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Where does *Aedes albopictus* (Diptera: Culicidae) really breed in a Mediterranean residential area? Results from a field study in Valencia, Eastern Spain*

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Abstract

Since its introduction in Spain in 2004, *Aedes albopictus* has rapidly spread across the country. Its aggressive biting behaviour causes nuisance, limiting outdoor activities. Also, its role as a vector of several arboviruses implies a major public health risk, with several cases of autochthonous dengue having been reported nationwide over the past few years. Control strategies usually focus on interventions in breeding sites. As such, accurate knowledge of the main larval habitats becomes a major priority in infested areas. A detailed identification of breeding sites of *Ae. albopictus* was carried out in the outdoors of 60 residential properties during July–August 2022 in El Vedat de Torrent (Valencia, Eastern Spain), an area recently colonised by this species. A total of 1444 real and potential breeding sites were examined. The most abundant potential larval habitat were plant pot plates (6.48 units/house), although a low infestation level was found, both for larvae (2.06% positivity, $\bar{x} = 30.5$ larvae/container), and pupae (0.51%, $\bar{x} = 2.5$ pupae/container). A total of 7715 larvae and 205 pupae were found in a disused flooded water pool depuration system. Animal drinkers, buckets and irrigation water containers were found to be the most common positive containers. No statistical difference was observed among the different container materials. A general statistical increase of 1 larva per 11.7 ml of water in breeding sites was detected. Breeding sites of other species such as *Culex pipiens* ($n = 2$) and *Culex modestus* ($n = 1$) were also rarely found in this residential area. To our knowledge, this is the first aedic index study carried out in Europe, and it provides valuable information about the main domestic breeding habitats of *Ae. albopictus*, which can greatly improve control programmes.

Introduction

The Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae), is one of the 100 most invasive species on Earth (Lowe *et al*., 2000). Indigenous to Southeast Asia, it has undergone an impressive expansion of its native range in the last few decades. In fact, it is currently present in large areas of every inhabited continent except Antarctica, in both tropical and temperate environments (Kraemer *et al*., 2015).

The first record of this mosquito outside its place of origin was registered in Europe, specifically in Albania in 1979 (Adhami and Reiter, 1998), although there were no reports in any other European country until 1990, when it was identified in Italy (Sabatini *et al*., 1990). This species is mainly spread passively by ground, aircraft and maritime traffic (Ibáñez-Justicia, 2020), highlighting pathways such as the trade of used tires or lucky bamboo plant cuttings in Europe (Scholte *et al*., 2010; Demeulemeester *et al*., 2014), where the species is currently established in at least 26 countries (ECDC, 2022).

Aedes albopictus causes an important nuisance given its biting behaviour, provoking discomfort in people when found in large numbers (Curcó *et al*., 2008), limiting outdoor activities. Bites can cause serious allergic reactions in sensitive individuals, especially in newly infested areas, which could also be considered an early warning of the introduction of this dipteran (Abramides *et al*., 2011). However, the major concern regarding this species is its capacity to transmit mosquito-borne diseases of public health importance (Näslund *et al*., 2021). In fact, the establishment of this vector in Euro-Mediterranean countries has led to autochthonous transmission events of several arboviruses; chikungunya cases were first reported in 2007 in Italy (Rezza *et al.*, 2007), dengue in 2010 in France (La Ruche *et al.*, 2010) and Zika in 2019, also in France (Giron *et al.*, 2019). Further cases of arboviruses linked to this species were also reported from other European countries such as Croatia or Spain (Gjenero-Margan *et al.*, 2011; MSCBS, 2018). Recently, two autochthonous confirmed dengue cases were reported in the Balearic Islands, Spain (Campelli *et al.*, 2023) and multiple transmission events in Lazio Region, Italy (De Carli *et al.*, 2023).

The skip-oviposition pattern of synanthropic *Aedes* implies egg dispersion in multiple and cryptic sites (Reinbold-Wasson and Reiskind, 2021), often small human-made reservoirs largely present in urban private areas (Stefopoulou *et al.*, 2018). In this sense, there is very limited information concerning larval habitat identification of *Ae. albopictus* in Europe (Baldacchino *et al.*, 2016). Better knowledge of the specific domestic breeding habitats occupied by this species in each region would provide important epidemiological and anthropological information, with direct implications for health education, environmental awareness and vector control (Alarcón-Elbal *et al.*, 2021, 2024). The use of entomological indicators such as the house index (HI), container index (CI), pupal index (PI) or Breteau index (BI) could be very useful for the proper monitoring of breeding sites of synanthropic aedine species (Reiter and Gubler, 1997; Focks, 2003), being *Aedes aegypti* (Linnaeus, 1762) particularly noteworthy among them. However, these tools have been scarcely used in Europe, although they are widely utilised in many countries of Central and South America, along with the Caribbean, where their employment is considered essential for the development of vector control programmes (Ministério da Sáude, 2009; César *et al.*, 2015; Bardach *et al.*, 2019).

