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Wageningen Academic Publishers

Journal of the European Mosquito Control Association, 2022; 40(01): 23-35

The first 6 years of surveillance of *Aedes albopictus* (Diptera: Culicidae) in Gibraltar

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Received: 18 February 2022 / Accepted: 7 June 2022

RESEARCH ARTICLE

Abstract

Following the expansion of the invasive mosquito *Aedes albopictus* (Diptera: Culicidae) through Spain along the Mediterranean coast, mosquito surveillance was established in Gibraltar in 2016. This paper reports on the first detection of *Ae. albopictus* in Gibraltar in 2017 as well as subsequent efforts to monitor the establishment of the species, including longitudinal data over several years since 2018, and results of a snapshot survey in 2021 on adult mosquito density. *Aedes albopictus* has become established across most of Gibraltar, with defined seasonality from August to October, slightly later than the peak in *Culex pipiens* densities. The larval habitats for *Ae. albopictus* remain largely enigmatic. Mosquito samples tested for chikungunya and West Nile virus were all found to be negative, and this paper includes recommendations for future control efforts and infectious disease risk assessment.

Keywords: *Aedes albopictus*, Gibraltar, Dengue, surveillance, *Culex pipiens*

1. Introduction

Aedes (Stegomyia) albopictus (Skuse, 1894), the Asian tiger mosquito, is an important invasive mosquito species that has rapidly colonised Europe over the last 30 years and has now been reported in 28 European countries (Medlock *et al.*, 2012; Osório *et al.*, 2018; Schaffner *et al.*, 2013). In recent years it has expanded in the Iberian Peninsula, including along the eastern Spanish coast and in the Algarve, Portugal (Obregon *et al.*, 2019; Osório *et al.*, 2020). Where the mosquito has established in Europe it has been responsible for the autochthonous transmission of three arboviruses important for public health: dengue, chikungunya and Zika viruses (Angelini *et al.*, 2007; ECDC, 2017, 2018, 2019a,b; Schaffner *et al.*, 2013).

In 2016, surveys as part of the VectorNet project (funded by ECDC/EFSA), were conducted by some of the authors (AGCV, FS, JMM) along the coast west from Malaga towards Gibraltar. During this work, the species was found at a cemetery in Marbella. Other studies reported *Ae. albopictus* also in Algeciras in 2015 (Collantes *et al.*, 2016), and at that time these were considered the most southwesterly records with no evidence of *Ae. albopictus* either in Gibraltar (Lucientes and Molina, 2017). At the same time, in 2016, collaboration was established between VectorNet experts, entomologists from the UK government and representatives of the Environmental Agency Gibraltar and the Gibraltar Botanic Gardens. The aims were to initiate surveillance for *Ae. albopictus* in Gibraltar, in order to monitor the presence or absence of this species, to provide evidence for public health disease risk assessments and to initiate virus screening in captured mosquitoes.

Where *Ae. albopictus* has established, there remains the risk of local transmission of viruses. This is as a result of returning infected travellers from other regions where the viruses are circulating. The first outbreak of chikungunya virus in Europe caused ~330 cases in Italy in 2007, vectored by *Ae. albopictus* which had started to colonise Italy from 1990 (Angelini *et al.*, 2007). At around the same time, *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) had also colonised the island of Madeira and was responsible for around 2,000 autochthonous cases of dengue by 2012 (Sousa *et al.*, 2012). Since these two outbreaks, there has been a great deal of recognition among public health officials that the concerns raised by entomologists over many years were correct. *Ae. albopictus* can rapidly establish itself in new territories and build populations capable of transmitting introduced viruses that were, until recently, confined to tropical regions. Since these two outbreaks there have been small clusters of cases of chikungunya in Croatia and France (from 2010 onwards), but also ~500 cases of chikungunya in Italy and 17 cases in France in 2017 (Delisle *et al.*, 2015; ECDC, 2017, 2018, 2019a,b; Marchand *et al.*, 2013; Nabet *et al.*, 2020). Although there was an epidemic of Zika virus in the Americas during 2015-2016 predominantly transmitted by *Ae. aegypti*, there were no local cases of Zika virus in Europe transmitted by *Ae. albopictus* at that time. However, in 2019, three autochthonous cases were reported for the first time in France, implicating European *Ae. albopictus* in local transmission (Giron *et al.*, 2019). In addition to the dengue outbreak in Madeira there have repeatedly been small clusters of locally acquired cases in France, and in 2018 four cases were reported in Spain, and therefore increasing concern for local transmission within Gibraltar should *Ae. albopictus* establish (ECDC, 2018, 2019a).

The impacts of this mosquito for Europe are significant, both for the public health burden of human case surveillance and treatment, the economic impact of morbidity on populations, and the cost of mosquito control to reduce the risk of virus transmission. Gibraltar, given its small size, geography, hydrology, and close community links, has the potential to be an ideal location to conduct an effective control strategy, focussing on source reduction and community engagement thereby minimising mosquito populations and the risk of virus transmission. Responding to and mitigating disease is contingent on data relating to mosquito distribution and abundance, and even limited surveillance on distribution, abundance and seasonality of *Ae. albopictus* can vastly enhance the efficacy of control efforts.

2. Methods

Gibraltar is a British Overseas Territory, located at the southern tip of the Iberian Peninsula, bordered by Spain to the north, and elsewhere by the Mediterranean Sea. It is a small territory, with an area of 6.7 sq. kms, and a population of 32,000 people. Much of the population lives in the densely populated town (36º8'N, 5º21'W) on the west side of the Rock of Gibraltar; one of the most densely populated areas in the world with ~5,000 people per sq. km. Gibraltar has a Mediterranean climate with mild, rainy winters and warm, dry summers. Average mean temperatures reach ~28 ºC in July and August, dropping to ~16 ºC in January. Rainfall is more common during the months October through to May, with hardly any rain from June to August. December is the wettest month (mean: ~170 mm). In the absence of rivers, streams or large bodies of water, historically water supply was provided by rainwater capture into cisterns, as well as the use of wells, water catchments, and reservoirs in the Rock's caverns. Nowadays, water is provided by desalination, with a separate saltwater system provided for sanitary purposes. The geology, like much of the Mediterranean is early Jurassic limestone, and monolithic promontory of the Rock dominates the territory to a height of 426 m.a.s.l. Apart from birds, many of which migrate through Gibraltar, there are very few wild land mammals, except the Barbary macaque (*Macaca sylvanus*); the only wild apes or monkeys found in Europe.

3. Surveillance strategies

In order to establish whether *Ae. albopictus* occurs in Gibraltar, and to gather data on its subsequent distribution, density and seasonality, a number of different strategies were adopted. This included gathering data from existing insect monitoring programmes, initiating active surveillance using a range of mosquito traps both in *ad hoc* locations and for long-term monitoring, establishing passive surveillance through a citizen science project on mosquito nuisance, and targeted enhanced surveillance to monitor species densities.

Nuisance biting reports

All complaints to Environmental Agency Gibraltar from the public, mostly by phone, are logged in a database and referred to environmental health officers (EHO) for that district who returns their call. The database records date, time, address and other relevant information. In respect of complaints referring to bites or stings, all EHOs have been trained to try and differentiate the nature of the bite, either bed bug, flea, tick or mosquito. This includes questions on time of day/night when they are bitten, location of bite, and their location when bitten; also if they saw the offending insect. A follow up visit by the EHO may take place, or a pest controller, if warranted. Only those that meet the typical criteria for *Ae. albopictus* are counted for these purposes. Calls are usually more common at the start of the season.

Passive surveillance and citizen science

Initial monitoring of mosquitoes in Gibraltar was confined to ongoing lepidoptera trapping using a Rothamsted light trap (RLT). This was supplemented by the establishment of a passive scheme for reporting nuisance biting mosquitoes to Environmental Agency in Gibraltar (see above), with all samples submitted to entomologists at the Gibraltar Botanic Gardens (GBG) for species identification using morphological keys (Becker *et al.*, 2010; Schaffner *et al.*, 2001). All data was recorded in an excel spreadsheet held as a shared file for all partners of the project. This database captured details of location name, sampling/report address, latitude/longitude, reporter, year, date, trap type, positive/negative sample, presence and numbers of eggs, larvae, pupae, ♂ or ♀ mosquitoes, species name, land use, host, identifier and notes. Data was later added by environmental health colleagues in the British Forces base in Gibraltar. This database has been maintained from 2016 through to current day (late 2021). Confirmed identifications of nuisance reports were used to populate a map of confirmed siting of *Ae. albopictus*.

