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SURVEILLANCE AND CONTROL OF MOSQUITOES IN SEWERS FROM AN URBAN MEDITERRANEAN AREA

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Abstract Urban areas provide suitable breeding areas for mosquitoes potentially affecting the epidemiology of many vector-borne diseases, through its effects on mosquito community composition. The invasive Asian tiger mosquito *Aedes albopictus* has colonized a large number of urbanized areas in Europe and is still increasing its distribution range. This species uses small water depots for laying their eggs and larval development, and anthropogenic areas offer multitude of potential breeding sites. Nowadays, most control programs of *Ae. albopictus* populations are based on the use of *Bacillus thuringensis* (BTI) although its efficacy under natural conditions have been traditionally neglected. As a part of an extensive study, we monitored the presence of larvae of mosquitoes including the invasive *Ae. albopictus* and the native *Culex pipiens* and *Culiseta longiareolata* in 73 neighbourhoods and 10 districts of the city of Barcelona from February to December during five consecutive years (2015-2019). Overall, more than 73000 sewers were inspected, which can be accessible breeding larval sites, in order to identify the annual and seasonal variation on the colonization process of sewers and the effects of water infrastructure characteristics. We found clear interannual differences in the presence of mosquito larvae in the area, reaching the maximum numbers during 2015 and 2018. In addition, the presence of mosquito larvae increased along the year, with the highest percentages of larvae in water infrastructures during August. Furthermore, our results highlight clear differences in the presence of mosquito larvae according to the typology of the sewers, being the sand sewers the one with the highest percentage of mosquito breeding activity. These results support that the typology of water infrastructures affects the presence of mosquito larvae, including those of the species *Ae. albopictus*, in a Mediterranean urban area and provide important information on the seasonality of mosquito reproduction useful for monitoring and surveillance programs of mosquitoes in the public sewage system.

Key words Asian tiger mosquito, invasive mosquitoes, larvae control, monitoring and surveillance of mosquitoes.

INTRODUCTION

Human population growth, globalization and climate change affect mosquito communities and the incidence of vector-borne pathogens (Ferraguti et al., 2016). The invasive Asian tiger mosquito (*Aedes albopictus*) is currently increasing its distribution range, and now occurs in a large number of urban areas in southern Europe (Cebrián-Camisón et al. 2020). In the invaded areas, *Ae. albopictus* has a wide ecological flexibility colonizing numerous human-made infrastructures. The success of this invasive species lies, at least in part, in its ability to use small accumulations of water for reproduction, and human inhabited areas offer multitude of potential breeding sites such as scuppers, drums, cemetery flower pots, and human-made ponds (Juliano et al., 2005). Besides the nuisance that bites by *Ae. albopictus* represent to humans (Faraji et al., 2014; Martínez-de la Puente et al., 2015; Cebrián-Camisón et al. 2020), the presence of *Ae. albopictus* has altered the epidemiological scenario for some mosquito-borne diseases (i.e. Dengue, Zika and Chikungunya) in the invaded areas representing a global health concern (Kraemer et al., 2015). In the city of Barcelona, the presence of dengue positive *Ae. albopictus* was repeatedly recorded in the last decade (Aranda et al., 2018; Montalvo, *pers. comm.*) and mosquitoes from this population are competent for Zika virus (Gutiérrez-López et al., 2019). Furthermore, events of local transmission of filarial worms have been recorded in the area (Laynez-Roldán

et al. 2018), for which *Ae. albopictus* are known vectors (Cancrini et al. 2003). Consequently, controlling the populations of *Ae. albopictus* in urban areas is necessary for public health and to reduce nuisance to citizens.