In this sense, the project 'New strategies for the control of the tiger mosquito in residential areas', also known as NESCOTIGER, was conducted in 2022 in Valencia, Spain, and aimed to explore various mosquito control strategies in both public and private settings. Within the scope of this research project, the aim of the current study was to identify and characterise the domestic breeding sites of *Ae. albopictus* in a recently invaded region of the Spanish Mediterranean. Ultimately, enhancing our understanding of this invasive mosquito species is crucial for the development of effective prevention and control measures.

Material and methods

Study area

The study was conducted in El Vedat (39°25′25″N 0°29′35″W), a residential area situated on the small mountain of the same name in Torrent, in the province of Valencia (Valencian Community, Eastern Spain). It is considered the municipality with the second highest population index of the province, with around 85,000 registered inhabitants in 2022, and it is located 9 km from the city of Valencia, the capital of the autonomous community and the third largest city in Spain. The area is characterised by a high economic level, with a mean annual homestead income ranging from 43,000€ to 61,000€ ($\bar{x}$ = 54,058€), Spain's average being 27,000€ to 32,000€ for the year 2020. Over 16–26% of the population is 65 years or older (INE, 2023). This large residential area sits 142 masl, on the edge of the Sierra Perenxisa, and it covers a surface area of 5.86 km^2, over 40.5% of which comprise urban green spaces, woodlands, pine trees and agriculture fields (mainly fruit trees, especially olives and orange trees). This high-income area had public water services and was characterised by big-size single-family homes (parcel size: $\bar{x} = 642\ m^2$; $\tilde{x} = 591\ m^2$; Mo = 591 m^2; min = 124 m^2; max = 2133 m^2; SD = 390 m^2), each usually having a private swimming pool and garden areas.

Geographic sectorisation and control interventions

In the NESCOTIGER project, a prior analysis of the study area was conducted, leading to the selection of six study sectors (0–5), each of which had simultaneously implemented a different *Aedes* control strategy (table 1). Such analysis was performed with the spatial analysis software QGIS, and through the employment of socioenvironmental variables such as type of housing, vegetation and water-catching systems in the area (unpublished data). Residents in the area were invited to participate in the project, and were provided with the control tools for its deployment in their private gardens.

Collection, processing and identification

A house-to-house cross-sectional entomological survey was carried out to detect larval breeding sites in outdoor areas (gardens, yards, terraces, etc.) of households in El Vedat. Houses were randomly selected among the voluntary participants in the NESCOTIGER project. For sampling, a team composed of the same two researchers visited each evaluated household, informed the residents of the purpose and procedures of the study and obtained informed consent from the head of the household.

The field survey was conducted in the period from July to August 2022. These weeks are the hottest and driest of the year, allowing for a more accurate identification of larval habitats strictly related to human activities. A mean temperature of 28.1°C (min. 19.6°C; max. 40.7°C) and an accumulated precipitation of 26.4 mm between the two studied months was recorded in the closest meteorological station during the study period (meteorological station 8414A 'Valencia, Aeropuerto', 6.2 km away from the study area) (AEMET, 2024). However, September is typically a rainy month in the Spanish Levante, leading to the formation of breeding sites also associated with water accumulation from rainfall.

In each house, every real and potential breeding site was recorded and evaluated. For each site, the container type, material (plastic, metal, ceramic, etc.), presence/absence of water, water-holding potential maximum capacity (ml) and presence of either mosquito's larvae or pupae were registered. For each mosquito-positive breeding site, all individuals were retrieved (when possible) with a plastic Pasteur pipette into plastic containers. For those reservoirs with high amounts of water, a fraction of the total was retrieved, and the total larvae and pupae density were calculated based on the estimated content of the container. Each household inspection lasted between 15 and 45 min, based on the number and size of reservoirs inspected.

In the laboratory, preimaginal stages were introduced alive into mosquito breeding containers (Bioquip Products, Rancho Domínguez, CA, USA) filled with the original breeding water. Larvae were reared under laboratory conditions until the 3rd/4th instar, afterwhich, individuals were euthanised by submersion in hot water (60°C) for 1 min. Subsequently, they were preserved in labelled vials filled with 70% ethanol until identification. The collected pupae were allowed to emerge into adults for taxonomic identification after being killed by placing them in a freezer (−20°C) for 30 min. Both immature and adult mosquitoes were identified using the e-taxonomy key of MoskeyTool (MediLabSecure, 2023).