Long-term and ad hoc adult mosquito monitoring

In addition to passive surveillance, a range of other traps were employed, including Mosquito Magnet® Executive mosquito traps (MosquitoMagnet, Lititz, PA, USA) baited with octenol (MM), BG-Sentinel 2® trap (BGS; Biogents, Regensburg, Germany), BG-Gravid *Aedes* Traps® (GAT; Biogents, Regensburg, Germany) and ovitraps. Ovitraps were constructed using black plastic pots (Ramona, 11 cm in width, 9 cm in height (Luwasa®, Interhydro AG, Allmendingen, Switzerland), containing water and with a polystyrene block (5×5×5 cm) provided for oviposition. Mostly these were deployed *ad hoc* in response to nuisance issues or in areas where *Ae. albopictus* was being reported. At GBG a MM was set up in May 2018 and run almost continuously to this day (late 2021) and checked periodically (every 1-2 weeks). All mosquitoes from all traps were identified by entomologists at GBG using morphological keys (Becker *et al.*, 2010; Schaffner *et al.*, 2001). Data generated from *ad hoc* trapping, as part of nuisance biting follow up studies, were used to supplement existing maps on the distribution of *Ae. albopictus*. Additional data on other mosquito species were also recorded. Longitudinal data from the MM at GBG was used to understand seasonality of both *Ae. albopictus* and *Culex (Culex) pipiens* Linnaeus, 1758 to assess biting periods and monitoring in density of mosquito species over time.

Enhanced mosquito trapping for comparable density measures

To better understand the comparative density of *Ae. albopictus* and *Cx. pipiens* across Gibraltar, a programme of enhanced surveillance was conducted during August and September 2021 at 21 sampling locations across the territory. At each site, BGS traps were deployed for 10-13 nights (although 3 traps remained in use for 33-34 nights). Where possible BGS traps were plugged into a mains electric source, otherwise they were run using a 12-volt 18Ah sealed lead acid battery. Each trap was baited with BG-Lure®, but no carbon dioxide. Traps were checked every two days with mosquitoes returned to the laboratory and identified by UKHSA and Wildlife Gibraltar entomologists at the GBG.

Testing for chikungunya virus (CHIKV)

Ninety-four pools of 10 *Ae. albopictus* were tested. The analysis of *Ae. albopictus* for the presence of CHIKV was performed using a primer/probe set targeting the E1 gene of CHIKV (Edwards *et al.*, 2007). Ten *Ae. albopictus* per Precellys tube (MK28, Labtech International, Heathfield, UK) were resuspended in 500 µl of RLT buffer + β-mercaptoethanol and homogenised (Precellys 24 High-Powered Bead Mill Homogenizer, Labtech International, Heathfield, UK) using 3×20 s bursts of 5000 rpm. After addition of 500 µl of 70% ethanol the samples were centrifuged at 5,000 rcf for 1 min and the supernatant processed through a QIAshredder (79656, Qiagen, Manchester, UK) at 16,000 rcf for 2 mins. Total RNA was extracted using the Kingfisher Flex Purification system (ThermoFisher Scientific Cat. No. 5400610) and the BioSprint 96 One-For-All Vet extraction kit, (Cat. No. 947057) in accordance with the manufacturer's instructions. RNA was eluted in 100 µl AVE buffer.

A synthetic RNA transcript covering a 127 bp region of the E1 gene from the CHIKV S27 prototype strain (National Collection of Pathogenic Viruses) was used as a positive control and for quantification (Edwards *et al.*, 2007). RT-PCR was performed using the Invitrogen SuperScript III Platinum One-Step qRT-PCR Kit (11732 088, Thermo Fisher Scientific, Hemel Hempstead, UK) on the QuantStudio 7 Flex Real-Time PCR platform, using standard cycling conditions for SSIII.

Testing for West Nile virus (WNV)

The same 94 pools of 10 *Ae. albopictus* were tested for WNV. For WNV detection, 500 µl of RLT (plus β-Mercaptoethanol) was added to each precellys tube (containing 10× *Ae. albopictus*) and samples homogenised using Precellys Tissue Homogenizer: 3× (20 seconds at 5,000 rpm). 500 µl of 70% ethanol was added to each tube and samples inverted 5× to mix. Tubes were centrifuged for 1 min at 5,000 rcf to pellet the contents and 700 µl of supernatant transferred to a QiaShredder and centrifuged at full speed (16,000 RCF) for 2 minutes. Supernatant was transferred to a KingFisher Flex S-block for RNA extraction, RNA eluted in 100 µl AVE buffer. Samples were analysed by qRT-PCR using the TaqMan™ Fast Virus One-Step Master Mix (# 4444436) with primers and probes targeting the NS5 gene of WNV (Hadfield *et al.*, 2001) (Hadfield *et al.*, 2001) and a 5 fold serial dilution of WNV synthetic RNA (4.0×10e5 to 25.6 copies μl^{-1}).

4. Results

Initial surveys 2016-2017

2016 Surveillance data

Mosquito specimens recorded in traps were first reported in Gibraltar in May 2016. Mosquito trapping was largely conducted as incidental trappings in the RLT at the GBG as part of routine lepidoptera trapping. This was supplemented from June 2016 by the initiation of GAT and BGS traps, as part of a visit by entomologists as part of a VectorNet project to build capacity for mosquito surveillance.

During the remaining part of 2016 four different species were trapped: *Aedes (Ochlerotatus) caspius* (Pallas, 1771), *Culex (Culex) laticinctus* Edwards, 1913, *Cx. pipiens* and *Culiseta (Allotheobaldia) longiareolata* (Macquart, 1838). The latter two species dominated the catch, with only 2 ♂ *Ae. caspius* trapped in early June in the RLT, and 13 larvae, 9♂ and 1♀ *Cx. laticinctus* collected/trapped around the animal enclosures through larval dipping in the wildlife park of the GBG.

During June to December 2016, 44♂ and 27♀ *Cx. pipiens* and 32♂ and 38♀ *Cs. longiareolata* were trapped. *Cx. pipiens* were most common during June (15♂ 7♀), July (10♂, 4♀) and August (16♂, 15♀), with only small numbers (1 or 2) trapped each month during the rest of the season. The majority of mosquitoes were mostly collected at the GBG in the RLT, but also using the sentinel and GAT traps. Elsewhere, *Cx. pipiens* was trapped in GAT traps at Four Corners. Almost all the trapped *Cs. longiareolata* were in the RLT at the GBG with the highest numbers in June (18♂ 24♀) and July (8♂ 6♀). Larval dipping using standard dippers in buckets, basins and barrels during 14-15 June yielded 353 larvae and 50 pupae, of which 13 larvae of *Cx. laticinctus*, 124 larvae and 22 pupae of *Cx. pipiens*, and 216 larvae and 28 pupae of *Cs. longiareolata*. There were no records of *Ae. albopictus* during larval or adult trapping.

2017 Surveillance data

The first record of *Ae. albopictus* was registered on 4th August 2017, as a ♀ flying over a patio in the Naval Hospital Hill area (36.122, -5.349). Throughout the rest of 2017, a further 2♂ and 83♀ *Ae. albopictus* were recorded, including 40♀ during August, collected in traps at the site of the first discovery, using various traps and methods: GAT (n=13), BGS (n=24) and human landing catches (n=6), and ovitraps of which one was found positive (>40 eggs). During the same month (August 2017), 1-2 ♀ *Ae. albopictus* were also recorded on the wing at the GBG, Vineyards area (36.122, -5.210), Trafalgar cemetery (36.151N, -5.352), and Stanley buildings (36.138, -5.351). *Aedes albopictus* continued to be active through September (2♂ 30♀) with specimens trapped in GAT traps at the GBG and Greenarc, including positive ovitraps (>100 eggs) at Greenarc and detection of 13 larvae and 4 pupae in bromeliads (*Bromeliaceae* spp.) in the GBG. During the following months, there were two further positive ovitraps in mid-October at Greenarc (30 and 36 eggs, respectively) and 3♀ in a GAT at Vineyards in early October. In December (5th), 4♀ were trapped in a BGS trap in Commonwealth Park. By the end of 2017, *Ae. albopictus* was evidently present, and possibly established in an area from the Naval Hospital Hill, Vineyards, the GBG, Trafalgar cemetery, Greenarc and Commonwealth Park.

