



## Spatial distribution and temporal dynamics of invasive and native mosquitoes in a large Mediterranean city

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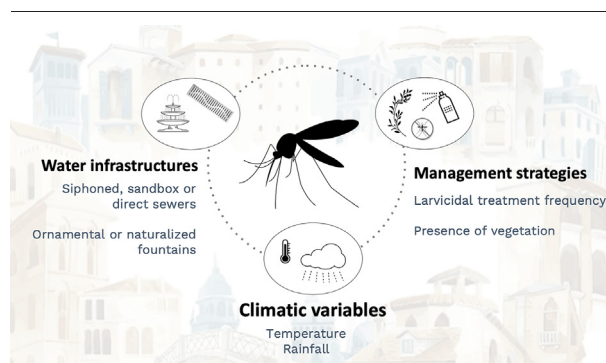
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### HIGHLIGHTS

- Mosquitoes use scuppers and ornamental fountains for breeding in urban areas.
- Sandbox scuppers harboured more often mosquito larvae than siphonic/ endless scuppers.
- Larvicidal treatments reduce mosquito larvae, but recolonization occurs within 10–25 days.
- Climatic, mainly temperature and accumulated rainfall, affect mosquito populations.
- Monitoring and control of mosquito larvae at the breeding sites may improve mosquito control in urban areas.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Mosquitoes, including invasive species like the Asian tiger mosquito *Aedes albopictus*, alongside native species *Culex pipiens* s.l., pose a significant nuisance to humans and serve as vectors for mosquito-borne diseases in urban areas. Understanding the impact of water infrastructure characteristics, climatic conditions, and management strategies on mosquito occurrence and effectiveness of control measures to assess their implications on mosquito occurrence is crucial for effective vector control. In this study, we examined data collected during the local vector control program in Barcelona, Spain, focusing on 234,225 visits to 31,334 different sewers, as well as 1817 visits to 152 fountains between 2015 and 2019. We investigated both the colonization and recolonization processes of mosquito larvae within these water infrastructures. Our findings revealed higher larval presence in sandbox-sewers compared to siphonic or direct sewers, and the presence of vegetation and the use of naturalized water positively influenced larval occurrence in fountains. The application of larvicidal treatment significantly reduced larvae presence; however, recolonization rates were negatively affected by the time elapsed since treatment. Climatic conditions played a critical role in the colonization and recolonization of sewers and urban fountains, with mosquito occurrence exhibiting non-linear patterns and, generally, increasing at intermediate temperatures and accumulated rainfall levels. This study emphasizes the importance of considering sewers and fountains characteristics and climatic conditions when implementing vector control programs to optimize resources and effectively reduce mosquito populations.

### 1. Introduction

Human-induced landscape alteration differently affects the transmission of vector-borne diseases, especially in the perspective of a general

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biodiversity loss due to an increase of human derived impacts on environment (Glidden et al., 2021). While urbanization tends to affect the abundance and composition of mosquito communities (Becker et al., 2020; Ferraguti et al., 2022; Ferraguti et al., 2016), a number of species are well adapted to exploit the artificial water sources in cities (McKinney, 2008; McKinney, 2002). This is specially the case of container-breeding species, like some *Aedes* species or *Culex pipiens*, which benefit from specific urban features where there is an abundance of artificial oviposition sites (Becker et al., 2020). For instance, the increasing implementation of various sustainable drainage systems in cities (Fletcher et al., 2014), raise concerns about their potential as breeding sites for mosquitoes. Although they do not offer optimal conditions for mosquito growth, pupae and larvae of *Ae. albopictus*, *Cx. pipiens* s.l., and *Cs. longiareolata*, have been found using this water infrastructures in France, especially during summer months (Valdelfener et al., 2018).

Nowadays, few studies have actually linked ecological drivers to changes in mosquito populations within urban environments, often resulting in contradictory conclusions. Some studies propose that alterations in top-down ecological dynamics may lead certain mosquito species to dominate urban areas due to reduced predation pressures (Carlson et al., 2004). Conversely, other research suggests that increasing levels of urban pollution can reduce the quality of larval habitats, thereby promoting the dominance of genera like *Culex*, known for their adaptability to temporary and degraded aquatic habitats (Carlson et al., 2004). Consequently, the epidemiology of mosquito-borne diseases can be significantly impacted (Johnson and Munshi-South, 2017; Rosenberg et al., 2018), resulting in severe outbreaks of diverse mosquito-borne pathogens, even in regions previously considered non-endemic. Additionally, this situation elevates the risk of the introduction and re-introduction of emerging infectious diseases (Gould et al., 2017; Poletti et al., 2011).

Among the most abundant mosquitoes in urban areas, invasive species such as the Asian tiger mosquito *Ae. albopictus* are of great concern, causing nuisance and posing global health risk (Ferraguti et al., 2022; Kraemer et al., 2015). The economic implications of their presence are also significant (Bonizzoni et al., 2013; Diagne et al., 2021). While *Ae. albopictus* is native to southeast Asia and typically found in natural habitats like bamboo stumps, tree holes, discarded tires, and flower vases (Higa, 2011), it has expanded its range globally (Eritja et al., 2005) becoming common in urban areas. The spread of *Ae. albopictus* has been facilitated through the trade of ornamental plants and used tires over long distances (Collantes et al., 2015), as well as passive transportation in cars over shorter distances (Eritja et al., 2017). Urbanization processes have also favored the proliferation of *Ae. albopictus* populations (Kolimenakis et al., 2021; Roche et al., 2015). In Catalonia (northeast Spain), *Ae. albopictus* was first identified as an invasive species in 2004, with the first records of its presence in the province of Barcelona (Aranda et al., 2006; Giménez et al., 2007) and in the city itself since 2005 (Montalvo et al., 2016). The species has found suitable conditions for reproduction along the entire eastern coast of Spain (Collantes et al., 2015).

Native mosquitoes such as *Cx. pipiens* and *Culiseta longiareolata* are also well-adapted species to urban habitats worldwide. The *Cx. pipiens* complex comprises six distinct taxa: *Cx. quinquefasciatus*, *Cx. pipiens f. pipiens*, *Cx. pipiens f. molestus*, *Cx. pipiens pallens*, *Cx. australicus* and *Cx. globocoxitus* (Aardema et al., 2020). However, in Spain, only the *pipiens* and *molestus* forms are present. These mosquitoes have a strong association with human-related habitats and structures (Farajollahi et al., 2011), and exhibit a pronounced preference for urbanized and built-up areas (Gangoso et al., 2020). *Culiseta longiareolata*, another mosquito commonly found in cities, overwinters as larvae and favors artificial habitats such as urban sewage and groundwater systems for oviposition (Becker et al., 2020). These species together with *Ae. albopictus* are commonly found in urban areas of Barcelona (Martínez-de la Puente et al., 2020a, 2020b), where they may use sewers as potential breeding sites. Overall, there are three types of sewers, i.e., siphoned, sandbox, direct, each characterized by different water accumulation patterns and levels. Understanding the characteristics of these water infrastructures is

crucial for implementing effective mosquito control strategies in urban environments.

Invasive *Aedes* species, along with *Cx. pipiens*, are well-known vectors of mosquito-borne diseases, which can have significant impacts on human health and the economy (Becker et al., 2020). In Europe, the invasion of *Ae. albopictus* played a crucial role in the first chikungunya outbreak in Italy in 2007, with over 200 cases traced back to a single infected returning traveller (Lindh et al., 2019). Local transmission of chikungunya and dengue by *Ae. albopictus* has also been reported in France since 2007 (Calba et al., 2017; Tomasello and Schlagenhauf, 2013), with a significant increase in dengue fever cases in 2022 (Cochet et al., 2022). In Spain, the presence of dengue-positive *Ae. albopictus* was recorded in 2015 (Aranda et al., 2018), and the first autochthonous cases of dengue were reported in Murcia and Barcelona in 2018 (González et al., 2017; Redondo-Bravo et al., 2019), and more recent cases in the Balearic Islands in 2022 (Centro de Coordinación de Alertas y Emergencias Sanitarias, 2023). Conversely, *Cx. pipiens* is involved in the transmission of more than 27 pathogens that affect humans (Wilkerson et al., 2021), including West Nile virus (Engler et al., 2013), St. Louis encephalitis virus (Reisen et al., 1992), Sindbis virus (Weitzel et al., 2014), and filarial worms (Bøgh et al., 1998). Additionally, *Cs. longiareolata* mosquitoes were included in the study due to their frequent presence in urban environments, although they do not usually pose a serious public health threat while being a nuisance to citizens (Becker et al., 2020).

To reduce both the nuisance to human populations and the local transmission of vector-borne pathogens, public health and municipal authorities are increasingly implementing mosquito control programs in urban areas (Bonnefoy et al., 2008; Caputo and Manica, 2020; Michaelakis et al., 2021). However, the negative impact of most biocides on biodiversity and environmental health necessitates the use of alternative strategies based on Integrated Pest Management principles (Martinou et al., 2020). These strategies promote the use of non-chemical alternatives to pesticides and emphasize focusing control actions on the larval phase, as highly effective and specific biological methods are available. The range of biocides approved for mosquito control is limited due to European legislation, and most preventive control programs rely on the use of *Bacillus thuringiensis israelensis* (*Bti*) for larvicide control (Killeen et al., 2002). Furthermore, a larval control strategy helps extend the efficacy of insecticides against adult mosquitoes by reducing the size of the population selected for resistance (Derua et al., 2018). The efficacy of *Bti* has been repeatedly tested under laboratory conditions, where larvae are exposed to varying concentrations (Becker, 2000). However, under field conditions, several factors may influence the effectiveness of larvicide treatments. These factors include climatic conditions (e.g., temperature and rainfall), the structure of urban water infrastructures (e.g., siphoned, sandbox, direct), and management strategies (e.g., frequency of treatments), which can affect the breeding capacity of mosquitoes in cities. For instance, Rakotoarivony and Schaffner (2012) demonstrated that mosquito presence is primarily influenced by an increase in rainfall and daytime surface temperature.