The list of biocides available for mosquito control is strongly limited by European legislation and most preventive control programs are based on the use of *Bacillus thuringensis* (BTI). The efficacy of BTI has been tested under laboratory conditions, exposing larvae to different concentrations of BTI (Becker, 2000), but under field conditions many factors may affecte the duration of BTI effectivity, i.e. presence of organic matter or temperature. We used data from a monitoring program on *Ae. albopictus* conducted during five consecutive years to analyse the importance of human-made water infrastructure characteristics on the occurrence of mosquito larvae in the city of Barcelona (Spain). In addition, we report information on the larvicides treatment (BTI) carried out in the area to control its populations. Finally, we derived recommendations to reduce the availability of breeding areas in the public sewage system and to increase the efficacy of larvicide treatments.

MATERIALS AND METHODS

From 2015 to 2019, the presence of mosquito larvae of the species *Ae. albopictus*, *Culex pipiens* and *Culiseta longiareolata*, was monitored in the framework of the mosquito surveillance and control program in the city of Barcelona conducted by the Agència de Salut Pública de Barcelona (ASPB) (https://www.aspb.cat/). Overall, more than 73,000 sewers from 73 neighbourhoods and 10 districts of the city of Barcelona were monitored, all of them available mosquito breeding in the public area. Structures where larval activity was found were periodically treated until the focus were controlled using a formulation of *Bacillus thuringiensis israelensis* (Vectobac G, Valent Biosciences Corporation, Libertyville, Illinois (Ill), US) and *Bacillus sphaericus* (Vectomax FG, Valent Biosciences Corporation, Libertyville, Illinois (Ill), US). Data used in this study includes 19,534 records of the colonization of mosquito larvae in three main typologies (i.e. siphoned sewer, sand and direct sewer; see Fig. 1) of water drainage infrastructures that may affect mosquito breeding suitability and the efficacy of the treatments. These records corresponded to months from March to December. Colonization processes was defined as records of the occurrence of mosquito larvae in sewers where larvae was absent in the previous inspection. Records were limited to sewers where the presence of water was observed, as this is indispensable for the development of mosquito larvae.

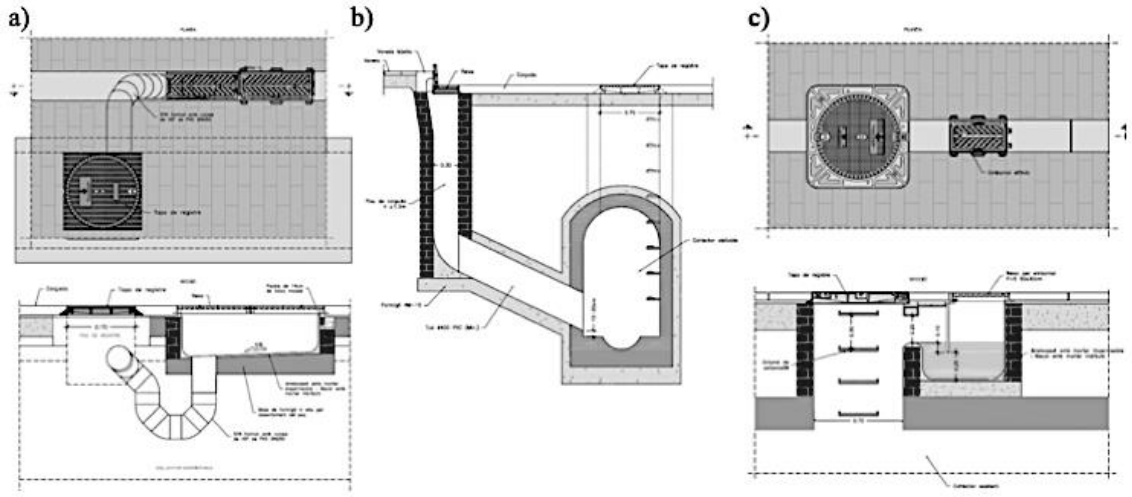

Figure 1. The three typologies of water infrastructure sewers were a) Siphoned sewer, b) Direct sewer and c) Sand sewer. **Source:** *Guia tècnica per al disseny de SUDS a Barcelona. Ajuntament de Barcelona 2020*