Three other species continued to be recorded. This included 60♂ and 79♀ *Cs. longiareolata* caught mostly in RLT at the GBG, recorded from January through to October, peaking in May (17♂, 20♀) and June (27♂, 29♀); 78♂ and 85♀ *Cx. pipiens* with the majority of ♂ collected in the RLT and majority of ♀ in BGS traps. Notably, during June and July 2017, BGS traps caught *Cx. pipiens* but no *Ae. albopictus*. *Cx. pipiens* numbers peaked in June and September, and small numbers (5♂, 2♀) of *Ae. caspius* were detected in RLT during July-October.

Enhanced trapping and long-term surveillance 2018-2021

During the following four years, increased reports of *Ae. albopictus* and *Cx. pipiens* were submitted along with *ad hoc* data on trapping and nuisance reports. A map of the earliest reports of *Ae. albopictus* is shown in Figure 1.

Aedes albopictus *long-term surveillance*

From May 2018 until the present day (end-August 2021) a MM trap has been running almost continuously at the GBG. Figure 2 shows the number of ♂ and ♀ *Ae. albopictus* collected by month. The traps were emptied roughly every week, and where the week crossed over two months, mosquito numbers have been attributed to the month with more nights (so numbers are rough estimates of mosquito abundance). In total 3,689 ♂ and 6,593 ♀ *Ae. albopictus* were trapped over the survey period. Peak months were July through to October, with minimal winter activity, although *Ae. albopictus* was recorded in every month of the year. Densities increased progressively from 2018 through to 2020, with almost 800 ♀ *Ae. albopictus* trapped during August 2020, possibly showing a clear increase in densities over time, as *Ae. albopictus* establishes. Over the four-year survey, the highest monthly mean densities of ♀ *Ae. albopictus* were in August (n=486 mosquitoes/month), September (n=390) and October (n=248). Lower mean numbers were caught in July (n=197), November (n=110), June (n=102), May (n=75), April and December (both n=24). The increase from July to August shows a 150% increase in densities, with a defined season of *Ae. albopictus* activity during the warmest months.

Culex pipiens *long-term surveillance (2018-2021)*

In total, 2,127 ♀ and 103 ♂ were recorded during the trapping period. The numbers of mosquitoes collected each month in the MM trap at the GBG is shown in Figure 3. Over the period of sampling there has been a general downward trend over time, with densities notably decreasing from peak densities of 224 ♀ *Cx. pipiens*/month in July 2018, to 104 in 2019, 16 in 2020 and 47 in 2021 (Figure 3). This data suggests that there is a less distinct seasonality during the last two years of survey. Combining these four years of data to generate mean densities of ♀ mosquitoes per month (Figure 4) it is clear that *Cx. pipiens* remains active during the winter (132 ♀*Cx. pipiens* collected in January 2019), with the main activity season from April through to a peak in July, with a decline in *Cx. pipiens* thereafter.

Comparison of Ae. albopictus *and* Cx. pipiens *data 2018-2021*

Considering the seasonality of the two species over the four years surveyed, the timing of the decline in *Cx. pipiens* numbers appears to occur just prior to the increase in *Ae. albopictus* densities (Figure 4). By comparing the data over time (Figure 5), the decline in *Cx. pipiens* densities seems to coincide with the increase in density of *Ae. albopictus*, with again the notable peaks in *Cx. pipiens* occurring during the periods of lower *Ae. albopictus* activity. During the 2018 and 2019 seasons, 59-63% of combined catches were *Ae. albopictus*, during 2020 and 2021, this had increased to 85-90%. However, this may reflect solely an increase in *Ae. albopictus* densities, without any change in *Cx. pipiens* densities. By comparing *Cx. pipiens* densities in 2020 to 2019, densities were only a third in 2020.

2018-2021 Other mosquito species

For the remaining four years, records of *Ae. caspius* and *Cs. longiareolata* were found every year (Table 1).

Additional results from larval sampling and ovitrapping 2018-2019

Very few *Ae. albopictus* larvae have been detected during larval sampling of container or natural habitats. In fact, larvae have only been reported on six occasions, three of them in bromeliads, and once each in a birdbath, a water butt and in stagnant water behind a water feature. *Aedes albopictus* larval sites remain extremely elusive in Gibraltar.

Ovitrapping has not been routine in Gibraltar over the last six years, however it is useful to report on data collected so far. Initial data from ovitraps during 2017 have been reported above. During 2018, ovitraps were at the GBG (317 eggs from 8 traps) and at GreenArc (200 eggs from 1 trap). Ovitrapping in 2019 was more extensive, with trapping at 7 sites between June-October, yielding 26 positive ovitrapping samples (5,392 eggs).

2021 enhanced surveillance data

During August/ early September 2021 BGS traps were run at 21 locations for at least 10-12 nights, although for three locations traps were run for longer, up to 34 nights (Table 2). In total >1,500 mosquitoes were trapped during the study, including *Ae. albopictus* (359 ♀, 205 ♂), *Cx. pipiens* (627 ♀, 214 ♂) and *Cs. longiareolata* (27 ♀, 4 ♂). To ensure comparability, densities of mosquitoes trapped by site were calculated as mosquitoes/trap night (TN).

From the 21 trapping locations, ♀ *Ae. albopictus* was absent from four of the locations, although two of these sites had small numbers of ♂ *Ae. albopictus* (Figure 6). These include two sites in the central area of Gibraltar (site 5, 11), including one that previously had records of *Ae. albopictus* since 2017 but had been subject to targeted mosquito control and source reduction. The remaining two sites included site 14 in the northern part of Gibraltar close to the airport, and site 9, which is isolated in the south at a slightly higher altitude to other locations.

Comparing relative densities (Figure 7) across the species. *Ae. albopictus* remains at very low densities in the north and northwest areas of Gibraltar, although densities of *Cx. pipiens* here remain comparable with other locations across the territory. Towards the southern tip, where *Ae. albopictus* is at low density, *Cx. pipiens* is also at low density. The main habitats for *Ae. albopictus* remain in the old town, the main parks and a few isolated locations on the south-east coast of the rock.

During the enhanced survey, *Cx. pipiens* was more common than *Ae. albopictus*. Plotting density of each species per trap night by location (Figure 8) there is no evidence to suggest that the presence of higher densities of one species is associated with a decline in the other, except in three locations: North Front Cemetery, the Hospital

Table 1. Density of other mosquito species trapped in the Mosquito Magnet trap at the Gibraltar Botanic Gardens, Gibraltar, 2016-2021.

Year	*Ae. caspius*		*Cs. longiareolata*	
	#♀	#♂	#♀	#♂
2016	0	2	38	32
2017	2	5	79	66
2018	0	0	8	5
2019	117	0	9	11
2020	79	1	3	3
2021	6	2	18	15