Despite mosquito-borne diseases are a significant public health concern, particularly in urban environments, it is crucial to understand how ecological drivers affect the population dynamics of mosquito species such as *Ae. albopictus* and *Cx. pipiens*, in order to develop effective management strategies to mitigate their spread. The main objective of this study is to identify differences in the occurrence of mosquitoes in water infrastructures (i.e., sewers and ornamental fountains) in the city of Barcelona regarding the (1) city district that may differ in the types of sewers present, (2) climatic conditions including temperature and rainfall, and (3) changes in management strategies, such as larvicidal treatments and habitat modification. To do that, we investigated the colonization process of areas where mosquitoes were initially absent and evaluate the effectiveness of treatments by monitoring the recolonization of infrastructures by mosquitoes where the presence of larvae and pupae was previously reported. Differences in the abundance of mosquito occurrence among districts can be anticipated based on the availability of different types of sewers. Specifically, sandbox scuppers are likely to provide more favorable conditions for mosquitoes

compared to the other two types (Montalvo et al., 2022). Furthermore, considering the environmental requirements of mosquitoes, a higher presence of larvae can be expected during the summer months (Ferraguti et al., 2013), characterized by intermediate temperature and rainfall levels. Lastly, it is expected that control management strategies will effectively reduce the abundance of mosquitoes in the area. The findings of this study provide valuable insights to public health and municipal authorities for the development of effective management strategies to control mosquito populations and reduce the risk of mosquito-borne diseases.

## 2. Materials and methods

### 2.1. Study area and mosquito surveillance protocol

This study relies on data from 2015 to 2019 provided by the Agència de Salut Pública de Barcelona (ASPB; <https://www.aspb.cat/>). This institution is responsible for mosquito and infectious disease surveillance in Barcelona. The ASPB plays a crucial role in monitoring imported mosquito-borne diseases, including dengue, Zika, and chikungunya (Millet et al., 2017). Furthermore, they actively work to mitigate the presence of mosquito vectors in the city, aiming to minimize the nuisance experienced by citizens and reduce the risk of introduction and transmission of emerging diseases (Ellena et al., 2020; González et al., 2017; Villalbí and Ventayol, 2016). The collaboration with the ASPB provides essential information for the investigation of mosquito populations and their associated health risks in the city of Barcelona.

The presence or absence of larvae and pupae of *Ae. albopictus*, *Cx. pipiens* and *Cs. longiareolata* was monitored in all 10 districts of Barcelona from February to December 2015–2019. These are the three predominant mosquito species breeding in sewers and fountains of Barcelona, as confirmed through morphological identification of numerous larvae and the

subsequent determination of emerged adults in laboratory settings, using serendipitously collected samples. However, it is important to acknowledge that we did not identify the larvae or pupae of mosquitoes to species level in this study. Therefore, we cannot completely rule out the sporadic breeding of other mosquito species such as *An. plumbeus*, *Ae. geniculatus*, *Cx. theileri*, and *Cx. laticinctus* in the city of Barcelona (Calder-Smith, 1965), representing a limitation of our research.

The mosquito control program implemented several components, including surveillance and control in public areas, addressing citizen complaints, and managing high-risk areas surrounding the residences of imported or autochthonous human cases of arboviruses such as dengue virus, chikungunya virus, Zika virus, and West Nile virus. In case of a confirmed human infections, all accessible mosquito breeding sites were identified and treated. Regular visits for surveillance and control of mosquitoes were conducted at the inspected water infrastructures by technicians of the ASPB as part of the surveillance and control mosquito program. The frequency of visits varied but generally occurred at least once per month, with some variation between years. Specifically, during 2019 visits were intensified due to the occurrence of a peak of dengue imported cases (ASPB unpublished data). During inspections, detailed information about the characteristics of the structures was recorded, including the type of sewers (e.g., siphoned sewers, sandbox sewers, and direct sewers; Fig. 1). Siphoned sewers had a siphon mechanism that prevented odors but resulted in stable water accumulation, making them problematic breeding sites. Sandbox sewers had a basin below the collector to prevent sand from entering the sewage system, but water could accumulate, providing abundant breeding sites. In contrast, direct sewers did not accumulate water posing no risk for the proliferation of mosquito larvae. Fountains were categorized as naturalized fountains, which lacked water recirculation or chlorination and had vegetation, and ornamental fountains, which could have vegetation, chlorination, and a recirculation system.

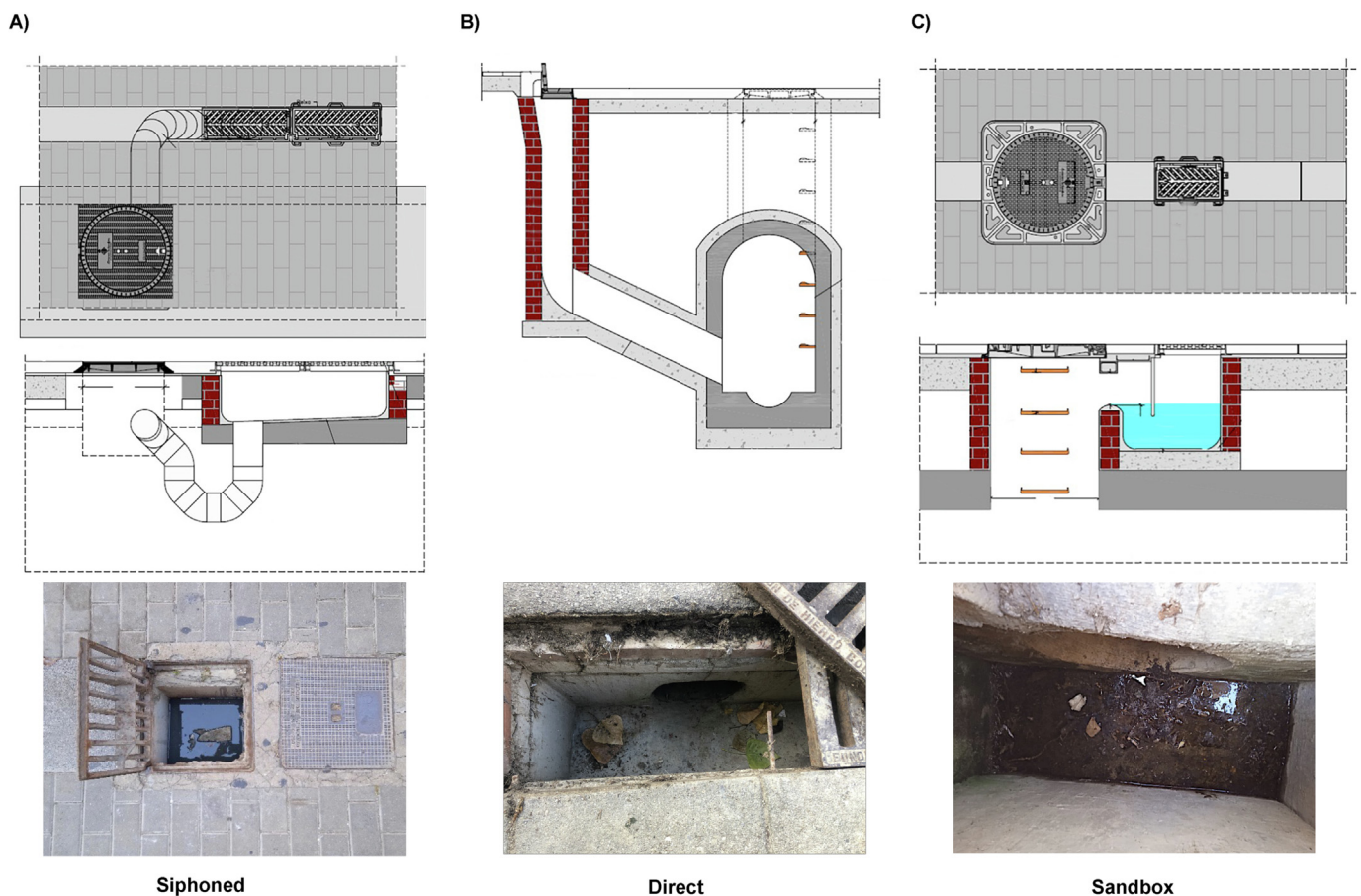


Fig. 1. Classification of the three types of sewers in the city of Barcelona.

Overall, the decision to apply the biocide treatment was based on the presence or absence of larval activity. The treatment involved using a formulation of *Bti* and *Bacillus sphaericus* (VFG: Vectomax FG, Valent Biosciences Corporation, Libertyville, Illinois, USA) or *Bti* alone (VG: Vectobac G, Valent Biosciences Corporation, Libertyville, Illinois, USA).

## 2.2. Climatic variables

Data regarding the mean, maximum, minimum temperature and rainfall during the study period were obtained from seven meteorological stations located in the city of Barcelona: Badalona - Museu, BCN - Zoo, BCN - Raval, BCN - Zona Universitaria, BCN - Port de Barcelona, BCN - Can Bruixa, and BCN - Font del Gat (accessible at: <http://www.meteo.cat/>). To analyse the relationship between these variables and mosquito occurrence, the data were aggregated on a weekly basis. Mean weekly values were calculated for both temperature and rainfall based on one, two, three, and four weeks prior to the monitoring date. Additionally, the accumulated rainfall was computed for different time intervals: 1–2, 1–3, 1–4, 2–3, 2–4, and 3–4 weeks before the monitoring date following Roiz et al. (2014), to account for the potential availability of breeding sites. Finally, for accumulated temperature, the mean temperatures of the corresponding weeks were summed up from one to four weeks prior to the monitoring date.

## 2.3. Statistical analysis

Independent analyses were conducted for urban sewers (scuppers and grids) and ornamental fountains, focusing on colonization and recolonization datasets. In order to establish the database, each sewer record was paired with the subsequent visit to the same sewer. The presence or absence of mosquito larvae or pupae mosquitoes (L1-L2, L2- L3, hereafter referred as mosquito larvae) during the second visit to each water infrastructures was used as the exploratory variable. Each record consisted of presence/absence data from the first and second visits, and multiple records could exist for each sewer. For inclusion in the study, the infrastructure had to have water present during the second visit, as it would be impossible to find live mosquito larvae or pupae in dry infrastructures. Two distinct datasets were created: one to examine the colonization process in areas without initial mosquito presence, and the other to assess the effectiveness of treatments by examining the recolonization of previously infested infrastructures. To avoid pseudo replication, only one randomly selected record per sewer was included in the analyses. Colonization was investigated in sewers and fountains that were negative for mosquito larvae in the first visit but positive or negative in the second visit of the same year, resulting in a final dataset of 19,534 and 1314 records for sewers and fountains, respectively. Recolonization was examined in sewers and fountains that initially had mosquito larvae, with 1059 records for sewers and 391 records for fountains. Additional variables included in the recolonization models were the type of treatment product used (VFG or VG), the time elapsed between the two visits, the larval density observed during the first visit in each sewer, and, for ornamental fountains, the presence of vegetation, the use of naturalized water, and the presence of a recirculation system. Furthermore, all models included the variables district, month, and year of sampling. Finally, after conducting exploratory analyses among mean, maximum, and minimum temperature, we decided to use the minimum temperature as it was consistently selected as the most influential variable in our analyses.

