number of eggs laid by a female in ASC-4 or ASC-5 is the outcome of a Poisson random draw with temperature-dependent probability p_{A_e} (Yang et al., 2009; Christophers, 1960a). Finally, females in ASC-5 die or move to ASC-2, where they will seek again for a blood-meal and, if surviving, re-enter the gonotrophic cycle as described above.

In addition to the development and survival cycle of events, host-seeking adult females (ASC-4) can also disperse actively (short-range dispersal) or passively (medium-range dispersal). In our model, active dispersal is defined as the probability that an adult mosquito flies at distance d_{ad} from the cell of origin and follows a log-normal dispersal kernel (Marcantonio et al., 2019). Thus, in each day t, the number of dispersing ASC-4 that move to a distance d from the origin cell i is an outcome of a binomial random draw with probability

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227    $p_{A_{ad}}$:

$$p_{A_{ad}} \sim LogNormal(meanlog = 4.95, sdlog = 0.66) \tag{3}$$

228   We assumed that females start dispersing from the center of the cell of origin i, there-

229 fore, given a cell size of 250 m, all mosquitoes dispersing less than 250 m do not leave i.

230 All other dispersing mosquitoes move to a cell i' (landing cell) which is chosen randomly

231 from the set of cells at distance d (i.e. non directional active dispersal).

232   In addition to active dispersal, the proposed model also considers dispersal aided by

233 car traffic along the main road network. This type of dispersal is thought to be amongst

234 the main drivers of medium-range geographical expansion for *Aedes* mosquitoes (Marcan-

235 tonio et al., 2016; Eritja et al., 2017). In the model, adults of all ages, except 1-day old,

236 that reside in cells intersecting roads may undergo this type of dispersal. We parameter-

237 ized passive dispersal using the probability of a car transporting *Aedes* mosquitoes $p_{A_{ad}}$

238 of 0.0051, as reported in Eritja et al. (2017), implying that, on average, only about 5 over

239 1,000 mosquitoes disperse passively every day from each cell of origin. Furthermore, to

240 define the probability of dispersing passively at different distances d, we used data on

241 driving patterns in Europe (Alemanno et al., 2012). We defined $p_{A_p dd}$ as the outcome of a

242 Gamma distribution with mean, md, equal to the average driving distance per trip in each

243 of the study areas:

$$p_{pd} \sim Gamma(\alpha = (md/(md * sd))^2, \beta = (md/(md * sd)^2) \tag{4}$$

244   where md was set to 18.43 km for Italy, 23.14 km for Spain and 28.14 km for the

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245 Netherlands (Alemanno et al., 2012). The standard deviation used to parameterize the

246 Gamma distribution was chosen to be very large (100 times the mean) in order to assign a

247 small probability density also to large dispersal distances. The matrix of distances between

248 cells along roads was calculated using the set of *v.net* modules in GRASS GIS 7.6 (Neteler

249 et al., 2012).

250 The model coded in the R statistical language (R Core Team, 2019) and adapted for

251 parallel computation is available in the Github repository: `https://github.com/`

252 `mattmar/euaeae`.

2.5 Experimental design and model validation

254 We simulated the introduction of 10, 50, 100, 250, 500 or 1000 eggs in a randomly chosen

255 cell randomly within the boundary of the corresponding port in each study area. Eggs

256 were chosen as the introduction propagule as they are the resistant life stage through which

257 *Aedes* mosquitoes overcome periods of adverse climatic conditions, such as cold winters

258 or dry summers. Each introduction was iterated 500 times to account for stochastic events

259 in both life cycle and dispersal, as the coefficient of variation of all outcomes of interest

260 stabilised around this number of iterations. The length of each simulated introduction

261 was set to three years in order to allow stabilisation of the simulated populations and

262 integrate inter-annual climatic variability. The introduction year was 2017. In addition,

263 we decided to run preliminary simulations to define the most favourable period of the year

264 for introducing *Ae. aegypti* in order to reduce the chance of false negative results. This set

265 of simulations began with 100 adults introduced on the 15th of each month of the year 2017

266 and lasted until the end of the same year. The introduction date in 2017 was thus chosen

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for each study area as the starting date of the scenario that recorded the highest number of adult mosquitoes in any of the simulated days. In the subsequent full model simulations, successful introductions were defined as those that yielded at least one individual in any life stage on the 20th of June 2018, which represents the end of the spring (and therefore the end of adverse climatic conditions for *Aedes aegypti*) the year after date of introduction (2017). Population demographic and dispersal trends for each introduction scenario were summarised using the inter-quartile ranges of the distribution of each outcome of interest across iterations for each simulated day.

To validate model outputs, we derived the proportion of each study area which experienced days below the 10°C isotherm for the whole 2017-2018 period, using ERA5 reanalysis (1979–2018) (Copernicus Climate Change Service, 2017). Temperatures around 10°C represent a theoretical thermal limit for *Ae. aegypti*, below which adult mosquitoes become torpid and unable to move (Christophers, 1960a; Reinhold et al., 2018). The invasion success ratio of simulated introductions was compared against the proportion of the study area below the 10°C isotherm to assess the credibility of model outputs.

Local sensitivity analysis was performed on model results by varying the values of four model parameters which were considered uncertain, namely: the probability of egg survival (p_{E_s}), the probability of egg hatching (p_{E_h}), the number of introduced eggs (E), the probability of adult dispersing passively ($p_{A_{pd}}$). We performed the sensitivity analysis by running 200 model iterations over an extensive range of values of each selected model parameter for the study area of Algeciras. The proportion of successful iterations, maximum population dispersal distance, female abundance and invaded area in hectares were plotted against each parameter range in order to assess the sensitivity of model outputs.

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290 Results of the local sensitivity analysis are reported in Appendix 2.