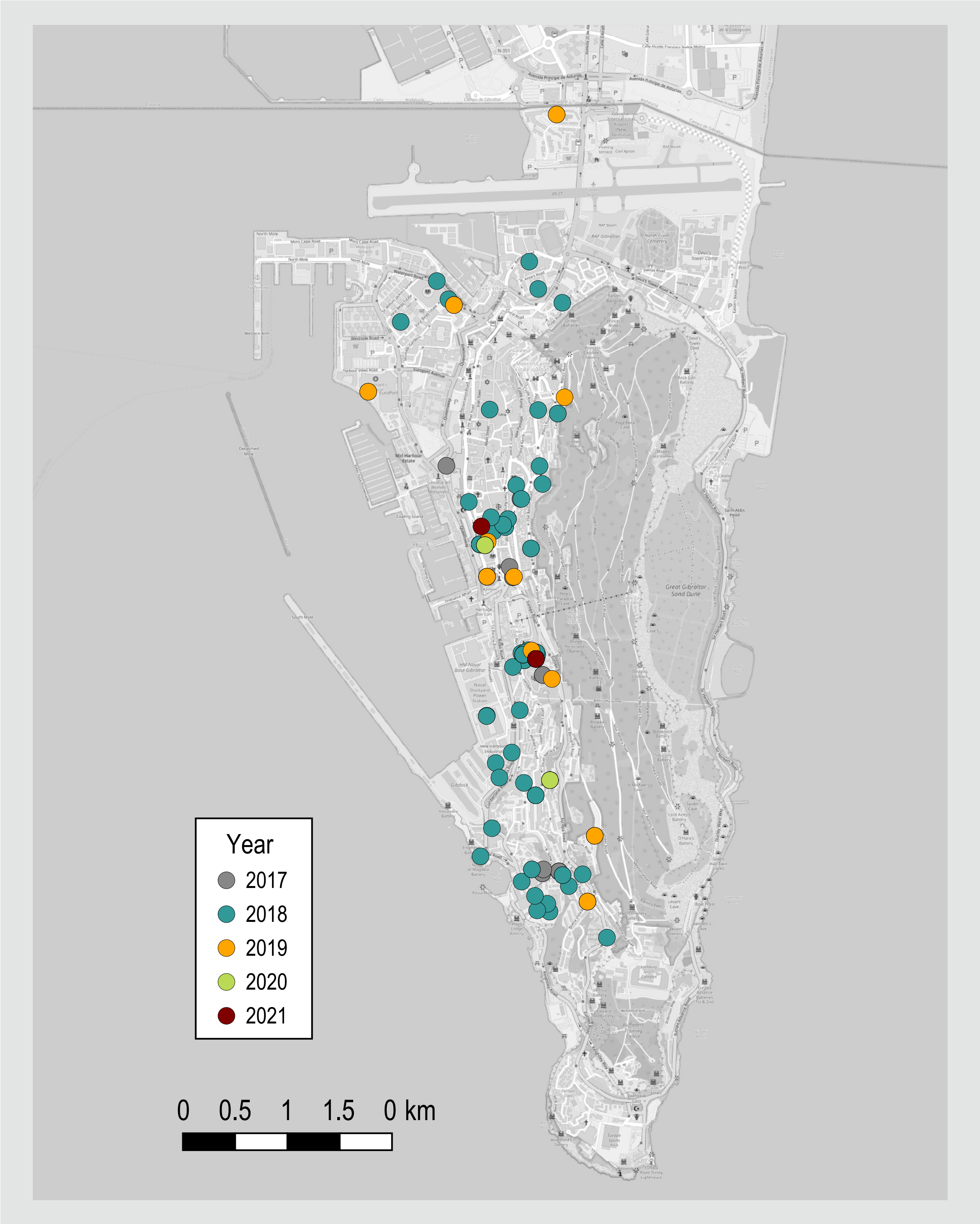

Figure 1. Locations of first reports of *Aedes albopictus* in Gibraltar, 2017-2021. Only the first report is shown where there are multiple reports for the same location over more than one year.

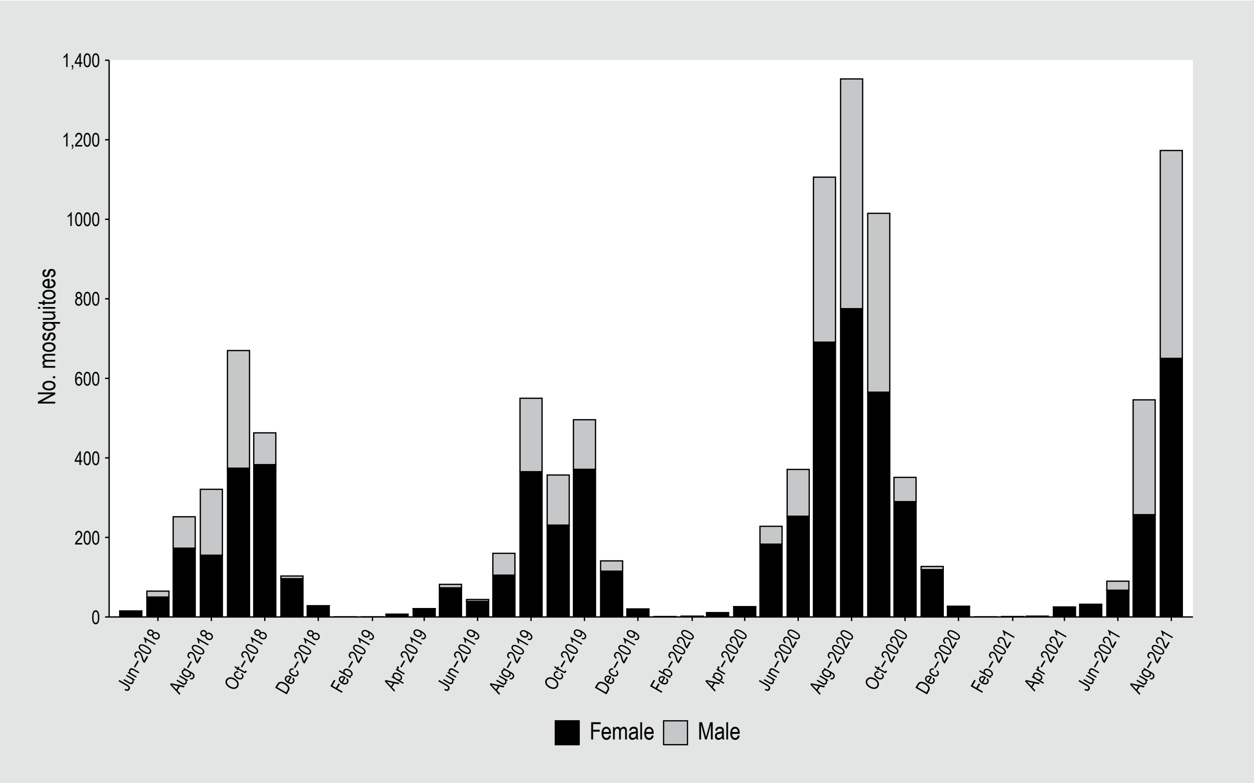

Figure 2. Seasonality of *Aedes albopictus* in Gibraltar based upon mosquito trapping (one Mosquito Magnet trap) at the Gibraltar Botanic Gardens, Gibraltar, May 2018 – August 2021.