Random forest (RF) analysis, a machine-learning algorithm known for its ability to identify non-linear relationships, was employed in a three-step variable selection strategy (Brieuc et al., 2018; Cutler et al., 2007). In the first step, variable importance was determined using RF regression analysis with 1000 trees (Breiman, 2001). The RF permutation-based score of importance was utilized for variable selection, employing a stepwise forward strategy for introducing variables. In the second step, relevant variables for result interpretation were selected based on the mean decrease in the Gini measure. This measure indicates the importance of a variable in estimating the value of the target variable across all trees in the forest.

Higher Gini scores reflect greater variable importance in the model. In the third step, predictive models consisting of 500 trees were fitted using the VSURF function, with a focus on prediction. The most important variables were selected based on relevant thresholding, and prediction steps. Given the overrepresentation of the absence of mosquitoes in the dataset (mosquito larvae accounted for only 0.72 % of observations), a combination of oversampling presences and undersampling absences was applied to weight this variable (Fernández et al., 2018; Fox et al., 2017). The accuracy of each RF model was assessed based on the percentage of observations correctly classified. Three metrics were used to evaluate the model performance:

1) Precision, a measure of the correctness achieved in positive predictions. It calculates the number of observations labelled as positive that are actually positive.

2) Recall (or sensitivity), as metric of the proportion of actual positive observations that are correctly labelled (predicted) by the model. It indicates how many observations of the positive class are accurately classified.

3) F1 score, a measure of a balanced assessment of the model's effectiveness by considering both precision and recall. It is the weighted harmonic mean of precision and recall, and it provides a single metric to evaluate the classification performance.

These three metrics are commonly used to assess the classification accuracy of models and provide insights into the model's effectiveness in correctly identifying positive cases (Powers, 2020).

To examine the relationship between each predictor variable in the model and the response variable, we constructed partial dependence plots (PDPs) using the `'pdp::partial()'` function. We specified the `'smooth'` argument to enable smoothing of the curves displayed in the resulting plots. The smoothing function displays a blue trend line that can be useful for highlighting general patterns in the PDP curves and reducing noise or variability in the data.

All statistical analyses were conducted in R version 4.1.1 (R Development Core Team 2005) using the packages: *arm*, *car*, *ggplot2*, *ggpubr*, *lme4*, *Matrix*, *MASS*, *MuMIn*, *multcomp*, *nortest*, *pdp*, *randomForest*, *Rcpp*, *ROSE*, *stats*, *tibble*, *tidyverse*, and *VSURF*.

## 3. Results

### 3.1. Phenology of mosquito activity at the sewers

Over the five-year period, a total of 234,225 visits were conducted through the ten district of Barcelona to examine 31,333 different sewers, along with 1817 visits were made to 152 fountains (Fig. 2). Out of these visits, only 0.72 % of them (1288 visits to 619 sewers and 397 visits to 68 ornamental fountains) resulted in the detection of immature stages of mosquitoes in the first visit. The sewers were classified as follows: 10.68 % were siphoned, 35.03 % were sandbox, and 54.29 % were direct, while 36.84 % of the fountains were naturalized and 63.16 were ornamental ones (Table 1).

The number of visits varied each year, with 39,844 visits in 2015, 53,494 visits in 2016, 35,949 visits in 2017, 44,308 visits in 2018, and 60,630 visits in 2019. The presence of mosquito larvae varied between years, with the highest percentage observed in 2015 (1.04 %), followed by 2016 (0.82 %), 2018 (0.75 %), and 2019 (0.52 %). In 2017, only 0.51 % of the monitored sewers showed the presence of mosquito larvae. Notably, clear differences were observed within each year, with no mosquito larvae recorded during the winter months (from December to February) (Fig. 3). Larval presence increased in the subsequent months, reaching its peak during the summer, particularly in May and July. Relatively high values were also observed in October.

### 3.2. Colonization models in sewers

Overall, 19,534 records were used to conduct the sewer colonization models. The out-of-bag (OOB) error for these colonization models was 6.73 %. After weighting the dependent variable, we got a balanced dataset

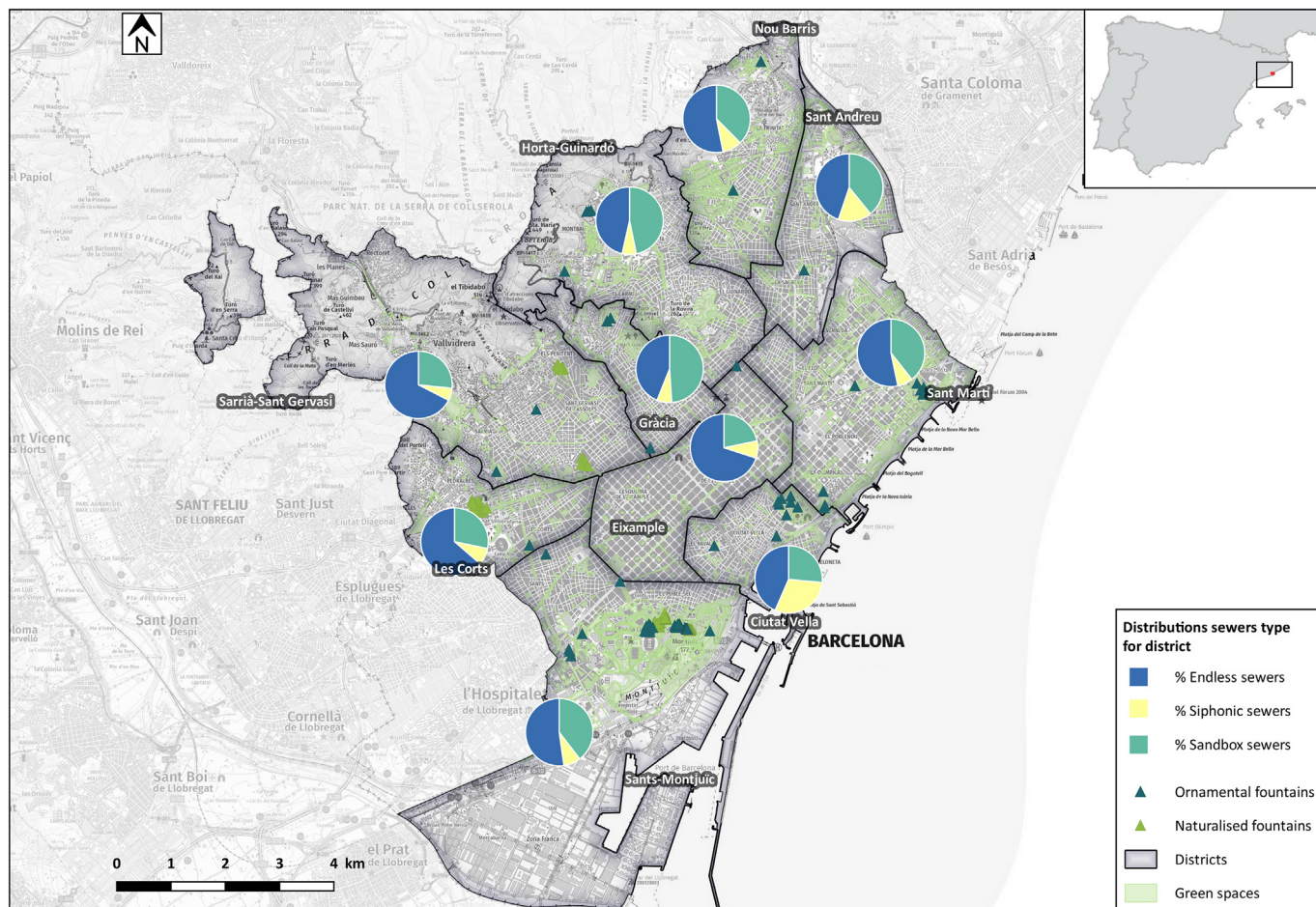


Fig. 2. Distribution map of Barcelona districts. The dots represent the precise locations of ornamental and naturalized fountains. Due to their high densities, the sewer dots are not displayed on the map. Instead, the proportion of sewers is depicted using pie charts.

with values of 0.991 for recall, 0.886 for precision and 0.936 for F1 score. The district and the year of sampling were, by far, the two most important variables (Fig. 4A).

According to the VSURF procedure, a total of ten variables were identified and ranked based on their level of importance. The presence of mosquito larvae was higher in the Horta Guinardo and Sarrià-Sant Gervasi districts (Fig. 5A), and there were fewer larvae reported in 2019 compared to the other years (Fig. 5B). The treatment significantly reduced the presence of larvae in the sewers (Fig. 5C), and the type of scuppers also had an effect on the occurrence of mosquito larvae. Specifically, sandbox scuppers had a higher presence of larvae compared to siphonic or direct scuppers (Fig. 5D). In addition, there were non-linear associations between

the presence of mosquitoes in the sewers and six different climatic variables. For instance, the presence of larvae and pupae of the mosquitoes initially increased with the average rainfall in the week and the second week prior to sampling, followed by a decline at higher accumulated precipitations (e.g., 40 mm in the former and around 60 mm in the latter, before increasing slightly and decreasing again after 80 mm of rainfall) (Fig. 6A,B). Furthermore, non-linear patterns were also evidenced with accumulated rainfall during the 2–3 and 2–4 weeks prior to the monitoring. In the first case, a decrease in the occurrence of larvae was observed at around 60 mm of water (Fig. 6C), while in the second case, there was an increase from 0 to 25 mm, followed by a decline at around 60 mm and a subsequent increase (Fig. 6D). The mean minimum temperature for one week and the

Table 1

Characteristics of the ten districts in the city of Barcelona. Total area, green space (including Parks, gardens, flowerbeds, tree surrounds and urban allotments, all municipally owned), and number of sewers and fountains by type.