3 Results

3.1 Likelihood of a successful introduction relative to time of the year

Results from the set of preliminary model simulations showed that the month of introduction which resulted in the highest median abundance of adult mosquitoes (relatively to the first year of introduction) was March for the port of Rotterdam, April for Barcelona, and May for the ports of Genoa, Venice and Algeciras. Introductions in any month of the year 2017 showed viable populations on the last day of the year (i.e., successful introductions) only in Barcelona, while Algeciras (10 months), Genoa (9), Venice (3) and Rotterdam (1) showed a decreasingly lower number of months suitable for successful introductions (Fig. 4).

3.2 Likelihood of a successful introduction relative to latitude

The percentage of full model simulations which resulted in established *Ae. aegypti* population was 100% for Barcelona, for a propagule pressure of at least 250 eggs. Algeciras showed somewhat similar results, however, 100% successful introductions was achieved only with a higher minimum propagule pressure of 1000 eggs (Table 2). On the contrary, we did not observe successful introductions for any propagule pressure either in Genoa, Venice or Rotterdam. In Rotterdam, mean daily temperatures during summer were never high enough (>20°C) to allow the accumulation of an adequate egg bank which would have permitted the overwintering of the population (see Fig. 3). Conversely, in Genoa and

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310 Venice, although introductions sometimes caused moderate population abundances (>50

311 adult mosquitoes per ha) during the most favourable months, very cold winter tempera-

312 tures, often under 0°C, caused extinction for all the introduced populations. The percent

313 success of invasion was well correlated with the percentage of area under the 10°C for

314 the period 2017-2018. Interestingly, a relatively small decrease of about 6% of area-days

315 under the 10°C isotherm between the ports of Barcelona and Genoa represented a climatic

316 divide for *Ae. aegypti* invasion success.

3.3 Space-time trend of introduced populations

318 With the exception of Rotterdam, all simulated populations were able to reproduce and

319 spread at local scale at least during the first summer after introduction. The peak of adult

320 mosquito abundance was reached in autumn in Algeciras, Barcelona and Genoa or in

321 late summer in Venice. Adult female abundances reached maximum overall values of

322 $584 \cdot ha^{-1}$ in Barcelona, but were considerably lower in the other study areas: Algeciras

323 yielded a maximum of $32 \cdot ha^{-1}$, Genoa $28 \cdot ha^{-1}$, Venice $20 \cdot ha^{-1}$ and Rotterdam $2 \cdot ha^{-1}$

324 (Fig. 5). The pools of eggs and immatures followed trends similar to adult mosquitoes but

325 lagging in time proportionally to differential developmental times, and reaching higher

326 abundances due to the inter-stage survival bottlenecks (Appendix Fig. 7). However, with

327 the onset of colder weather conditions at the end of summer, mosquito populations de-

328 clined rapidly in all the study areas. The decline was more evident as soon as daily average

329 temperature was constantly under 10°C, and even in Algeciras and Barcelona, winter den-

330 sities dropped to a minimum to 1 female/ha during winter months. In these months, the

331 consistent egg bank accumulated during the warmer months ensured population overwin-

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tering. On the contrary, after the first summer, simulated populations from Genoa, Venice and Rotterdam were not able to overwinter (Fig. 5). The 10°C isotherm computed for the whole Europe from the first of September 2017 to the 31th of May 2018 showed that these three study areas, but not Algeciras and Barcelona, presented mean temperatures values below 10°C for at least 1 full winter month.

The spatial spread of introduced populations was limited during the simulated period, as the median dispersal distance was never higher than 4 km from the port of introduction (Fig. 5). The two study areas which showed viable populations after the first simulated year reported different dispersal trends. *Ae. aegypti* populations in Algeciras showed a more seasonal spatial spread, dispersing more during summer and contracting during winter. The simulated populations in this study area moved farther just after introduction, dispersing more than 2 km and invading a maximum of 638 hectares, but in the following years the invaded area contracted. On the contrary, Barcelona's populations remained in the surrounding of the introduction location for more than one year before showing a more marked spread. During 2019, this population was able to spread to a maximum of 2434 ha, doubling the area invaded in 2018 (Fig. 5).

4 Discussion

The overarching aim of this study was to examine both the likelihood and the dynamics of a putative re-introduction of *Ae. aegypti* in European ports under current climatic conditions. To do so, we developed a spatially explicit process-based model tailored to the physiological requirements of *Ae. aegypti*. We applied the model to simulate and study

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353 the introduction of *Ae. aegypty* in five major European ports with high potential propagule

354 pressure and along a gradient of latitude. Our modelling framework accounted for: i) the

355 effect of spatial and temporal temperature variation on population dynamics, ii) the dis-

356 persal at local and regional scales as well as iii) stochastic events following introduction.

357 The approach we adopted has the advantage to be mechanistically linked to *Ae. aegypti*

358 biology which stems in more ecologically-realistic estimates of the likelihood of invasion

359 in already sensitive areas.

360   According to model results and given current climatic conditions, a relatively low *Ae.*

361 *aegypti* propagule pressure has the potential to cause species establishment, high local

362 densities and slow initial dispersal in the two southernmost study areas: Algeciras and

363 Barcelona. Overall, mosquito densities reported in model outputs, with peaks of 584 fe-

364 males per hectare in Barcelona study area, are in line with previous observations in areas

365 where *Ae. aegypti* is well established (Focks et al., 1981; Ritchie et al., 2013; Garcia et al.,

366 2016).

367   Barcelona was the most suitable to invasive *Ae. aegypti* than Algeciras, allowing also

368 for higher abundance and more rapid spatial spread of simulated invasive populations.