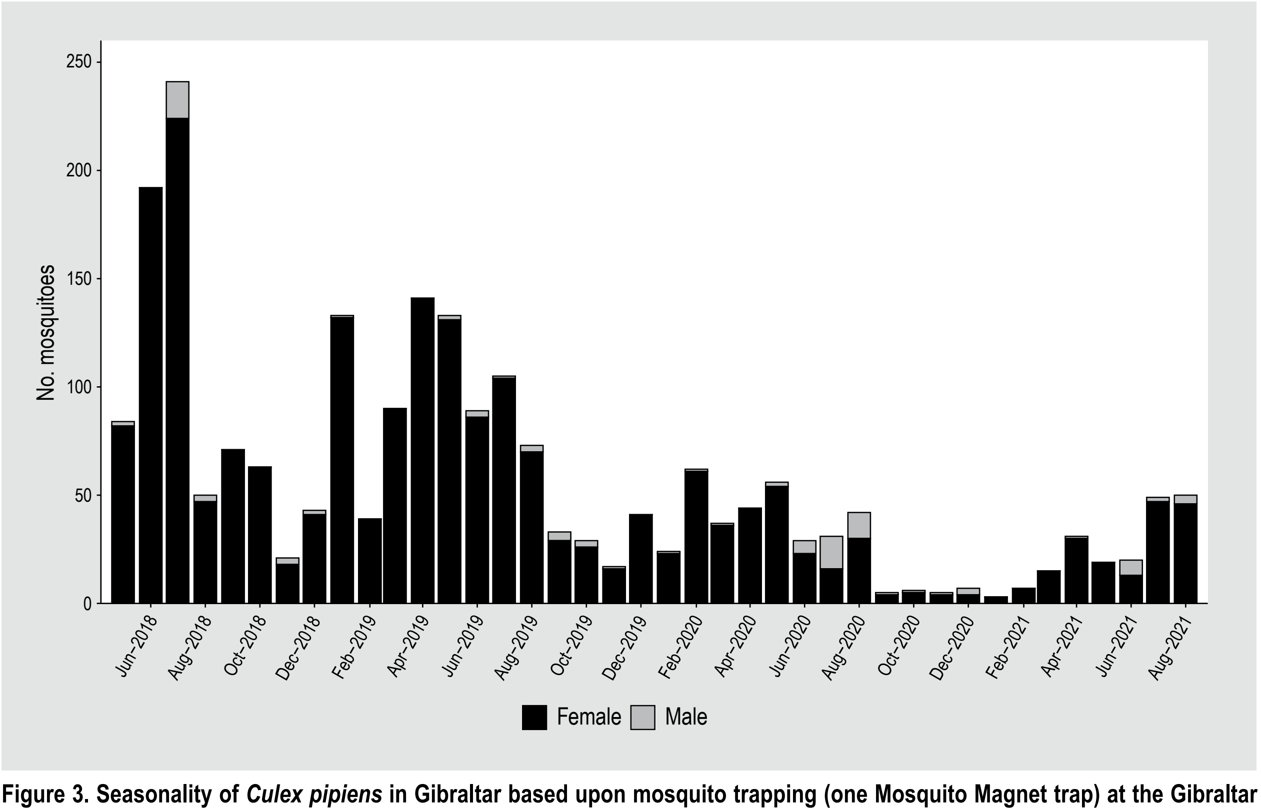

Figure 3. Seasonality of *Culex pipiens* in Gibraltar based upon mosquito trapping (one Mosquito Magnet trap) at the Gibraltar Botanic Gardens, Gibraltar, May 2018 – August 2021.

Table 2. Results of enhanced mosquito surveillance using BG-Sentinel 2 traps during August/September 2021 at 21 locations across Gibraltar.

No.	Location	Latitude	Longitude	Nights survey	Main (M)/ Battery (B)	*Aedes albopictus*/TN		*Culex pipiens*/TN		*Culiseta longiareolata*/TN	
						♀	♂	♀	♂	♀	♂
1	Caleta hotel	36.138	-5.341	13	M	3	1	5	10	0	0
2	Monteverde yard	36.141	-5.341	8	B	16	8	10	24	0	0
3	St. Bernard's Hospital	36.142	-5.360	11	B	8	17	68	28	0	0
4	Commonwealth Park	36.140	-5.356	34	M	44	36	30	17	0	0
5	Emile hostel	36.145	-5.354	11	B	0	0	18	0	1	0
6	Garrison library	36.139	-5.352	11	B	20	8	25	42	10	4
7	Gibraltar Botanic Gardens	36.131	-5.352	13	M	48	9	68	8	1	0
8	Michael Dobinson way	36.117	-5.343	11	B	55	1	48	26	6	0
9	Commander British Forces Gibraltar	36.123	-5.348	12	B	0	6	10	0	0	0
10	Castle Road	36.141	-5.351	11	B	29	23	27	26	0	0
11	Bella vista	36.117	-5.349	11	B	14	10	7	9	0	0
12	The Mount	36.125	-5.349	10	M	36	10	105	1	46	0
13	St. Theresa's church	36.148	-5.346	12	B	2	0	10	15	0	0
14	North Front Cemetery	36.149	-5.344	12	B	0	0	41	9	0	0
15	Trafalgar Cemetery	36.134	-5.352	11	B	43	57	42	67	0	0
16	Aquagib	36.142	-5.349	34	M	15	9	6	2	3	0
17	Green arc	36.136	-5.353	11	B	0	1	19	0	0	0
18	Whitham's air station	36.128	-5.351	12	M	10	8	11	0	1	0
19	University of Gibraltar	36.110	-5.345	12	M	2	0	13	6	0	0
20	GibralFlora	36.146	-5.356	33	M	11	1	61	44	0	0
21	Bleak House	36.113	-5.350	12	M	3	0	3	0	1	0

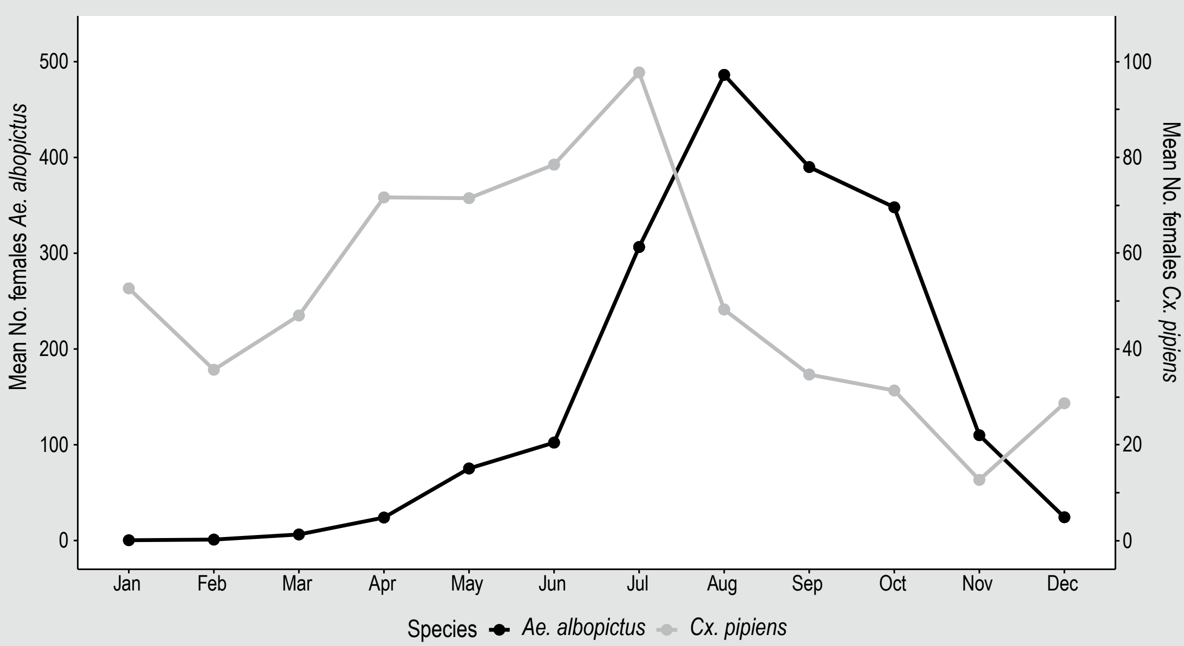

Figure 4. Mean densities of *Aedes albopictus* (y-axis 1) and *Culex pipiens* (y-axis 2) trapped in Gibraltar, depicting typical phenology and abundance during 2018-2021.