Barcelona district	District area (m <sup>2</sup> )	Green spaces (m <sup>2</sup> ) (%)	Siphoned sewer	Sandbox sewer	Direct sewer	Naturalized fountain	Ornamental fountain
Ciutat Vella	4,204,930.80	587,085.06 (13.96)	1039	916	1504	0	9
Eixample	7,464,303.21	550,591.69 (7.38)	319	843	2716	0	2
Gracia	4,224,277.84	566,662.27 (13.41)	155	1076	965	0	3
Horta Guinardo	11,919,630.83	1,436,152.89 (12.05)	175	1220	1211	0	8
Les Corts	6,010,769.11	737,361.84 (12.27)	161	562	1265	1	14
Nou Barris	8,056,467.80	1,180,701.18 (14.65)	233	942	1332	0	2
Sant Andreu	6,592,479.94	561,761.67 (8.52)	349	852	975	0	1
Sant Marti	10,437,809.60	1,635,475.31 (15.67)	369	1983	2682	5	4
Sants Montjuic	22,879,850.05	2,722,291.51 (11.90)	373	1798	2375	42	45
Sarria Sant Gervasi	19,915,566.17	822,614.23 (4.13)	174	784	1985	8	8

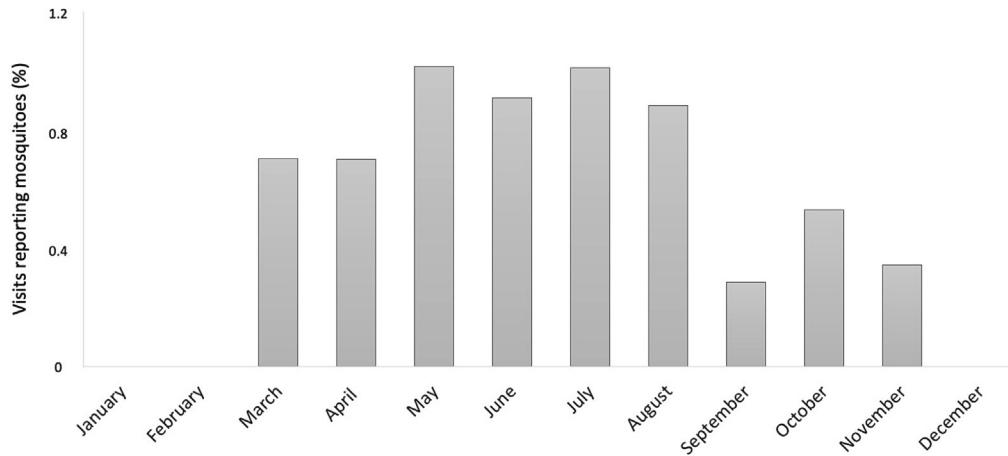


Fig. 3. Monthly variation in the percentage of visits to sewers and fountains reporting the presence of mosquito larvae or pupae. The data presented covers the period from 2015 to 2019 in Barcelona.

fourth week prior to the monitoring affected the occurrence of mosquitoes with a similar pattern characterized by an initial increase followed by a decline (Fig. 6E,F). In the case of the mean minimum temperature during the

fourth week prior to the monitoring, a second peak in mosquito occurrence was observed at values close to 550 °C of accumulated temperature (Fig. 6F).

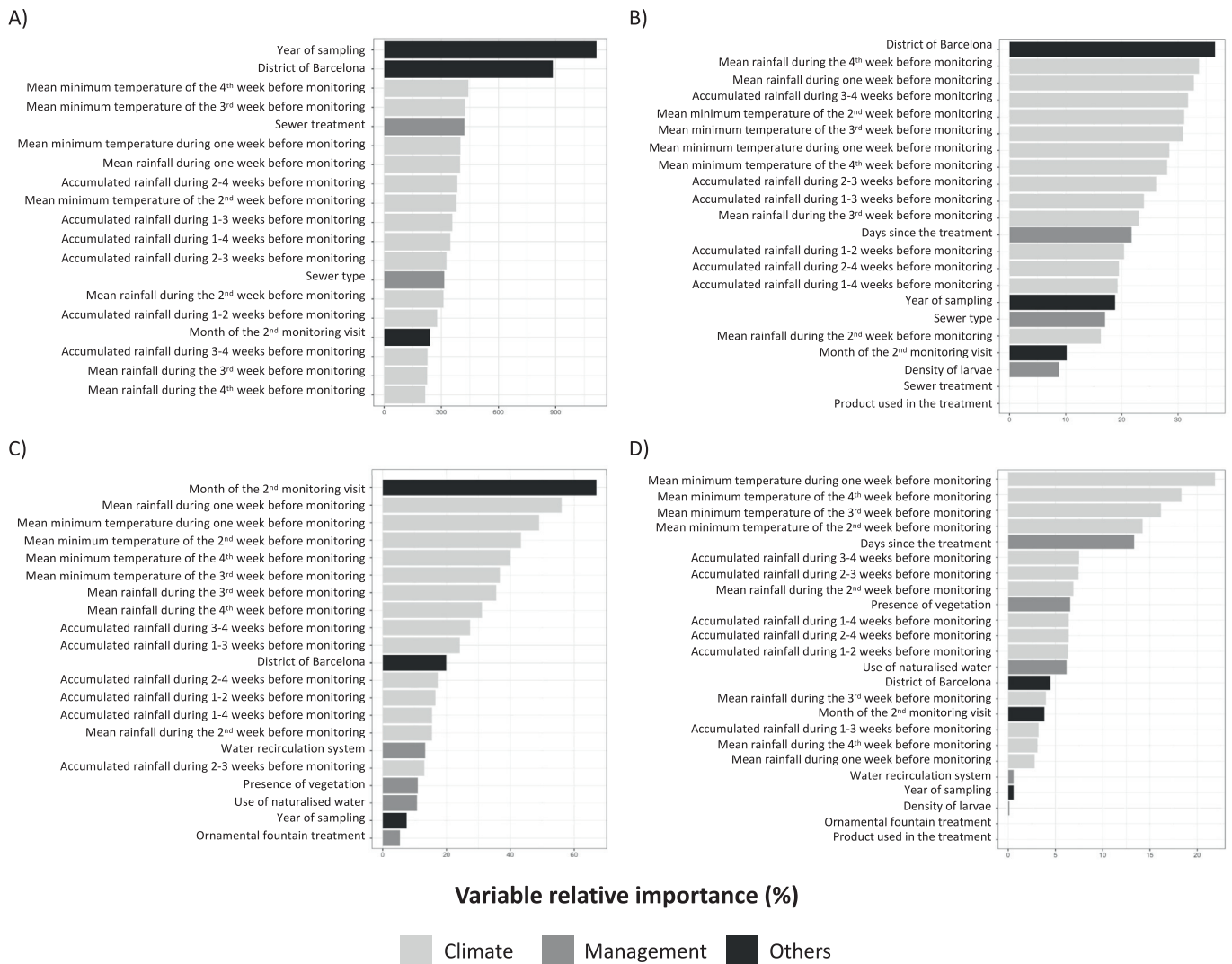
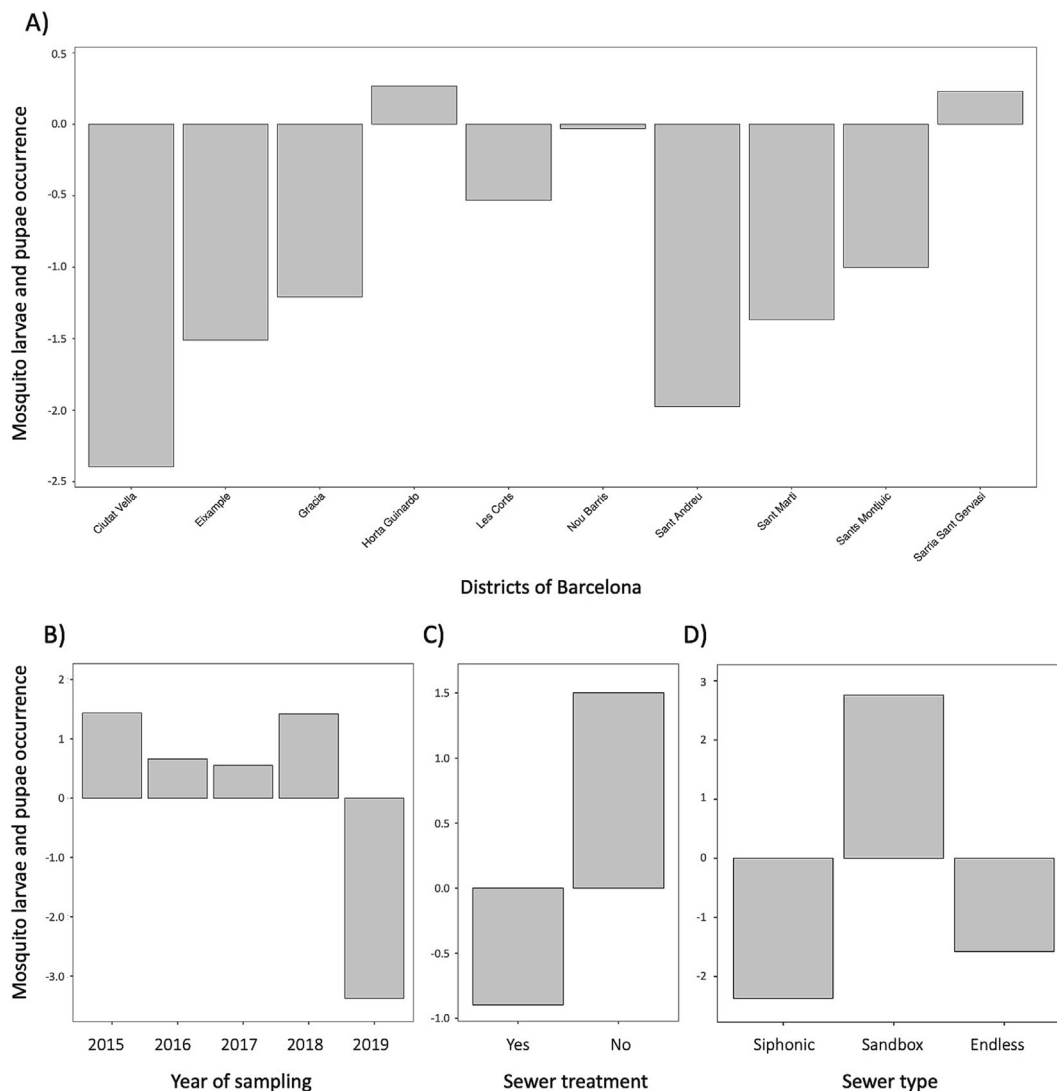


Fig. 4. Quantification of the importance of the variables included in the A) sewer colonization, B) sewer re-colonization, C) ornamental fountain colonization, and D) ornamental fountain re-colonization models. Variables are ordered following the mean decrease Gini measure, based on the Gini impurity index used for the calculation of splits in trees. A higher Gini's score indicates higher variable importance.



**Fig. 5.** Partial dependence plot (PDP) illustrating the relationship between mosquito larvae occurrence and categorical variables in the sewer colonization models. A) District of Barcelona city, B) years of sampling, C) treatment, D) typology of the sewer. The PDP show the relative logit contribution of the variable on the class probability as perceived by the model.

### 3.3. Recolonization models in sewers

The dataset includes 1059 records for the sewer recolonization models. The OOB error for these recolonization models was 3.97 %. After weighting the dependent variable, we got a balanced dataset with values of 0.986 for recall, 0.935 for precision and 0.961 for F1 score. The most significant variable was the district of Barcelona, although the percentage of relative importance was not significantly different from the other significant variables, which were all related to climate (Fig. 4B).