369 These results are associated with the different layout of urban areas around the two ports

370 as well as local climatic conditions. Algeciras is relatively isolated from other urban areas,

371 therefore dispersing mosquitoes need to disperse relatively far to find suitable urban envi-

372 ronments, which may have favoured a more seasonal dispersal pattern. This pattern may

373 have only rarely allowed for enough propagules to "jump" to and colonise new distant ar-

374 eas, resulting in local extinction and invaded area contraction during winter months (Roche

375 et al., 2015). On the contrary, the massive urban sprawl of Barcelona seamlessly connects

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376 the port area with neighbouring suitable urban areas along the coast either North, West or

377 South (Fig. 5). As a result, invasive propagules could gradually form new invasion fronts,

378 favouring a longer and more gradual dispersal. Both Algeciras and Barcelona fall in the

379 hot-mediterranean climatic zone, which is characterised by warm summer and mild win-

380 ters, meaning optimal conditions for *Ae. aegypti* life cycle. The relatively higher invasion

381 success and population densities simulated for Barcelona can be explained considering

382 this area warmer temperatures between May and September, which also correspond to the

383 core of the mosquito growing season. Although we did not consider water availability

384 for breeding sites in our model, it is worth highlighting that this would not represent a

385 limiting condition in both the suitable study areas, as yearly precipitations are well above

386 values of 400-500 mm/yr known to hinder *Aedes* population persistence (Caminade et al.,

387 2012). Mosquito populations introduced either in the ports of Genoa, Venice and Rotter-

388 dam, despite persisting for short-to-longer periods and dispersing in the surrounding areas

389 of each ports, were never able to establish overwintering populations. These three areas

390 have longer and harsher cold temperature during winter months which are at least for one

391 consecutive month below the isotherm of 10°C, making it impossible for introduced *Ae.*

392 *aegypti* to overcome the adverse period of the year.

393 Taken together these results indicate that, regardless of the propagule pressure, the

394 likelihood of *Ae. aegypti* invasion along European coasts is high in the southern Mediter-

395 ranean basin, is less likely at latitudes between the ports of Barcelona and Venice, and

396 becomes extremely unlikely further up North. However, where the current temperature

397 regime is too cold to allow species survival over winter, either microclimatic refugia un-

398 der current climatic conditions or climate change, in the near future, could still allow *Ae.*

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399 *aegypti* establishment. Microclimatic refugia are widespread in urban areas and can be

400 caused by urban heat-island effects or by urban infrastructures and have been shown to

401 be exploited by *Ae. aegypti* (Tsunoda et al., 2014). An example of local conditions de-

402 tached from the regional climatic regime which has allowed *Ae. aegypti* persistence is the

403 city of Washington, DC, USA, where *Ae. aegypti* was able to establish viable populations

404 by overcoming the otherwise unsuitable climatic conditions sheltering in the myriad of

405 underground refugia of the metropolitan area (Gloria-Soria et al., 2018).

406 Microclimatic urban refugia are by definition limited to small extensions, whereas

407 global change has the potential to expand dramatically the geographical extent suitable

408 for *Ae. aegypti* as well as *Aedes*-borne virus transmission in Europe. Mosquito physio-

409 logical rates are shortened by increasing environmental temperatures in the range of 10-

410 35°C (Mordecai et al., 2019), and the 10°C isotherm of the coldest month is often used as

411 a proxy to assess *Ae. aegypti* geographic suitability. Europe is currently characterized by a

412 10°C isotherm of the coldest month extending to most of the south inland areas of Spain,

413 Italy and Greece, which underpins how three out of five coastal areas that we tested were

414 not suitable for *Ae. aegypti* establishment. However, the current direction of global change

415 is predicted to bring warmer temperatures in most of Europe that, coupled with unchanged

416 or increased precipitation along coastal areas, will impact the extent of regions where *Ae.*

417 *aegypti* introduction could cause invasions (Kraemer et al., 2019; Liu et al., 2019; Ryan

418 et al., 2019). That said, our findings suggest that simulated populations introduced in the

419 region of Genoa persisted for most of the winter to became extinct only at the beginning

420 of spring. Some model iterations showed individuals persisting till the month of April

421 2018. This result suggests that, according to our model, Genoa is close to suitability for

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422 *Ae. aegypti.* Thus, areas such as the region of Venice and Genoa, which have already acted

423 as ports of introduction for *Ae. albopictus* (Sabatini et al., 1990; Knudsen et al., 1996),

424 another invasive mosquito, should already be targeted for limiting the likelihood of *Ae.*

425 *aegypti* establishment in the near future.

426 The potential establishment of populations of *Ae. aegypti* in the southernmost study

427 areas considered in this study does not consequently imply an arbovirus outbreak. How-

428 ever, assuming a putative introduction of *Aedes*-borne pathogens and the lack of adequate

429 surveillance, the socio-environmental conditions of Algeciras and Barcelona may be suit-

430 able to sustain transmission of such introduced pathogens in the human population. In the

431 past twenty years, the chikungunya outbreaks in southern european countries such as Italy,

432 Spain and Croatia results from the establishment of *Ae. albopictus* in highly dense pop-

433 ulated areas coupled with the introduction of pathogens via infected travellers returning

434 from areas with high incidence rates (Liumbruno et al., 2008; Lindh et al., 2019).

435 Our modelling exercise is not exempt from caveats. We did not consider evolutionary

436 processes which may affect mosquito physiological rates allowing *Ae. aegypti* to be locally

437 adapted to different set of environmental conditions (Reinhold et al., 2018). For example,

438 variability in the quiescence strategy to prolong embryonic viability has been observed in

439 different *Ae. aegypti* populations (Oliva et al., 2018). Moreover, potential interspecific

440 competition between individuals of *Ae. albopictus* and *Ae. aegypti* is another factor not

441 considered in this study which may limit the invasion success of *Ae. aegypti* (Carrasquilla

442 and Lounibos, 2015). Finally, we assumed that both human host density and the number

443 of breeding sites are not limiting factors. We believe these assumption realistic, because:

444 1) we selected main European ports surrounded by urbanized areas as introducing sites,

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445 which are expected to have a high density of available hosts; 2) due to the domestic be-

446 haviour of *Ae. aegypty*; it is well know that *Ae. aegypti* has preference for man-made

447 containers as breeding sites, and discarded objects such as cans, jars, pots are expected to

448 be overabundant in urban areas. We also acknowledge that we have considered urban areas

449 as homogeneous land use features, which is far from reality. Urban areas are dynamical

450 landscape features, whose heterogeneity has been recognized as an emerging character-

451 istic in pathogen circulation dynamics. The importance of urban areas for vector species

452 lays in the very diverse microclimatic and habitat conditions they can provide, which cre-

453 ates emerging interfaces for interaction between humans and other species (Vanwambeke

454 et al., 2019).