RESULTS AND DISCUSSION

The presence of mosquito larvae differed between years, with the highest percentage of positive sewers found in 2015 (5.05%) followed by 2018 (4.76%), 2017 (3.26%) and 2016 (3.19%). In 2019, only 0.69% of the sewers monitored had mosquito larvae. Clear differences were found within years, with a total absence of mosquito larvae recorded in March,

increasing during the next months to reach the maximum values during summer, especially in August (Table 1). Interestingly, relatively high values were also recorded during November and December. Results from monitoring programs like this conducted in Barcelona provide valuable information on requirements of mosquito species, including invasive ones, to breed in the area, allowing to identify the breeding activity patterns of these insects. The patterns reported here suggests that environmental characteristics may strongly affect the presence of mosquito larvae in the area, with clear differences found between and within years. For instance, temperature is a major driver of mosquito populations strongly affecting the rate of development of mosquitoes during their larval stages, and these variables have been demonstrated extremely important in the case of *Ae. albopictus*. Apart from temperature, annual precipitation may determine the maintenance of larval habitats affecting the ability of mosquitoes to reach high levels of abundance in anthropized areas (Romi et al., 2008). For instance, regional differences in precipitation affect the distribution of *Ae. albopictus* in the U.S.A. (Alto and Juliano, 2001), while precipitation patterns have been proposed as a limiting factor in Mediterranean areas due to its seasonality (Eritja et al., 2005) Thus, variations in temperature and precipitation during the study period may strongly affect the availability of breeding habitats for these mosquito species. This should be contextualized based on the fact that, for example, *Ae. albopictus* is able to adapt to changing environmental conditions. For example, the eggs of this species are resistant to desiccation and can survive for more than a year and this species is able to use artificial breeding sites such as tyres and flower pots, which together with their public health relevance, support the necessity to maintain a constant surveillance and control of their populations.

Table 1. Percentage of sewers where presence of mosquito larvae was recorded per month, during the 2015-2019.

	March	April	May	June	July	August	September	October	November	December
Sewers with larvae (%)	**0**	**1.04**	**5.06**	**4.11**	**3.54**	**6.97**	**1.66**	**2.61**	**0.75**	**1.72**

We further investigated those factors potentially affecting the presence of mosquitoes in urban water infrastructures. The percentage of mosquito larvae differed between sewer types, with a higher percentage found in siphoned grids (0.96%), followed by direct sewers (3.64%) and sandbox sewers (4.62%). These results may be related to the breeding requirements of mosquitoes. This is especially relevant for the case of *Ae. albopictus* which is known to breed in a variety of small containers such as rain-water drums, tyres and small clay pots, where the sand sewers may accumulate rainwater enough for mosquito breeding. Also, during the course of this study, applications of BTI were recorded in these water infrastructures and further analyses will allow us to identify the impact of these treatments on the mosquito populations in the area. These results may allow authorities to optimize the protocol to control measures including the frequency of the treatments. Thus, it is important to consider the environmental characteristics along the seasons including temperature, precipitation and humidity, which may affect the efficacy of the protocols in the Mediterranean area (Proestos et al., 2015; Generalitat de Catalunya, 2016; Jourdain et al., 2019).

CONCLUSIONS

The study of the main drivers of mosquito population colonization, including the invasive *Ae. albopictus*, has important consequences under a public health perspective. Our results suggest that mosquito surveillance measures should be conducted considering the water infrastructure characteristics, focusing the monitoring and control efforts in those months with the highest mosquito activity. Based on these results, together with the facts that *Ae. albopictus* and *Cx. pipiens* have been involved in the local transmission of arboviruses in Europe (Calba et al., 2017; Lindh et al., 2019) and the relative high number of imported infected people reaching the study area (Millet et al., 2017), it is essential to develop further entomological surveillance / control programs like those carried out here for the prevention of autochthonous transmission of mosquito-borne diseases. Thus, under the current global change scenario, the mosquito colonization process involved in the transmission of various pathogens definitely merits further studies.

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