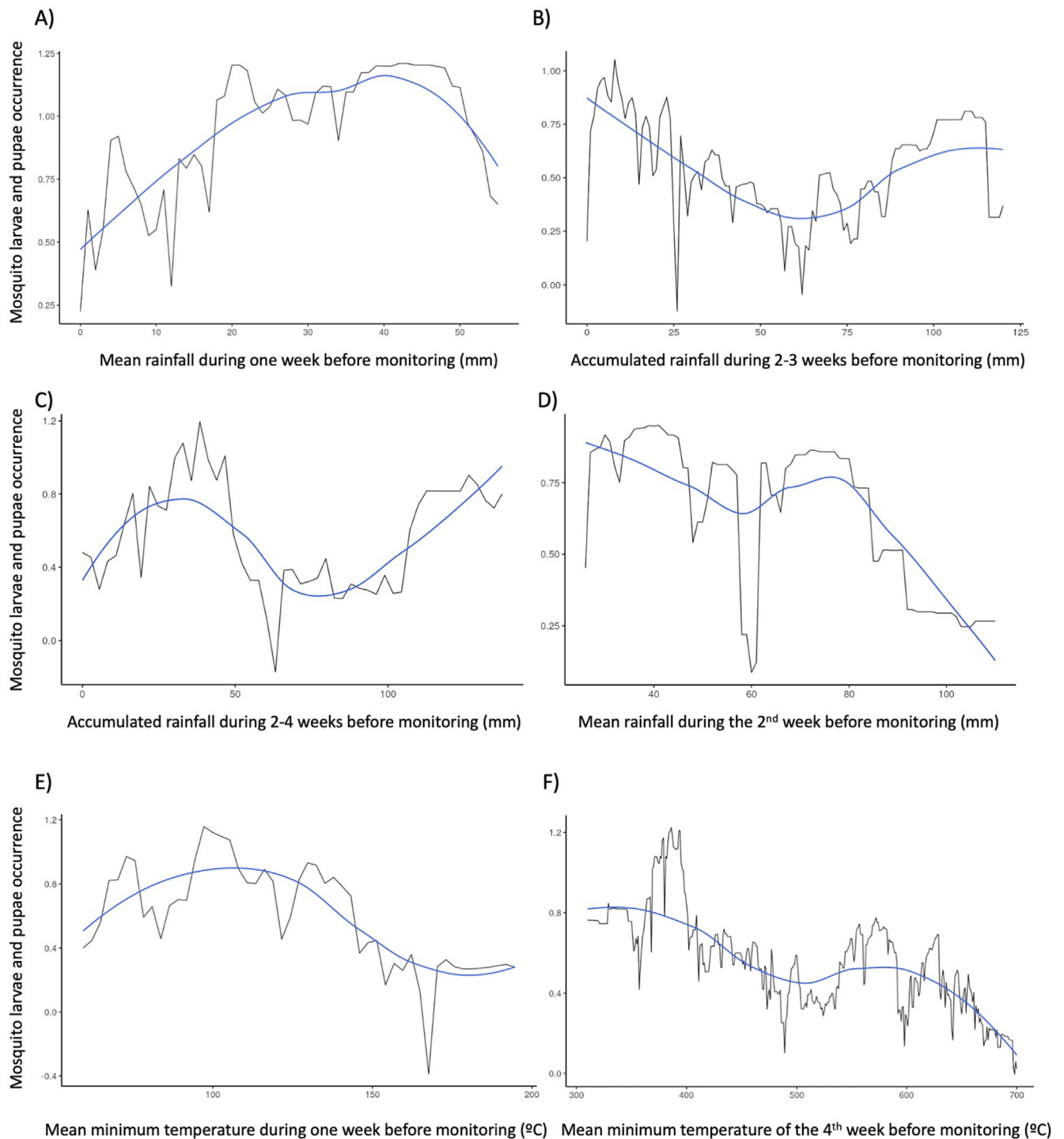
Based on the VSURF procedure, seven variables were selected and ranked by importance. The presence of mosquito larvae was higher in the Horta Guinardo and Sarrià-Sant Gervasi districts (Fig. 7A), and there was a decrease in larvae occurrences in 2019 compared to the other years (Fig. 7B). Furthermore, there were non-linear association patterns between the occurrence of the mosquitoes in the sewers and four climatic and one management variables. Mosquitoes exhibited different patterns in relation to the mean rainfall of the week prior and the fourth week prior to the mosquito monitoring, increasing in the first case until stabilizing at 10 mm of rainfall (Fig. 8A), and decreasing in a U-shaped pattern in the second case, with the minimum value around 40 mm (Fig. 8B). Additionally, non-linear relationships were observed between the accumulated rainfall of the 1–3 weeks before monitoring and the occurrence of mosquitoes,

where mosquitoes increased until reaching a peak at approximately values of 75 mm, and then decreased (Fig. 8C). Mosquito occurrence also showed an initial decrease related to the mean minimum temperature of the week before monitoring, followed by an increase with a peak at the accumulated values of 160 °C (Fig. 8D). Finally, we observed a negative impact of the number of days since treatment on the occurrence of mosquito larvae during the second visit. This relationship is depicted by the smoothed PDP curve (Fig. 8E), which shows a decrease in the occurrence of mosquito larvae as the number of days since treatment increases, particularly after 10 days post-treatment.

### 3.4. Colonization models of ornamental fountains

Overall, 1314 records were used for the colonization models from ornamental fountains. The OOB error for these models was 6.32 %. After weighting the dependent variable, we got a balanced dataset with values of 0.987 for recall, 0.892 for precision and 0.938 for F1 score. The variable with the highest importance in the model was the month of the second monitoring visit to the sewers, followed by climate-related variables (Fig. 4C).

According to the VSURF procedure, a total of seven variables were selected and ranked based on their level of importance. Results indicated



**Fig. 6.** Partial dependence plot (PDP) illustrating the relationship between mosquito occurrence in sewers colonization process and continuous variables. The examined variables include A) mean rainfall one week before the monitoring, B) accumulated rainfall 2–3 weeks before the monitoring, C) accumulated rainfall 2–4 weeks before the monitoring, D) mean rainfall during the 2nd week before the monitoring, E) mean minimum temperature one week before the monitoring, and F) mean minimum temperature of the 4th week before the monitoring. The PDPs show the relative logit contribution of each variable to the class probability as perceived by the model. The blue trend line in each PDP represents the smoothed relationship between the independent variable and the response variable, highlighting general patterns while reducing noise or variability in the data.

*Note:* Negative values on the y-axis indicate that the positive class is less likely for that value of the independent variable shown on the x-axis, according to the model. Similarly, positive values indicate that the positive class is more likely for that value of the independent variable. A value of zero implies that the independent variable has no average impact on the class probability, as predicted by the model.

that mosquito larvae were more commonly found in fountains from the Horta Guinardo and Sants Montjuic districts (Fig. 9A), as well as in fountains without any surrounding vegetation (Fig. 9B). The application of larvicide treatment had a positive impact on the occurrence of mosquito

larvae in the fountains (Fig. 9C). The occurrence of mosquito larvae was lower in naturalized water fountains (Fig. 9D). In addition, there were non-linear associations between the occurrence of the mosquitoes in the fountains and three climatic variables, all of them related to ambient



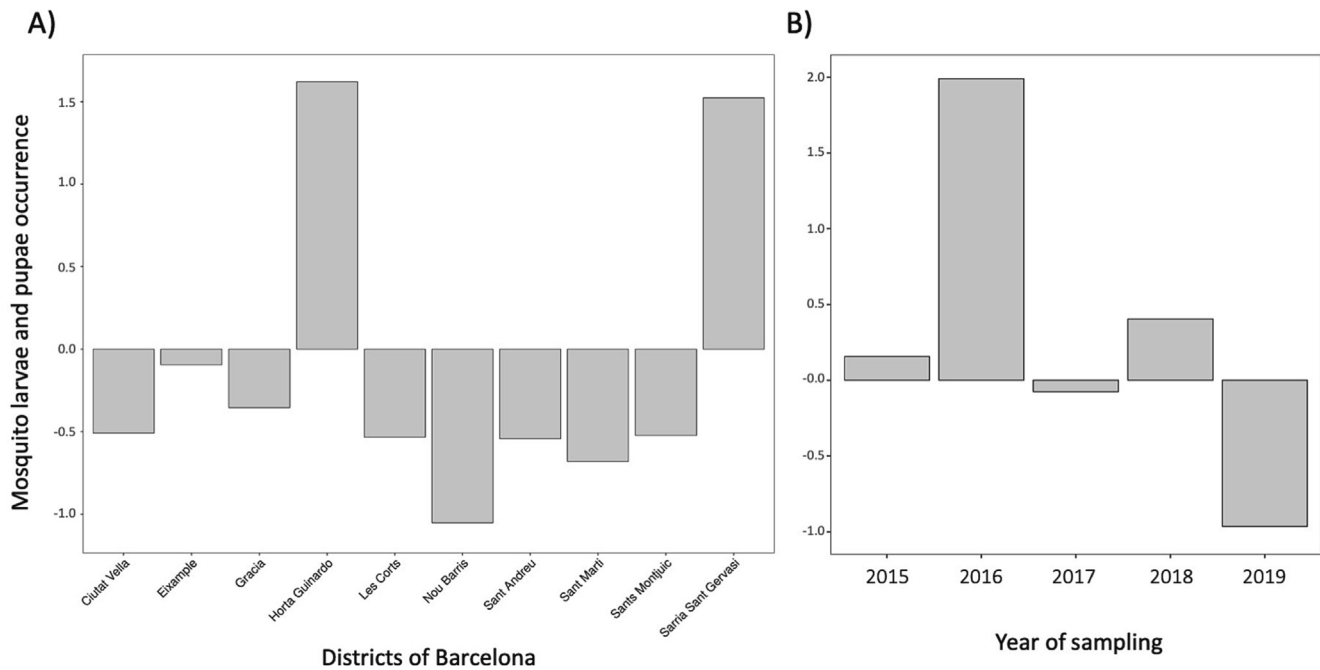


Fig. 7. Partial dependence plot (PDP) illustrating the relationship between mosquito larvae occurrence and categorical variables in the sewer recolonization models. A) District of Barcelona city, B) years of sampling. The PDP show the relative logit contribution of the variable on the class probability as perceived by the model.

temperature. Overall, the occurrence of mosquitoes increased with the mean minimum temperature during the week before monitoring (Fig. 10A), as well as during the third (Fig. 10B) and fourth weeks (Fig. 10C), followed by a subsequent decrease and a second increase in the latter two cases.