5   Conclusions

456 Environmental conditions in Southern European urban areas such as Algeciras and Barcelona

457 suggest potential for colonization of these areas by *Ae. aegypti*. We found that the spread

458 pattern in time and space following establishment could be a continuous gradual spread

459 or a more seasonal stepping-stone pattern, dictated by the specific characteristics and con-

460 nectivity of each urban areas. Moreover, in case of establishment, *Ae. aegypti* populations

461 in these areas were found to reach abundances similar to regions of the world with ac-

462 tive transmission of *Aedes*-borne viruses. European areas located at higher latitudes, such

463 as the port of Genoa, were found not suitable for colonization by these mosquitoes, but

464 Global Change could modify conditions for *Ae. aegypti* invasion also in these regions.

465 Finally, colonization of Northern European urban areas, aside short-lived and limited en-

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466 croachment due to urban refugia, was found to be very unlikely.

467   It is commonly accepted that targeted monitoring of sensitive areas and early control

468 actions are the most effective methods to prevent the establishment of invasive species in

469 new areas. Maintaining *Ae. aegypti* populations at low densities and possibly limiting

470 their spatial spread is crucial to avoid the risk of pathogen transmission. Our findings and

471 model framework may support surveillance initiatives for those European coastal urban

472 areas which have a known high propagule pressure and a high probability of *Ae. aegypti*

473 establishment.

474   The model we applied for our simulations is made available as an R function, in the

475 hope to foster reproducibility, further development and application onto different scenar-

476 ios, geographical areas or spatial scales.

477 6   Acknowledgements

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Tables

Table 1: A summary of model processes happening in day t and in cell i, with associated probability density distribution and parameters.

Stage	Process description	Process ppd	Parameters	References
Egg	**Survive**: Probability that an egg survives in day t, cell i.	$E_s \sim Uniform(p_{E_s})$	$p_{E_s} = 0.99$	Otero et al. (2008)
	Hatch: Probability that an embrionated egg hatches in day t, cell i.	$E_h \sim Binomial(p_{E_h})$	$p_{E_h} = Beta(a,b)$; $a = 1.45, b = 6.51$	Soares-Pinheiro et al. (2016)
Immature	**Survive**: Probability that an immature survives in day t, cell i.	$I_s \sim Binomial(p_{I_s})$	$p_{I_s} = \mathcal{P}_6(temp)$	Yang et al. (2009)
	Emerge: Probability that an immature >=6 days old emerges in day t, cell i.	$I_e \sim Binomial(p_{I_e})$	$p_{I_e} = \mathcal{P}_7(temp)$	Yang et al. (2009)
Adult	**Survive**: Probability that an adult survives in day t, cell i.	$A_s \sim Binomial(p_{A_s})$	$p_{A_s} = \mathcal{P}_5(temp)$	Yang et al. (2009)
	Determine sex: Probability that a newly emerged adult is female in day t, cell i.	$A_f \sim Binomial(p_{A_f})$	$p_{A_f} = 0.5$	-
	Mature eggs: Probability that a blood-fed female matures eggs in day t, cell i.	$A_{em} \sim Binomial(p_{A_{em}})$	$p_{A_{em}} = \mathcal{P}_5(temp)$	Yang et al. (2009)
	Oviposit: Number of eggs that an adult female, which underwent eggs maturation, lays in day t, cell i.	$A_{el} \sim Poisson(\lambda_{el})$	$\lambda_{el} = \mathcal{P}_4(temp)$	Yang et al. (2009); Christophers (1960b)
	Disperse a-distance: Probability that an host-seeking adult female disperses in day t from cell i to cell i' at distance d.	$A_{ad} \sim Binomial(p_{A_{ad}})$	$p_{A_{ad}} = LNormal(m, sd)$; $m = 4.95$ $sd = 0.66$.	Marcantonio et al. (2019)
	Disperse p: Probability that an host-seeking adult female in day t in cell ii adjacent to a road intercepts a car.	$A_{pd} \sim Binomial(p_{A_{pd}})$	$p_{A_{pd}} = 0.0051$	Eritja et al. (2017)
	Disperse p-distance: Probability that a passively dispersing adult A_{pd} disperses in day t from cell ii to cell ii' at distance d.	$A_{pdd} \sim Binomial(p_{A_{pdd}})$	$p_{A_{pdd}} = Gamma(\alpha, \beta)$ $\alpha = (c/10000)^2$ $alpha = c/10000^2$ $c = \{18.43, 23.14, 28.14\}$	Alemanno et al. (2012)

Table 2: Number of introduced eggs and corresponding percentage of successful introductions for each of the considered study areas. The last column represent the proportion of each study area that fell below the isotherm of 10°C for the years 2017-2018 (ERA5 reanalysis (1979–2018)).