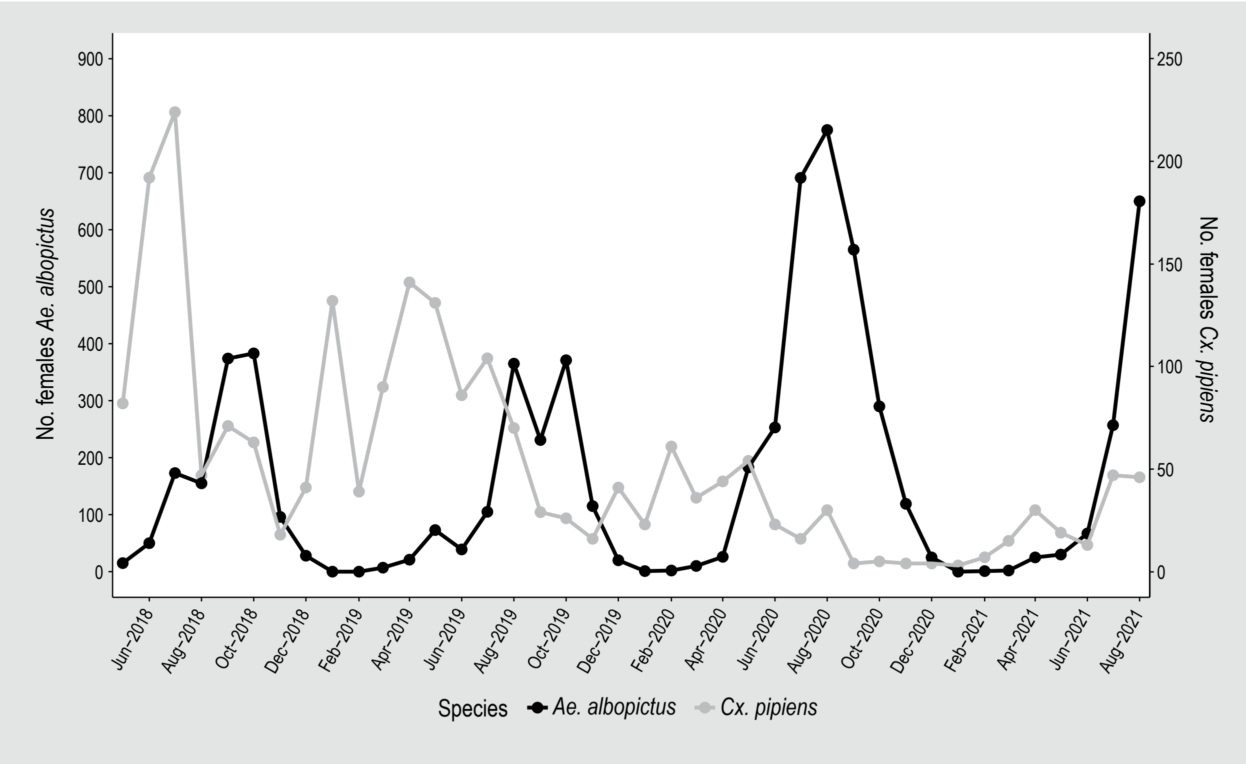

Figure 5. Comparison of densities of female *Aedes albopictus* (y-axis left) and *Culex pipiens* (y-axis right) trapped in the Mosquito Magnet trap at the Gibraltar Botanic Gardens, Gibraltar, May 2018 – August 2021.

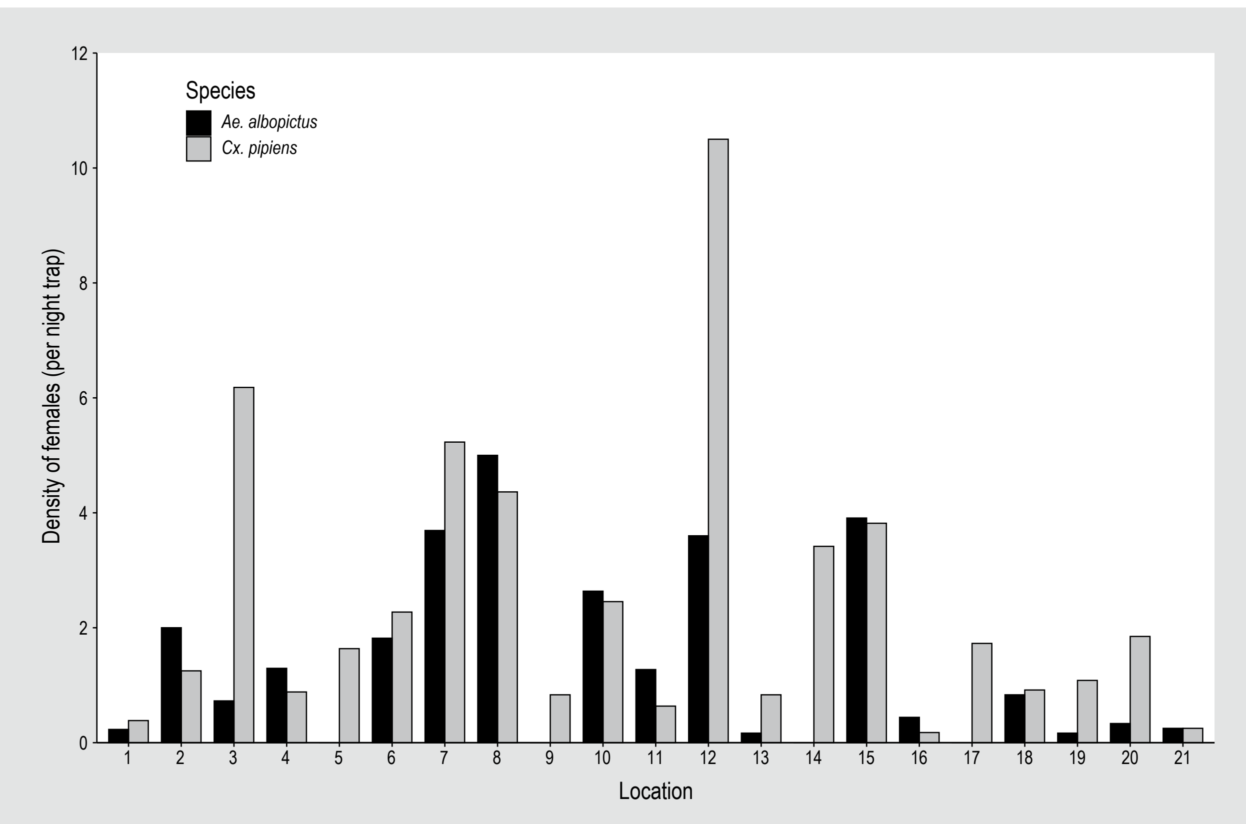

Figure 6. Mean density of female *Aedes albopictus* (black) and *Culex pipiens* (grey) trapped per night by BG-Sentinel 2 traps during August 2021 in various locations in Gibraltar.

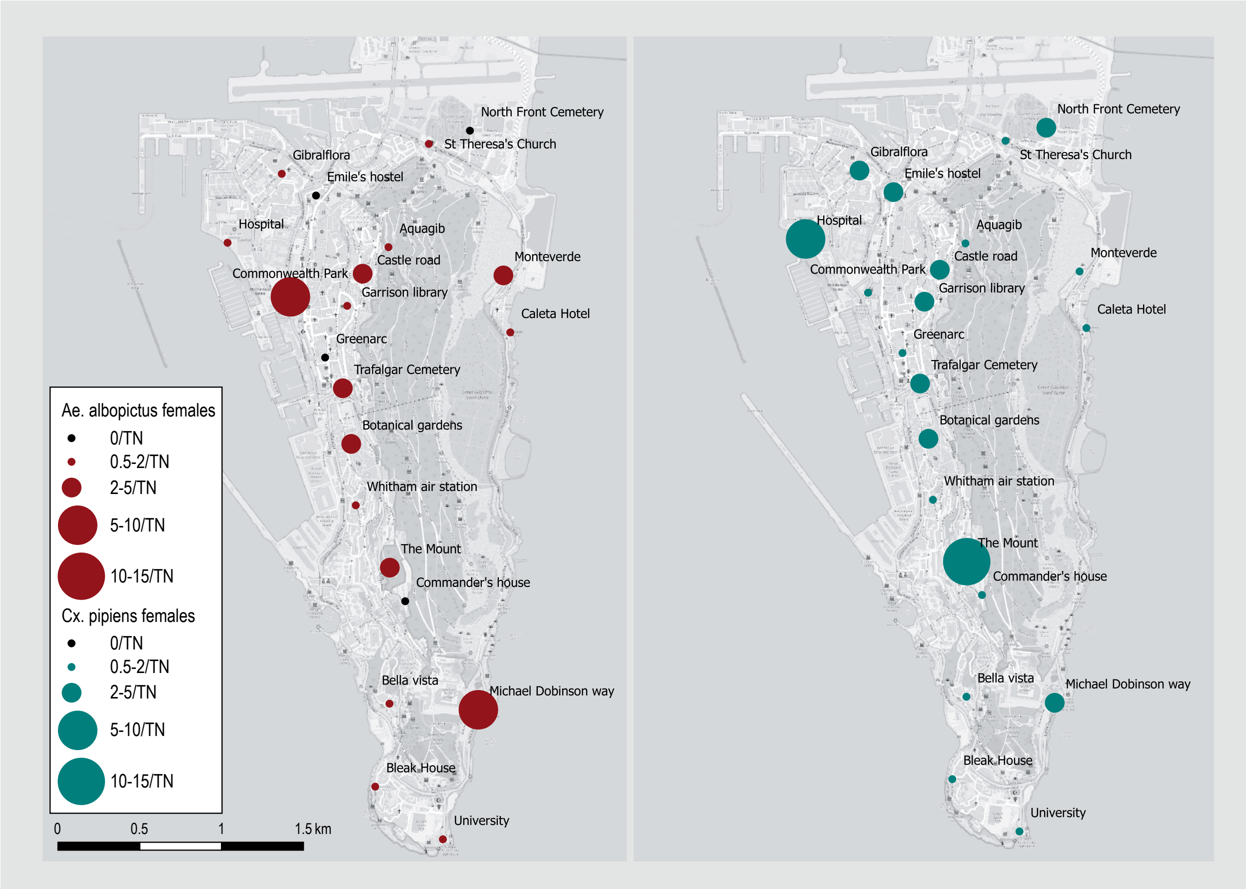

Figure 7. Spatial representation of mosquito densities observed by BG-Sentinel 2 traps across Gibraltar: *Aedes albopictus* (left), *Culex pipiens* (right).

and the Mount, where higher densities of *Cx. pipiens* are disproportionate to the densities of *Ae. albopictus*.