### 3.5. Recolonization models of ornamental fountains

The dataset used in this model includes 391 records for ornamental fountains. The OOB error for these recolonization models was 7.67 %. After weighting the dependent variable, we got a balanced dataset with values of 0.951 for recall, 0.881 for precision and 0.914 for F1 score. The most important variables were all related to mean minimum temperatures and to management strategies (Fig. 4D).

Based on the VSURF procedure, five variables were selected and ranked by importance. The number of days since treatment had a negative impact on the occurrence of mosquito larvae during the second visit. The relationship observed in the smoothed PDP curve (Fig. 11A) suggest that the occurrence of mosquito larvae decreases as the number of days since treatment increases. Two peaks can be observed in the curve, occurring at approximately 13- and 25-days post-treatment. Furthermore, the occurrence of mosquitoes in fountains exhibited an upward trend with increasing mean minimum temperature during the week prior to monitoring (Fig. 11B) and during the fourth week prior to the mosquito monitoring (Fig. 11C), with subsequent declines followed by a second increase in the latter case. The inverse pattern characterized by an initial decline followed by a rebound was observed for the association between the occurrence of mosquito larvae and both the mean minimum temperature during the third week before monitoring (Fig. 11D) and the accumulated rainfall during 1–4 weeks before monitoring (Fig. 11E).

## 4. Discussion

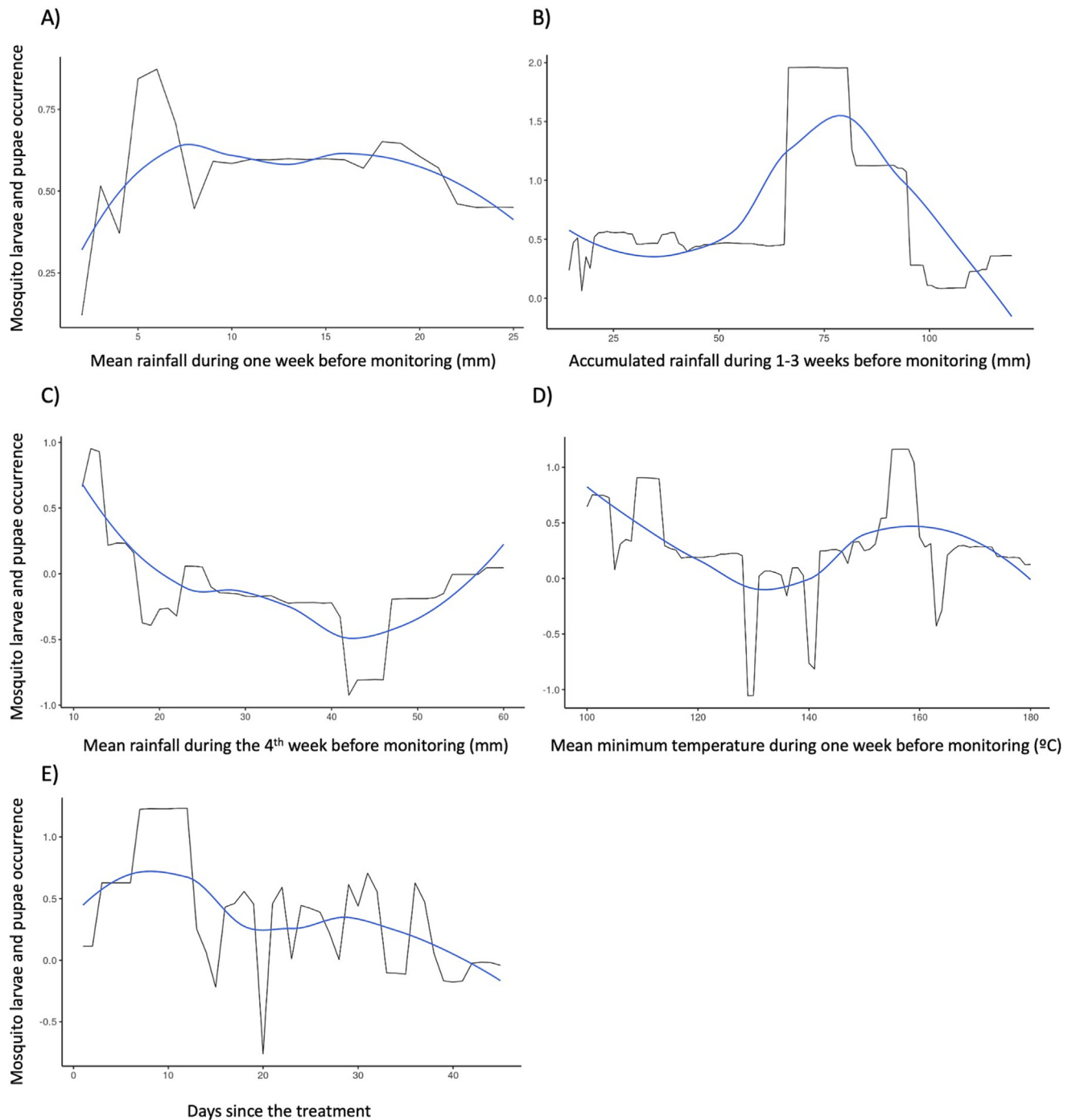
Mosquitoes play a central role in the epidemiology of a large number of vector-borne diseases. Environmental characteristics strongly affect the population dynamics of mosquitoes and, therefore, the risk of emerging diseases. Our study investigated the effect of urban water infrastructures, climate conditions, and management practices

on the occurrence of mosquitoes in public areas, sewers, and fountains in Barcelona.

Predictive models of mosquito distribution allow for targeted management and prioritization of prevention efforts, thereby reducing the cost of vector surveillance and control programs like the one managed by the ASPB. Effective mosquito management is essential to prevent the transmission of arboviruses that pose a significant threat to public health (Millet et al., 2017). Developing optimal vector surveillance and control programs is the responsibility of municipalities, and understanding the risk and vulnerability factors in each city is crucial for their effective implementation. Our analysis revealed that only a small fraction of monitored sewers presented mosquito larvae, allowing for more effective and cost-efficient control measures.

### 4.1. Geographical and interannual differences and effects of water infrastructure characteristics

The incidence of mosquito-borne pathogens affecting humans is often directly related to the spatial distribution and abundance of the main mosquito species (Hamer et al., 2011). Research on vector control management plans is an emerging area of community ecology in many countries (Juliano, 2009; Juliano and Lounibos, 2005; Martinou et al., 2020; Valdelfener et al., 2018). The patterns we found here suggest that environmental characteristics may strongly influence the presence of *Ae. albopictus*, *Cx. pipiens* and *Cs. longiareolata* larvae in the area. Clear differences were also found between and within years. Similar results were observed in a study conducted in a large metropolis in France (Valdelfener et al., 2018), where the authors observed that the density and distribution of the same mosquito species varied based the presence of artificial habitats, water, vegetation, and climate. Our models revealed two important districts of the city of Barcelona, Horta Guinardo and Sarrià-Sant Gervasi, that were positively associated with mosquito occurrence in both sewer colonization and recolonization models. Our analysis identified two districts in the city of Barcelona, namely Horta Guinardo and Sarrià-Sant Gervasi, that consistently showed a higher occurrence of mosquitoes in both sewer colonization and recolonization models. These districts are characterized by a significant level of mosquito activity each year and have a notable presence of sandboxes, while having a lower number of siphoned sewers compared to

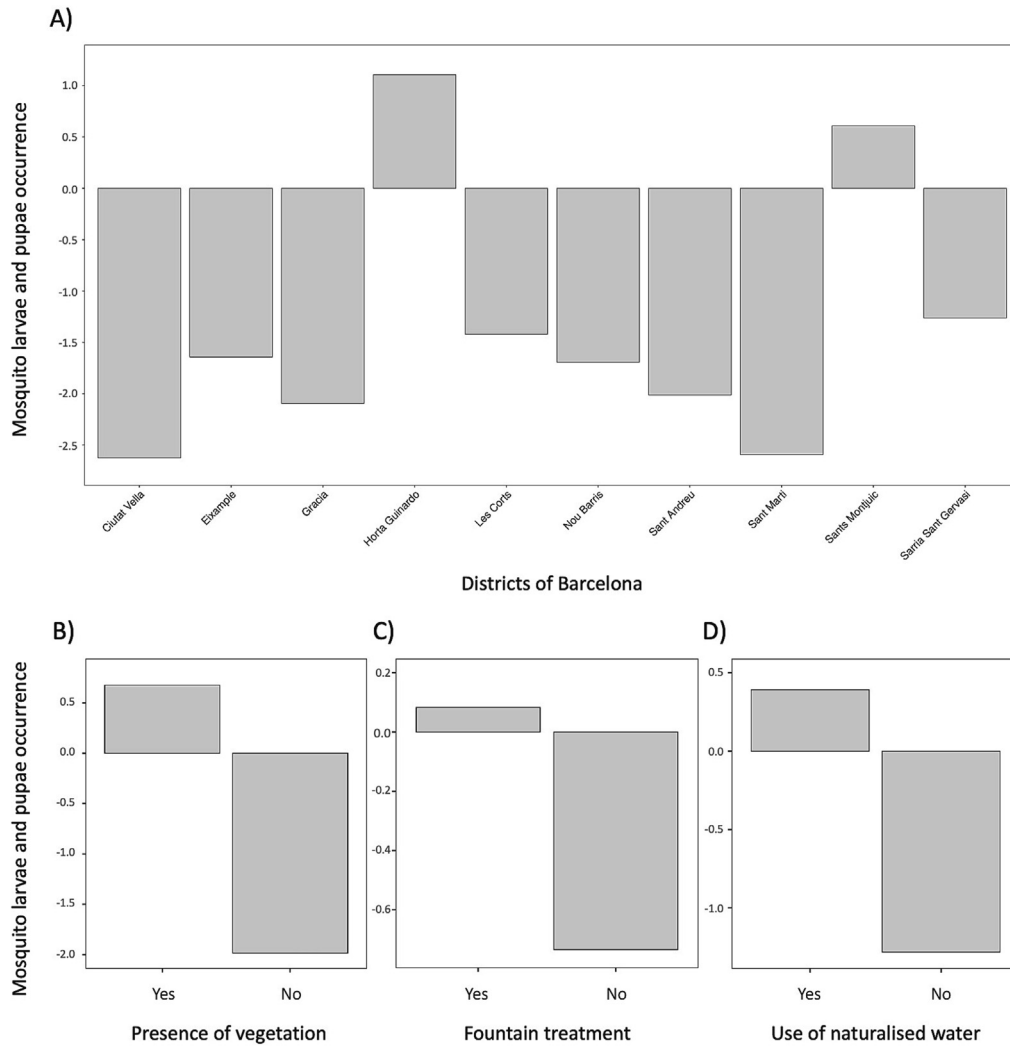


**Fig. 8.** Partial dependence plot (PDP) illustrating the relationship between mosquito occurrence in sewers recolonization process and continuous variables. The examined variables include A) mean rainfall during one week before the monitoring, B) accumulated rainfall during 1–3 weeks before the monitoring, C) mean rainfall during the 4th week before the monitoring, D) mean minimum temperature during one week before the monitoring, and E) days since the treatment. The PDPs show the relative logit contribution of each variable to the class probability as perceived by the model. The blue trend line in each PDP represents the smoothed relationship between the independent variable and the response variable, highlighting general patterns while reducing noise or variability in the data.