Site	Introduced eggs	Introduction success %	Proportion of the study area below 10°C %
Algeciras	10,50,100,250,500,1000	6,11,16,48,74,100	4.8
Barcelona	10,50,100,250,500,1000	4,26,72,100,100,100	15.3
Genoa	10,50,100,250,500,1000	0	21.7
Venice	10,50,100,250,500,1000	0	24.6
Rotterdam	10,50,100,250,500,1000	0	29.4

Figures

a)   b)
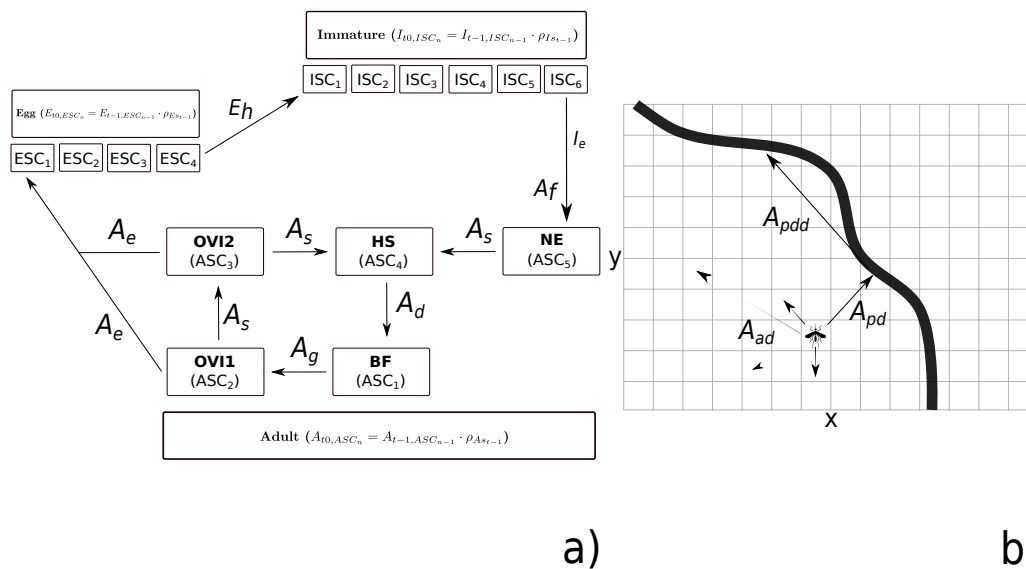
Figure 1: Graphical representation of model structure: in A) we showed the life cycle of *Ae. aegypti*, and in B) a representation of active and passive dispersal processes. A more detailed description of all model parameters reported in the figure is provided in Table 1.
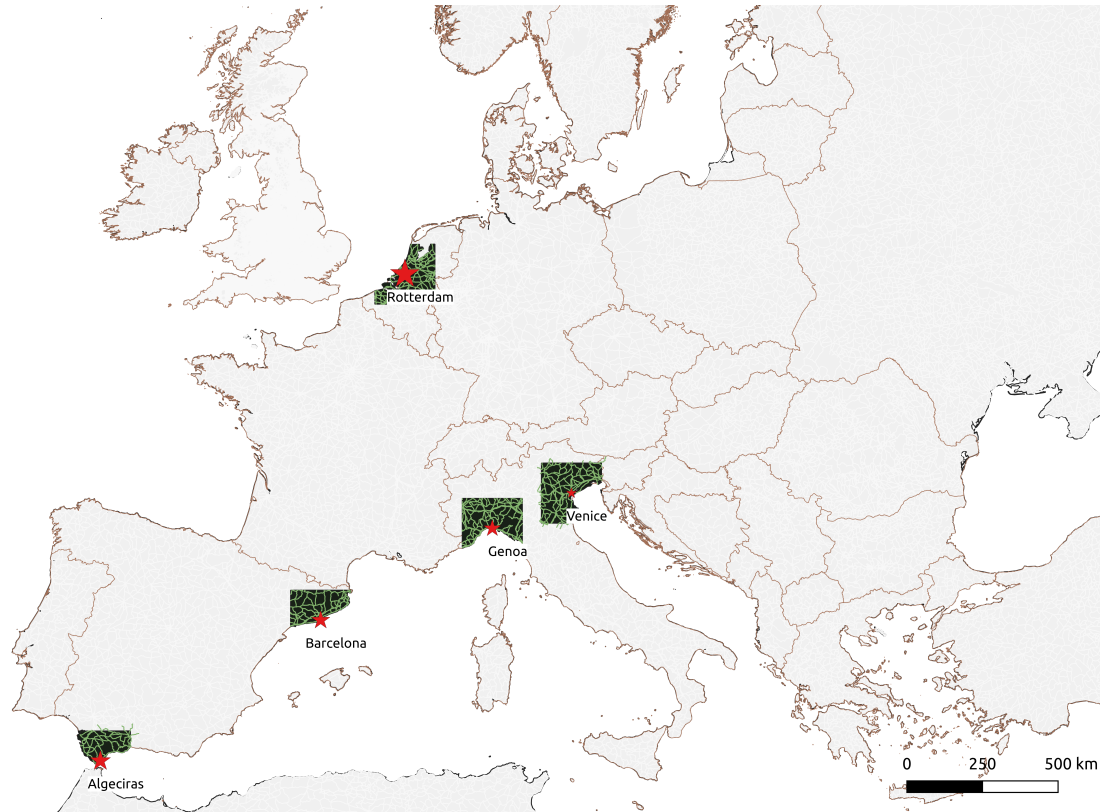
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Figure 2: Locations of the five ports (red stars) investigated along a latitudinal gradient and corresponding study area (black). The network of primary roads is reported in green. The size of the "port" pin is proportional to the quantity of twenty-foot equivalent unit (TEU) received in the corresponding port for the year 2018 (Amsterdam=13.6, Algeciras=4.8, Barcelona=3.4, Genoa=2.5, Venice=0.6 TEU).