Testing of mosquito samples for arboviruses

The assay reproducibly detected down to 128 copies/µl of CHIKV RNA as determined by the synthetic standard control. All *Ae albopictus* extracts tested negative for the presence of Chikungunya virus. Synthetic WNV RNA was reproducibly detected down to 25.6 copies μ^{-1}. No WNV RNA was detected in any of the *Ae. albopictus* samples.

5. Discussion

This paper presents the first evidence of *Ae. albopictus* in Gibraltar, which first appeared in the south-central part of Gibraltar in August 2017. Since then through a variety of ongoing surveillance strategies, *Ae. albopictus* has been detected throughout the western populated areas of Gibraltar, and more recently on the east side of the Rock. Longitudinal datasets suggest that densities of *Ae. albopictus* are increasing over time, with much of the adult activity increasing during August to October. This appears from some of the data to coincide with an apparent decline in *Cx. pipiens*, both over time, as well as in their annual seasonality. Adult *Cx. pipiens* remains far more active during the cooler, winter months then *Ae. albopictus*, and has generally peaked by the time that the latter species becomes more noticeable. There is anecdotal evidence that *Ae. albopictus* biting becomes more apparent with the arrival of the levant winds, which is accompanied with increased humidity. Prior to August the prevailing wind is from the west, and changes to the easterly levant at that time. Increased biting at this time may be to do with a change in human behaviour/response to a warmer, more humid environment, although the data from the trapping suggest that *Ae. albopictus* becomes more abundant. It is more likely therefore that the microclimate becomes more favourable for mosquito breeding and activity, even though rainfall at this time of year remains scarce.

The larval habitats of *Ae. albopictus* in Gibraltar remain elusive, and so far, there is little evidence of preferred breeding sites, except the small number of container habitats and bromeliads from which larvae have been found. The latter are largely confined to the Botanic Gardens, whereas containers can occur anywhere. In contrast, *Cx. pipiens* have been found in a range of artificial containers,

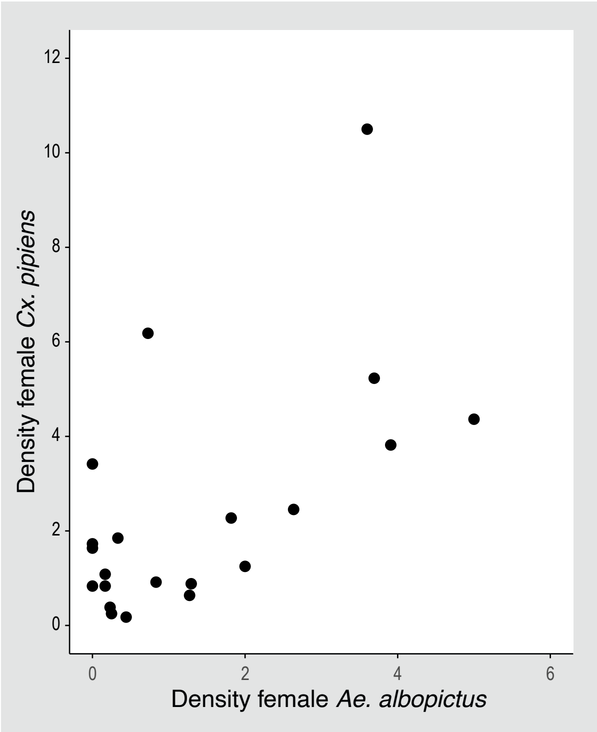

Figure 8. Comparison of densities of *Aedes albopictus* and *Culex pipiens* using BG-Sentinel 2 traps across 21 locations of Gibraltar during 2021.

buckets, tree-holes, vases, wheelbarrows, barrels, catch basins and sewage tanks. In general, gardens and yards are well tended, and there is usually very little garbage in the streets or the well-tended municipal gardens. Perhaps the humid environment in summer leads to less evaporation of water from cryptic and non-cryptic larval breeding sites. Certainly, the watering of plants, either in private or public gardens, would contribute to the larval breeding sites. Extensive sampling of drainage systems has found no *Ae. albopictus*, and this may be on account of their design, or that saline waters (inimical for *Ae. albopictus*) are used in drainage systems. Although some houses do have underground cisterns, which are known for harbouring *Ae. albopictus* elsewhere, they are no longer widely in use.

Aedes albopictus may remain a biting nuisance as it continues to establish. Local measures to mitigate biting issues by source reduction and targeted larvicidal control can impact on local mosquito densities. However, larval habitats remain cryptic. There is too little information on the larval breeding sites of *Ae. albopictus* in Gibraltar. It is known that gardens and areas of vegetation/shrubs/botanical gardens are associated with adult mosquito biting, but often it is not possible to identify breeding sites. This knowledge is crucial to mount an effective larvicidal control programme when faced with disease transmission. Given the levels of low precipitation during summer and high rates of evaporation, it seems that regular rainfall is insufficient for regular re-wetting of larval habitats (i.e. containers, litter etc.), therefore water storage and/or watering is likely to be crucial. Many homes have plants, and many of these have water trays. If regularly filled, then these can provide some habitats for breeding. However, for much of the summer these dry too quickly to be suitable. Plant container trays may become more suitable during the levant weather conditions, when humidity is high, and evaporation is reduced. Further work to identify larval habitats across Gibraltar for *Ae. albopictus* should be instigated.

Densities of both *Ae. albopictus* and *Cx. pipiens* remain low in Gibraltar, nevertheless, there still remains the potential for transmission of arboviruses, given the potential for imported cases from the rest of Europe and overseas to lead to local transmission. This concern will increase if local transmission persists in nearby parts of Spain, as large numbers of people travel across the border on a daily basis. So far though, local dengue cases in Spain have been very focal, and are subjected to rigorous control programmes. There is no endemic transmission of any of *Ae. albopictus*-transmitted arboviruses currently in Europe, and so spread from a European endemic zone currently seems unlikely. Gibraltar has strong links to many countries, particularly via the bunkering industry, as ship's crews are often rotated via flights arriving at the airport, so imported cases from anywhere globally is a possibility. Warmer, more humid environments will permit conditions for both the mosquito and for virus development in mosquitoes.

To ensure that swift and efficient responses are enacted in the event of imported or local cases, it is important to develop a contingency plan. This may detail stakeholders, actions and responsibilities, and identify gaps in preparedness and response. The aim of a contingency plan is to lay out actions and responsibilities in the event of a human arbovirus case detected in Gibraltar. Crucially, it is first necessary to determine whether cases are locally acquired, or travel associated, and so travel history information must be sought. Both scenarios can lead to onward transmission and so bite prevention measures and isolation are required during the infectious period.

If locally acquired, identifying breeding sites within 300 metres of cases and investigating other human local cases to an index case is the priority. In addition to limiting movement of an infected case, breeding sites and mosquito densities should be reduced in the vicinity of the case. This will act to reduce the prospects of onward transmission. Larval control can reduce increases of new potential vectors and adulticiding may be considered in knocking down active host-seeking ♀ mosquitoes, possibly pathogen-infected. Insecticidal residual spraying may also be considered around the case's home. Human surveillance of other cases around the index local case may be needed to establish wider transmission and for locating other foci. If travel associated, vector control will be needed around the case(s) to prevent transmission.

Public communication on bite risk prevention and source reduction around the home remains the primary and most effective sustainable and cost-effective tool to avoid bites and minimise mosquito densities. In a compliant community such as exists in Gibraltar, with a strong community linked through social networks, advice could be disseminated rapidly. Advice on reducing larval habitats and reducing exposure to mosquito biting ought to be ongoing, but particularly so during an outbreak. Public health messages should be prepared ahead of time.