*Note:* Negative values on the y-axis indicate that the positive class is less likely for that value of the independent variable shown on the x-axis, according to the model. Similarly, positive values indicate that the positive class is more likely for that value of the independent variable. A value of zero implies that the independent variable has no average impact on the class probability, as predicted by the model.

other districts (see Table 1). It should be noted that the presence of sandy sewers is especially intended to prevent sand from parks from entering the sewage system, so urban parks with soil will always have more sandy sewers than other spaces. However, our models showed how mosquito larvae colonized sandbox sewers more than siphoned or direct ones. Additionally, these parks usually have abundant vegetation, which can serve as a refuge for mosquitoes (Ferraguti et al., 2022). Here, Horta Guinardo and

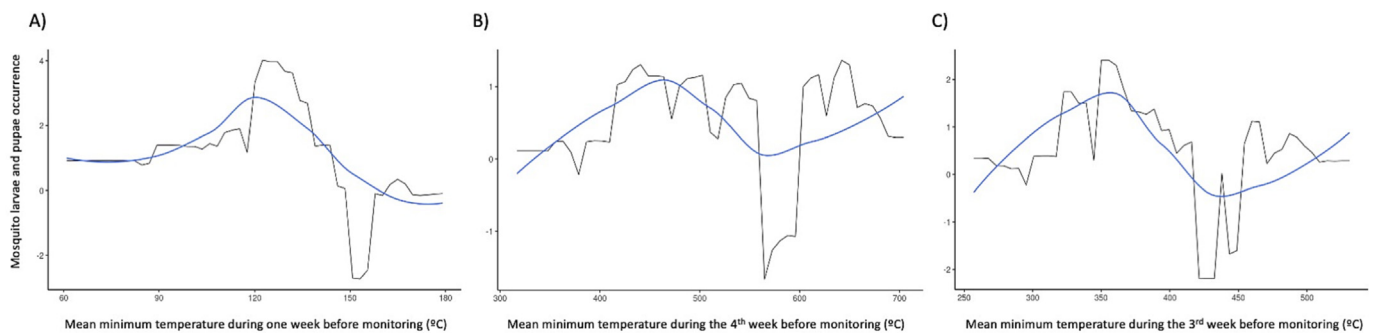
Sarriá-Sant Gervasi districts are known for their higher residential density and abundant green spaces, particularly in Sarriá-Sant Gervasi, which include various outdoor areas such as courtyard, terraces, and gardens. These residential areas provide ample breeding sites for mosquitoes, significantly contributing to the dispersion of mosquitoes to public areas and affecting the colonization and/or recolonization processes (Bellini et al., 2020).



**Fig. 9.** Partial dependence plot (PDP) illustrating the relationship between mosquito larvae occurrence and categorical variables in the fountain colonization models. A) District of Barcelona city, B) presence of vegetation, C) treatment, D) use of naturalized water. The PDP show the relative logit contribution of the variable on the class probability as perceived by the model.

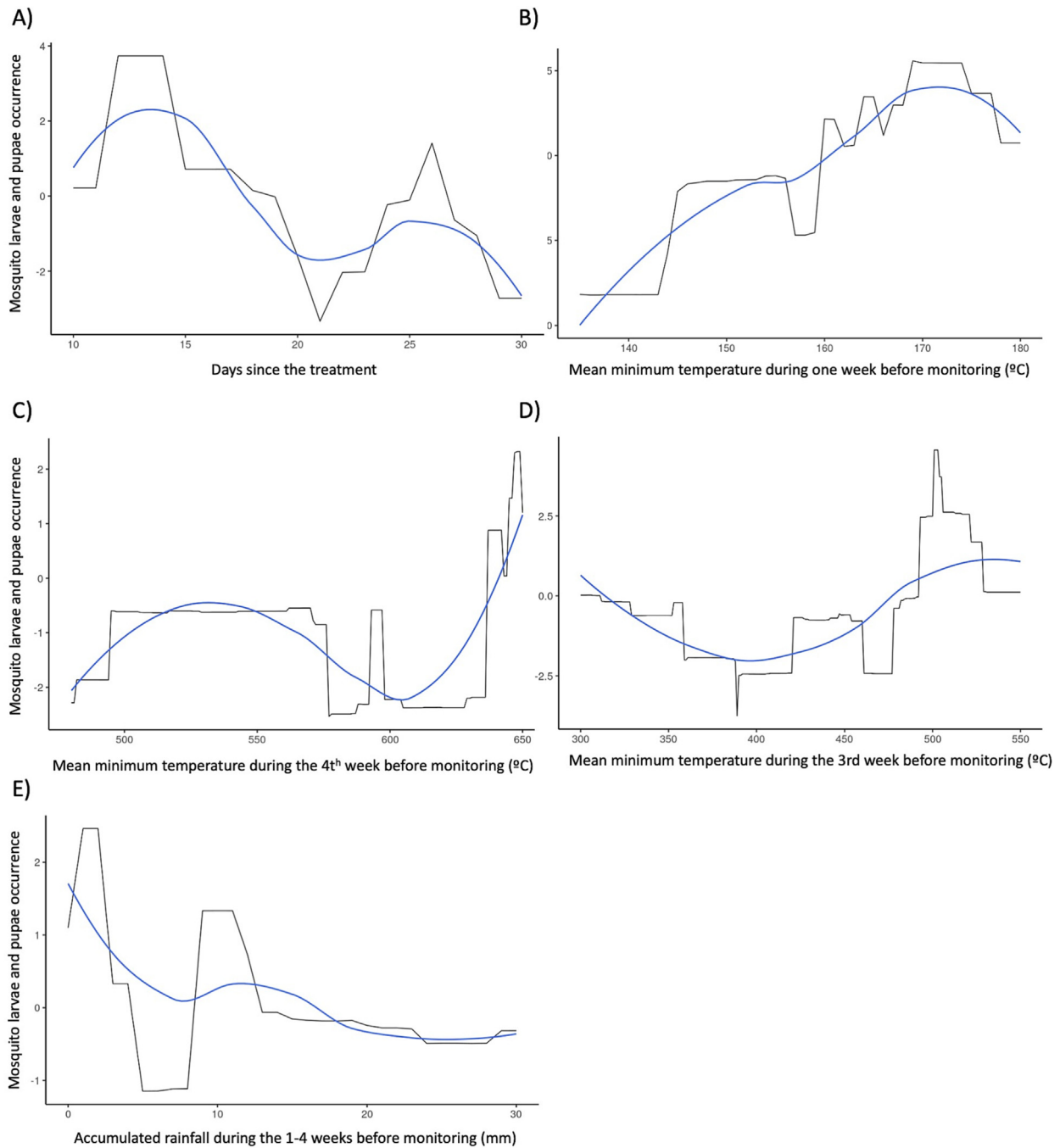
In the case of ornamental fountains, the district variable was the only included as a predictor in the colonization model. Among the districts, Horta Guinardo and Sants-Montjuic were found to have the highest likelihood of

larvae occurrence. Notably, the Sants-Montjuic district exhibited a greater number of fountains compared to other districts, as it is the most naturalized area of the city and includes the Montjuic mountain, which has natural



**Fig. 10.** Partial dependence plot (PDP) illustrating the relationship between mosquito occurrence in fountain colonization process and continuous variables. The examined variables include A) mean minimum temperature during one week before the monitoring, B) mean minimum temperature during the 4th week before the monitoring, and C) mean minimum temperature during the 3rd week before the monitoring. The PDPs show the relative logit contribution of each variable to the class probability as perceived by the model. The blue trend line in each PDP represents the smoothed relationship between the independent variable and the response variable, highlighting general patterns while reducing noise or variability in the data.

*Note:* Negative values on the y-axis indicate that the positive class is less likely for that value of the independent variable shown on the x-axis, according to the model. Similarly, positive values indicate that the positive class is more likely for that value of the independent variable. A value of zero implies that the independent variable has no average impact on the class probability, as predicted by the model.



**Fig. 11.** Partial dependence plot (PDP) illustrating the relationship between mosquito occurrence in fountain recolonization process and continuous variables. The examined variables include A) days since the treatment, B) mean minimum temperature during one week before the monitoring, C) mean minimum temperature during the 4th week before the monitoring, D) mean minimum temperature during the 3rd week before the monitoring, and E) accumulated rainfall during the 1–4 weeks before the monitoring. The PDPs show the relative logit contribution of each variable to the class probability as perceived by the model. The blue trend line in each PDP represents the smoothed relationship between the independent variable and the response variable, highlighting general patterns while reducing noise or variability in the data.

*Note:* Negative values on the y-axis indicate that the positive class is less likely for that value of the independent variable shown on the x-axis, according to the model. Similarly, positive values indicate that the positive class is more likely for that value of the independent variable. A value of zero implies that the independent variable has no average impact on the class probability, as predicted by the model.

values in terms of fauna and flora that led municipal managers to prioritize the location of a large number of naturalized fountains (around 50 %) in this area (Table 1, Fig. 2). Indeed, naturalized fountains, characterized by the absence of water recirculation and chlorination, present a higher risk for mosquito breeding. This can be attributed to difficulties associated with sustaining an ecological equilibrium in urban settings, where human activities and disturbances such as dogs bathing and children playing in the fountains hinder the maintenance of a balanced ecosystem.