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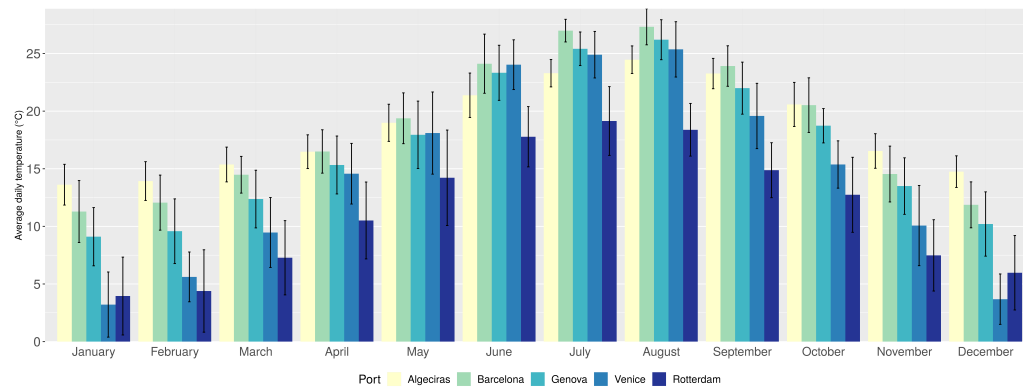
Figure 3: Monthly average temperature and standard deviation (error bars) for the years 2017-2019 in the five study areas.
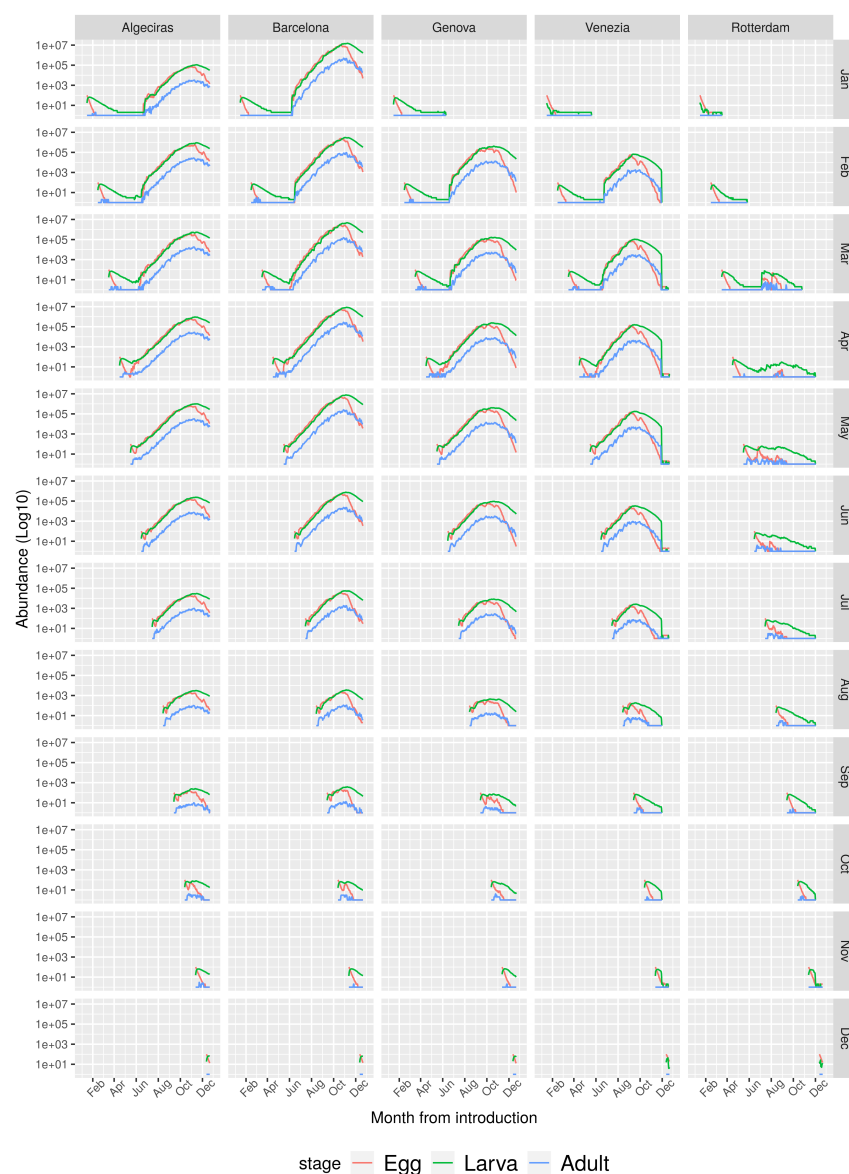
Figure 4: Time trend of *Ae. aegypti* population abundances from preliminary model iterations simulating the introduction of 250 eggs starting on the 15th of each month of 2017 and ending on the 20th of December 2017 for the five study areas. Blue lines represent adult females, green lines larvae and red lines eggs. Values on the y-axis are transformed using the common logarithm.
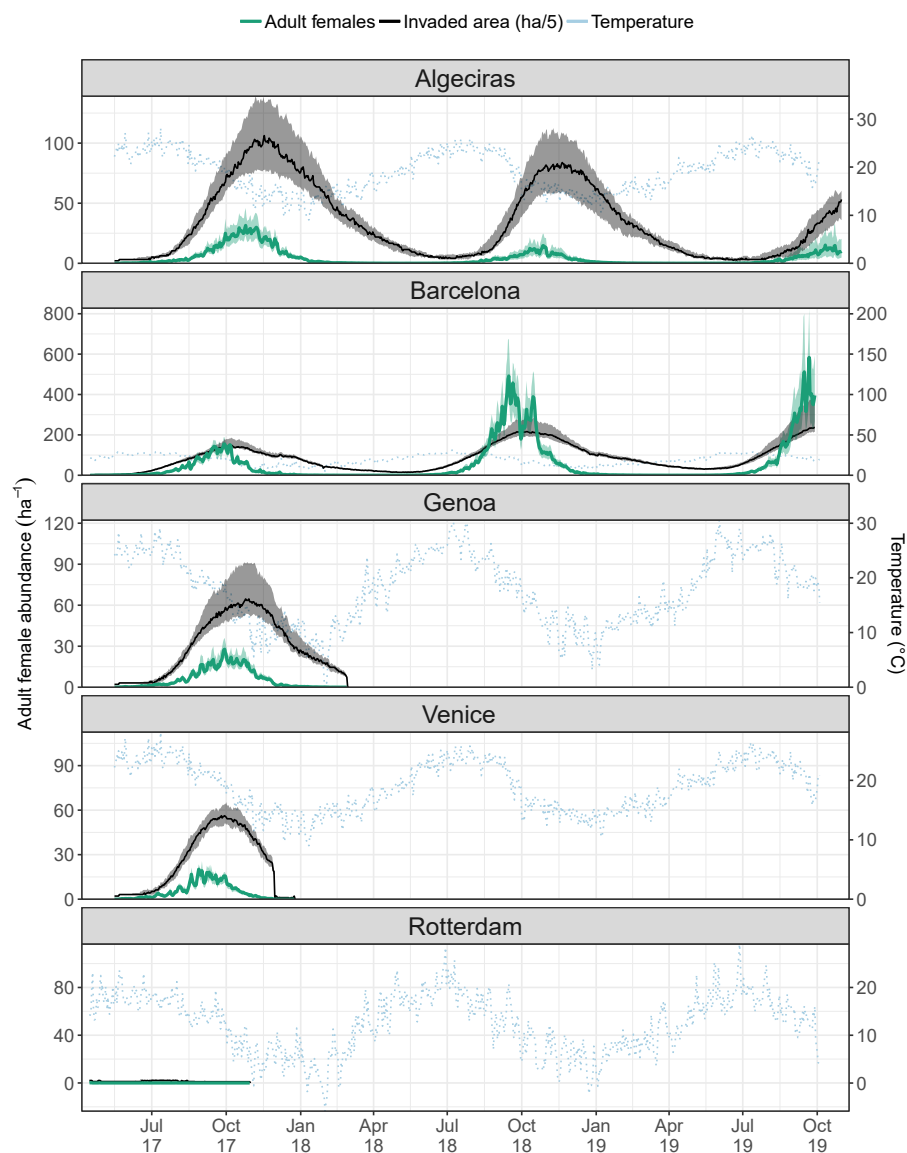
Figure 5: Temporal trend describing the outputs of model simulations for the five study areas. We reported both median (green lines) and inter-quartile (green ribbons) population densities of adult females per hectare and median (black line) and inter-quartile range (grey ribbons) of the invaded area in hectares (divided by a factor of 5 for scale reasons), derived from 500 iterated introductions of 250 *Ae. aegypti* eggs in each of the 5 study areas. The light-blue dotted line reports the trend in average daily temperatures and refers to the values in the right y-axis.
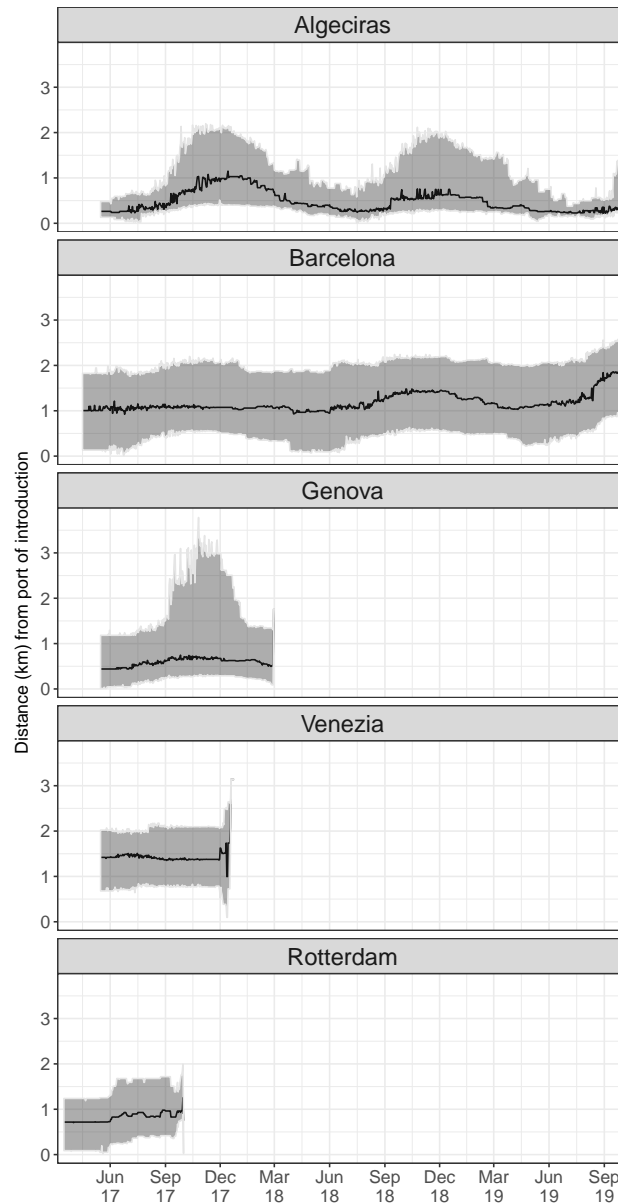
Figure 6: Median (black line) and inter-quartile (grey ribbons) dispersal distance (km) of simulated mosquito populations derived from 500 iterated introductions of 250 *Ae. aegypti* eggs in each of the 5 study areas. Dispersal distance was calculated as the euclidean distance between the centroid of each port of introduction and centroids of cells with at least one *Ae. aegypti* individual in any developmental stage.