As larval habitats remain enigmatic, it may be necessary to deploy house to house surveys, and thorough investigations of breeding sites (including possible cryptic sites) to enable larval control. This should include areas of overgrown hillsides between buildings which may harbour water-filled litter providing habitat for mosquitoes. This land between buildings is often steep and without clear ownership boundaries and should be investigated for cryptic larval breeding sites. There are likely to be very few areas within the urban area where this occurs, but where land is left unmanaged and perhaps harbours litter, a targeted annual programme of litter picking of specific locations could be conducted to reduce the impact of these areas on mosquito breeding. Consideration should also be given to water collection within homes, as well as balconies, yards, gardens, and drainage systems. Residential balconies and terraces are often well tended, with many citizens watering plants in pots and troughs. Plant pots often sit on a dish, and if regularly watered, these pools of water could maintain mosquito larvae, even in the summer months, but perhaps only for a few days before the water evaporates, and many plants are adapted to the semi- arid conditions. Some plants themselves, such as bromeliads, may contain water and may also support mosquito larvae. Household plants in pots on terraces were one of the greatest challenges for mosquito control in Madeira during the 2012 dengue outbreak. It is possible that mosquitoes collected during an outbreak could be tested for virus, and so building local laboratory capability could be a priority. Finally, if there is sustained transmission or further local cases, the logistics around deployment of adulticides should be considered.

Acknowledgements

The UK Overseas Territories (UKOT) programme is funded by the Foreign, Commonwealth and Development Office through the Conflict, Stability and Security Fund (CSSF) governance programme

Conflict of interest

The authors declare no conflict of interest.

References

Angelini, R., Finarelli, A.C., Angelini, P., Po, C., Petropulacos, K., Silvi, G., Macini, P., Fortuna, C., Venturi, G., Magurano, F., Fiorentini, C., Marchi, A., Benedetti, E., Bucci, P., Boros, S., Romi, R., Majori, G., Ciufolini, M.G., Nicoletti, L., Rezza, G. and Cassone, A., 2007. Chikungunya in north-eastern Italy: a summing up of the outbreak. Eurosurveillance 12: E071122.

Becker, N., Petrić, D., Boase, C. and Lane, J., 2010. Mosquitoes and their control. Springer, Heidelberg, Germany, 577 pp.

Collantes, F., Delacour S., Delgado, J.A., Bengoa, M., Torrell-Sorio, A., Guinea, H., Ruiz, S., Lucientes, J. and Mosquito Alert, 2016. Updating the known distribution of Aedes albopictus (Skuse, 1894) in Spain 2015. Acta Tropica 164: 64-68.

Delisle, E., Rousseau, C., Broche, B., Leparc-Goffart, I., L'ambert, G., Cochet, A., Prat, C., Foulongne, V., Ferré, J.B., Catelinois, O., Flusin, O., Tchernonog, E., Moussion, I.E., Wiegandt, A., Septfons, A., Mendy, A., Moyano, M.B., Laporte, L., Maurel, J., Jourdain, F., Reynes, J., Paty, M.C. and Golliot, F., 2015. Chikungunya outbreak in Montpellier, France, September to October 2014. Eurosurveillance 20(17): 21108.

Edwards, C.J., Welch, S.R., Chamberlain, J., Hewson, R., Tolley, H., Cane, P.A. and Lloyd, G., 2007. Molecular diagnosis and analysis of chikungunya virus. Journal of Clinical Virology 39: 271-275.

European Centre for Disease Prevention and Control (ECDC), 2017. Rapid risk assessment: cluster of autochthonous chikungunya cases in France. ECDC, Technical report., Stockholm, Sweden. Available at: https://tinyurl.com/2h5jykwu

European Centre for Disease Prevention and Control (ECDC), 2018. Local transmission of dengue fever in France and Spain – 2018. ECDC, Stockholm, Sweden. Available at: https://tinyurl.com/mjmjh8yn

European Centre for Disease Prevention and Control (ECDC), 2019a. Autochthonous cases of dengue in Spain and France. ECDC, Stockholm, Sweden. Available at: https://tinyurl.com/2p94cvsa

European Centre for Disease Prevention and Control (ECDC), 2019b. Rapid risk assessment: Zika virus disease in Var department, France. ECDC, Stockholm, Sweden. Availablet at: https://tinyurl.com/yfz9xtmc

Giron, S., Franke, F., Decoppet, A., Cadiou, B., Travaglini, T., Thirion, L., Durand, G., Jeannin, C., L'ambert, G. and Grard, G., 2019. Vector-borne transmission of Zika virus in Europe, southern France, August 2019. Eurosurveillance 24: 1900655.

Hadfield, T., Turell, M., Dempsey, M., David, J. and Park, E., 2001. Detection of West Nile virus in mosquitoes by RT-PCR. Molecular and Cellular Probes 15: 147-150.

Lucientes, J. and Molina, R., 2017. Informe de las actividades desarrolladas por la Universidad de Zaragoza durante el año 2017 en virtud del procedimiento abierto N°2015/507 PA004 para la realización del trabajo técnico que lleva como título: 'Vigilancia entomológica en aeropuertos y puertos frente a vectores importados de enfermedades infecciosas exóticas, y vigilancia de potenciales vectores autóctonos de dichas enfermedades'. Universidad Zaragoza, Zaragoza, Spain, 127 pp. Available at: https://tinyurl.com/8yr9k7hn

Marchand, E., Prat, C., Jeannin, C., Lafont, E., Bergmann, T., Flusin, O., Rizzi, J., Roux, N., Busso, V., Deniau, J., Noel, H., Vaillant, V., Leparc-Goffart, I., Six, C. and Paty, M., 2013. Autochthonous case of dengue in France, October 2013. Eurosurveillance 18: 20661. https://doi.org/10.2807/1560-7917.ES2013.18.50.20661

Medlock, J., Hansford, K., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H. and Van Bortel, W., 2012. A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. Vector Borne and Zoonotic Diseases 12: 435-447. https://doi.org/10.1089/vbz.2011.0814

Nabet, C., Chaline, A., Franetich, J.F., Brossas, J.Y., Shahmirian, N., Silvie, O., Tannier, X. and Piarroux, R., 2020. Prediction of malaria transmission drivers in *Anopheles* mosquitoes using artificial intelligence coupled to MALDI-TOF mass spectrometry. Scientific Reports 10: 11379. https://doi.org/10.1038/s41598-020-68272-z

Obregon, R., Flores, E. and Jordano, D., 2019. First report of the Asian tiger mosquito, *Aedes (Stegomyia) albopictus* Skuse, 1984 (Diptera, Culicidae) in Cordoba (southern Spain). New challenges for the administration and citizens of Cordoba. Journal of the European Mosquito Control Association 37: 28-33.

Osório, H., Zé-Zé, L., Neto, M., Silva, S., Marques, F., Silva, A. and Alves, M., 2018. Detection of the invasive mosquito species *Aedes (Stegomyia) albopictus* (Diptera: Culicidae) in Portugal. International Journal of Environmental Research and Public Health 15: 820. https://doi.org/10.3390/ijerph15040820

Osório, H.C., Rocha, J., Roquette, R., Guerreiro, N.M., Zé-Zé, L., Amaro, F., Silva, M. and Alves, M.J., 2020. Seasonal dynamics and spatial distribution of *Aedes albopictus* (Diptera: Culicidae) in a temperate region in Europe, Southern Portugal. International Journal of Environmental Research and Public Health 17: 7083.

Schaffner, F., Angel, G., Geoffroy, B., Hervy, J.P., Rhaiem, A. and Brunhes, J., 2001. The mosquitoes of Europe. Identification and teaching software. Institut de Recherche pour le Développement & EID Méditerranée, Montpellier, France.

Schaffner, F., Medlock, J.M. and Van Bortel, W., 2013. Public health significance of invasive mosquitoes in Europe. Clinical Microbiology and Infection 19: 685-692. https://doi.org/10.1111/1469-0691.12189

Sousa, C.A., Clairouin, M., Seixas, G., Viveiros, B., Novo, M.T., Silva, A.C., Escoval, M.T. and Economopoulou, A., 2012. Ongoing outbreak of dengue type 1 in the Autonomous Region of Madeira, Portugal: preliminary report. Eurosurveillance 17: 20333.