Environmental characteristics of the surrounding areas, such as the presence of vegetation, could also have played an important role in insect colonization due to mosquito resting behavior (Becker et al., 2020). Vegetation favors mosquito larval development as it provides shelter and a source of organic matter and nutrients. Similarly, the importance of these variables has been investigated in other metropolises such as New York, Chicago, and Houston, where the presence of vegetation was positively associated with the human risk of mosquito-borne diseases (Brownstein et al., 2002;

Nolan et al., 2012). By contrast, environments with more vegetation and natural soils were poorly colonized by the same three mosquito species that we investigated, as shown by a study conducted in Lyon, France (Valdelfener et al., 2018). This aligns with the negative relationship between the presence of vegetation and the occurrence of mosquitoes in our study (Fig. 9B). Among the possible reasons explaining this relationship, in the case of naturalized fountains where no recirculation or chlorination of water occurs, the presence of fauna such as amphibians and heteropteran may act as natural predators against mosquito larvae. Similarly, ornamental fountains with water recirculation and chlorination create an environment where mosquito colonization becomes unfeasible. By considering these factors, we can better understand why the presence of vegetation and the specific characteristics of the fountains affect mosquito occurrence in our study. However, despite its importance, the predictive ability to identify and effectively manage growing vector populations remains limited at small spatial and temporal scales, although evidence from our study and other published research shows that colonization is heterogeneous at much finer scales (Harrigan et al., 2010; Honório et al., 2009; Kitron et al., 2006; Rochlin et al., 2011; Ruiz et al., 2007). To our knowledge, this is the first time that the greater need for control in naturalized and vegetated areas of ornamental fountains has been highlighted in urban areas.

Interannual differences were observed in both colonization and recolonization models, with a lower presence of mosquitoes in 2019. This pattern could be due to differences in breeding sites on private properties, affecting colonization and/or recolonization dynamics in public areas. Additionally, a higher level of surveillance and control was implemented in 2019 due to a large number of imported cases of arboviruses (i.e., dengue, chikungunya, and Zika infections) in Barcelona from May to November. More than 175 cases occurred (ASPB unpublished data), and intensive control and treatment were required in public and private areas to minimize the potential risk of transmission of imported arboviruses. Climatic conditions could also affect these interannual dynamics, although this fact was not explored in the study. In addition, intra-annual variation in the presence of mosquitoes have been found, with higher probabilities of occurrence during the summer months, although difference in mosquito abundance throughout the year may vary among species. However, different mosquito species have their own distinct biology, including variations in overwintering strategies and resistance to desiccation (Becker et al., 2020), which could impact the interpretation of our results, particularly in understanding recolonization dynamics and the effects of environmental factors on different species. Future studies should therefore consider the differential effects of variables in each of the mosquito species present to gain a more comprehensive understanding of their responses to management strategies. Nonetheless, given our focus on the most abundant species within the *Aedes*, *Culex*, and *Culiseta* genera, it is reasonable to assume that other closely related species would exhibit similar responses. As a result, our findings can be applicable to mosquito management and surveillance programs targeting these genera.

#### 4.2. Temporal dynamics of Bti treatment efficacy and implications for mosquito control strategies

The re-appearance of mosquito larvae in sewers was negatively influenced by the time interval between *Bti* treatment application and the subsequent visit (Fig. 5C). Conversely, the effectiveness of treatment in fountains appears to be lower compared to sewers, as mosquitoes in these structures showed a moderate likelihood of recolonizing the treated areas (Fig. 9C). This can likely be due to mosquitoes showing a greater propensity to colonize fountains that were previously occupied, probably associated with the dosage and distribution of the larvicide product, which may be influenced by the larger water volume of fountains compared to sewers.

The mosquito occurrence peaks identified by our predictive models indicate a temporary increase in mosquito larvae occurrence during specific time points, followed by a subsequent decline as more time elapses since the treatment. This trend could be attributed to the fact that visits to these areas were more frequent when mosquito presence was detected.

Previous studies using biocide have reported that factors including temperature, rainfall, and humidity may affect the efficacy of *Bti*-based control in the Mediterranean area (Jourdain et al., 2019; Proestos et al., 2015), and particularly in the studied area (Generalitat de Catalunya, 2016). The results of our predictive models show that treatments need some time to be effective and decrease in efficacy after approximately 20 days. These temporal patterns should be considered in future control strategies for mosquitoes, especially in these sewers that concentrate most mosquito larvae detections. Overall, our findings highlights the importance of optimizing the frequency of visits to different water infrastructures in order to enhance the effectiveness of control measures and better predict the colonization of mosquitoes.

#### 4.3. Climatic effects on mosquito occurrence and implications for vector control strategies

Climatic conditions affect the abundance of mosquito populations, and the rise in environmental temperature may increase the rate of development, productivity, and abundance of mosquito populations, thus affecting the transmission of the pathogens they vector (Chaves, 2016; Poh et al., 2019). Indeed, environmental temperature has usually been identified as a major driver of mosquito populations, probably related to its effects on the variation in development rate of mosquito larvae (Ewing et al., 2016). Together with precipitation, temperature has important consequences for mosquito productivity and abundance (Chuang et al., 2011).

Our results show that mosquito occurrence during both colonization and recolonization processes of sewers decreased with increasing overall values of minimum temperature during one and four weeks before the monitoring. These non-linear patterns with rainfall may reflect different processes affecting habitat quality in sewers. Intense rainfall immediately before sampling may produce a flushing effect in the breeding sites, washing out immature mosquitoes from the sewers (Benedum et al., 2018; Koenraadt and Harrington, 2008; Paaajmans et al., 2007). Similarly, while mosquito larvae and adult activity tend to increase with rising temperatures following the winter season, higher temperatures can lead to sewer desiccation, potentially reducing mosquito activity. The highest occurrence of larvae is typically observed from May to July but begins to decrease by August, likely due to habitat drying (Fig. 3). Previous studies have demonstrated the negative impact of habitat drying on container-dwelling mosquitoes, including species of the genera *Culex* and *Aedes* (Bradshaw and Holzappel, 1988; Juliano and Stoffregen, 1994; Sota and Mogi, 1992). Extreme temperatures and drying conditions may increase mortality rates of mosquitoes (Alto and Juliano, 2001), resulting in higher egg desiccation in *Culex* (Reeves et al., 1994) and *Aedes* (Mogi et al., 1996), and adversely affecting the habitat quality for larvae and pupae (Aspbury and Juliano, 1998).

In the case of ornamental fountains, mosquito occurrence showed more variable trends, although high mosquito occurrence was observed when temperatures reached higher values in the weeks preceding monitoring, likely due to the availability of water in these structures being independent of rainfall patterns.

Accumulated rainfall serves as an ecological indicator for identifying areas where mosquito populations can thrive, as it influences the availability of larval habitats. While water sources in urban areas may become available independent of rainfall, such as through street cleaning operations, rainfall can significantly increase the availability of habitats suitable for larval development. This relationship has been proposed as a limiting factor in Mediterranean regions, including Spain, due to the seasonality of mosquitoes (Eritja et al., 2005). In both colonization and re-colonization models, it was observed that the occurrence of mosquito larvae and pupae in sewers and urban fountains increased non-linearly with rainfall in the one to four weeks prior to the inspector visit, until reaching a threshold beyond which it sharply declined. This dynamic can be explained by the initial increase in larvae following the first rains, which may activate the hatching of existing clutches or create new breeding habitats for surviving adults. However, heavy rainfall can also have a flushing effect, reducing mosquito presence by expelling larvae from their habitats (Benedum et al., 2018;

Koenraadt and Harrington, 2008; Paaijmans et al., 2007). In addition, excess simulated rainfall has been found to negatively affect the retention of *Ae. albopictus* larvae and pupae, particularly in small habitats (Dieng et al., 2012). It is noteworthy that the effects of precipitation on mosquito populations should be considered in future vector management plans, especially in the context of climate change and the associated risks of pathogen transmission, such as dengue (Benedum et al., 2018) or malaria incidence (Wu et al., 2017).

Our findings have implications for optimizing vector control strategies, emphasizing the importance of considering climatic characteristics in coordinating management measures and control protocols. Surveillance efforts, therefore, should be intensified during summer, particularly from during May to August. By targeting these periods, resources and monitoring efforts can be optimized to reduce mosquito vector populations more effectively. This approach can contribute to more efficient and targeted interventions in mosquito control programs.

## 5. Conclusion

Mosquito monitoring programs, such as the one conducted in Barcelona, provide valuable insights into the breeding requirements and activity patterns of mosquito species, including invasive ones. Our findings demonstrate the importance of accurate monitoring during vector management operations, as it enables adaptive improvements to mosquito surveillance and control programs. Through the surveillance of mosquito larvae, we were able to identify the areas of the city most affected by mosquitoes. By understanding the relationship between larvae presence and environmental, management, and vector-related factors, we can enhance measures aimed at reduce or eradicating vector activity, if necessary. The mosquito surveillance program in Barcelona relies on effective coordination among ecologists, epidemiologists, entomologists, and technical experts in water management to mitigate the local risk of mosquito-borne diseases in a city with strong connections to arbovirus-endemic countries. Additionally, the support of the community is instrumental in reducing the incidence of mosquito-borne diseases in private areas. The findings of this study have led to the initiation of a program by the ASPB to convert all sandboxes into direct scuppers. This decision is based on the results of a pilot study (Montalvo et al., 2022), which demonstrated that the addition of a concrete layer at the bottom of scuppers effectively prevents water accumulation and mosquito breeding. The implementation of this program is expected to have a significant impact on reducing mosquito populations in the affected areas. Additionally, our study has provided valuable insights into the tendency of mosquitoes to breed in specific scuppers, enabling the prioritization of scuppers for modification and an increase in the frequency of biocide application. These targeted measures will further contribute to mosquito control efforts and enhance the effectiveness of the management program. A close collaboration between public health officials and city managers responsible for maintaining and constructing naturalized sources can play a crucial role in preventing the proliferation of mosquitoes. By implementing building and management criteria aimed at reducing mosquito breeding, these stakeholders can effectively mitigate mosquito populations. The information collected during the control program becomes instrumental in modifying and adapting surveillance and control strategies to maximize their effectiveness while minimizing economic costs. This approach allows management practices to generate valuable scientific data that can be utilized to continuously improve mosquito management strategies. By integrating scientific knowledge with practical management efforts, it is possible to achieve more efficient and sustainable mosquito control outcomes.

## Credit authorship contribution statement

**J.F.:** Conceptualization, Data curation, Writing- Reviewing and Editing, Supervision. **M.F.:** Conceptualization, Data curation and analysis, Software, Writing- Original draft preparation. Writing- Reviewing and Editing. **J.M.P.:** Conceptualization, Supervision, Writing- Reviewing and Editing. **S.B.:** Data curation. **J.P.M.:** Writing- Reviewing and Editing. **C.R.:** Project

administration, Data curation. **A.V.:** Data acquisition. **T.M.:** Conceptualization, Project administration, Data acquisition and Curation, Supervision, Writing- Reviewing and Editing.

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## Data availability

Data will be made available on request.

## 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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## Ethics

This work involved no human subjects.

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