Appendix 1: Temporal and spatial trends of the simulated mosquito populations
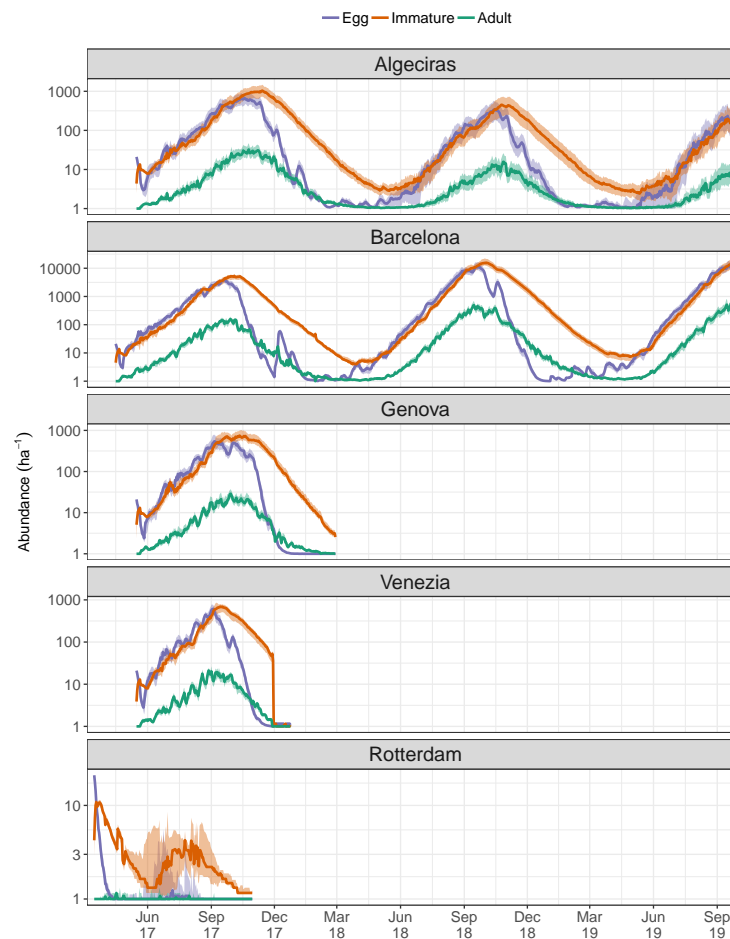
Temporal trend for all life stages

Figure 7: Temporal trend reporting: i) median (lines) and inter-quartile (ribbons) population abundances for each stage (adults in green, immatures in orange and eggs in purple) for introduced *Ae. aegypti* populations. Values on the y-axis are transformed using the common logarithm.

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Appendix 2: Sensitivity analysis on uncertain model parameters

In an attempt to assess and report the effect on model result of those model parameters which were judged more uncertain (i.e., lacking experimental or field estimates) we run a global sensitivity analysis by exploring the parameter space of: the probability of egg survival (p_{E_s}), the probability of egg hatching (p_{E_h}), the number of introduced eggs (E), the probability of adult dispersing passively ($p_{A_{pd}}$; Table 3). We performed the sensitivity analysis iterating 200 times the introduction of 250 eggs (except for E) on the 15th of May on the study area of Algeciras.

Table 3

Model parameter	Parameter space	Value used in the model
Daily probability of egg survival	(0.90,1.00)	0.99
Daily probability of egg hatching	(0.01, 1.00)	0.076
Daily probability of passive dispersal	(0.0018, 0.0180)	0.0051
Number of introduced eggs	(10,1000)	250

Summary statistics of model results were plotted against the range of parameter values to provide a simple and straightforward assessment of model sensitivity (Fig. 8).
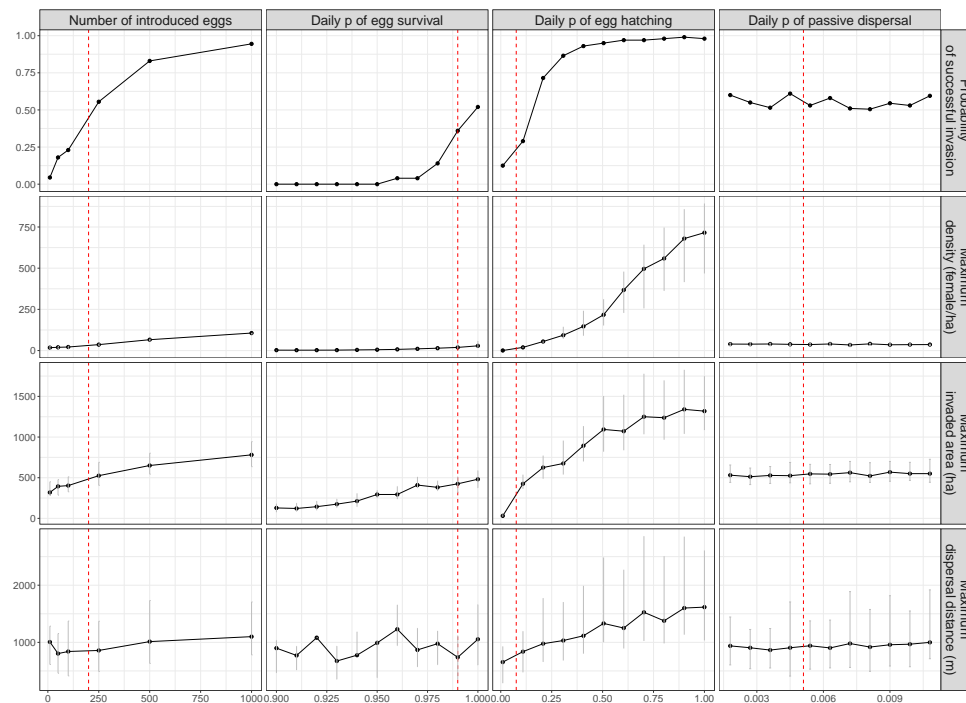
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Figure 8: Matrix of scatterplots showing the effect of the four model parameters (column) selected for sensitivity analysis on different model outputs (rows). The black dots represent median values across the 200 iterations while vertical grey lines the inter-quartile range for each type of output, except for the probability of successful invasion which is cumulative across iterations. The red dashed vertical lines indicate the parameters value used in model simulations.

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