Table 1. Description of implemented interventions in El Vedat de Torrent during the NESCOTIGER project classified by study sectors (0–5)

Description	Insecticide composition	Sectors					
		0	1	2	3	4	5
Workshops							
Workshops concerning *Aedes albopictus* biology, ecology, and control strategies were conducted in primary schools and neighbouring local associations (Alarcón-Elbal *et al*., 2024).	None	■	■	■	■	■	■
Painted water catch-basins							
Public water catch basins in the study area were coated with the insecticide paint Inesfly 5A IGR NG.	Alphacypermethrin, 0.7%;D-Alletrin, 1.0%; Pyriproxyfen, 0.063%		■	■	■	■	■
Sticky trap							
Ovitraps were equipped with a sticky surface for capturing gravid females	None		■			■	
Lethal ovitrap							
Ovitraps coated on the inside with the insecticide paint Inesfly 5A IGR NG	Alphacypermethrin, 0.7%; D-Alletrin, 1.0%; Pyriproxyfen, 0.063%			■		■	
Larvicide							
Larvicide sprays (Inesfly LARVA IGR) that could be employed by citizens to coat potential breeding sites in their homesteads. Additionally, empty black plastic containers for spraying and filling with water for acting as an oviposition trap were also given to citizens	Pyriproxyfen 0.2%				■	■	

Grey markings represent the sector in which each intervention was implemented.

Data analysis

Data analysis was performed with the statistics programs RStudio (R version 4.1.2) and Microsoft Excel. Based on the gathered data, the HI (n houses infested with larvae and pupae/n houses inspected × 100), CI (n containers infested with larvae and pupae/n containers inspected × 100), BI (n containers infested with larvae and pupae/n houses inspected × 100) and PI (n pupae/n inspected houses × 100) were calculated for El Vedat as well as for each independent studied sector. A χ^2 test comparing the BI among sectors was performed, employing sector 5 as the control. A Kruskal–Wallis test was developed to evaluate the effect of material type over the larval abundance, employing the 'kruskal.test()' function from base R. Finally, a linear model was carried out, employing the 'lm()' function from base R to evaluate the effect of total water in containers over *Ae. albopictus* larvae abundance. Statistical differences were accepted for $p < 0.05$.

Results

A total of 60 households (10 houses per sector) were surveyed for the presence of immature mosquitoes in El Vedat (table 2). A total of 1444 potential breeding sites were examined, of which 35 (2.4%) harboured immature mosquitoes. A total of 13,125 larvae and 385 pupae of three mosquito species were captured: *Ae. albopictus* (10,476 larvae and 385 pupae in 33 foci); *Culex pipiens* (Linnaeus, 1758) (2648 larvae in 2 foci); and *Culex modestus* (Ficalbi, 1889) (2 larvae in 1 focus). The following results only reflect *Ae. albopictus* breeding sites for both larvae and pupae (tables 2 and 3).

Aedic indices

The mean HI showed that 40% of the houses in the studied area had presence of *Ae. albopictus* larvae and that 2.9% of the evaluated containers (CI) were infested by *Ae. albopictus*. Statistical

Table 2. *Aedes* entomological indices stratified by sector in El Vedat, Valencia, Spain, July–August 2022.

Sector	pos_houses(n)	pos_cont(n)	ins_houses(n)	ins_cont(n)	HI	CI	BI	S5 (%)	PI
0	4	5	10	234	40	2.14	50	83.33	570
1	6	7	10	158	60	4.43	70	116.67	2530
2	4	8	10	179	40	4.47	80	133.33	140
3	3	3	10	186	30	1.61	30	50	150
4	3	4	10	168	30	2.38	40	66.67	390
5	4	6	10	218	40	2.75	60	NA	20
Total	24	33	60	1143	40	2.89	55	–	633

pos_houses (n), no of positive houses; pos_cont (n), no of positive containers; ins_houses (n), no of inspected houses; ins_cont (n), no of inspected containers; HI, house index; CI, container index; BI, Breteau's index; S5(%), percentual difference between BI for each sector and Sector 5; PI, pupal index; NA, not applicable.

differences for the BI were recorded among the different sectors ($\chi^2 = 31.81$, df = 5, $p < 0.001$), with a mean value of 55%. The highest BI was found in sectors 1 and 2 (BI = 70 and 80%, respectively) (table 2), both being sites where no larvicide treatment was implemented, in contrast to sectors 3 and 4, where a reduction of the BI compared to sector 5 was seen (50.0 and 66.7%, respectively) (fig. 1). Sectors 1 and 2 recorded higher CI values than that of the control zone (sector 5), while sector 3 accounted for

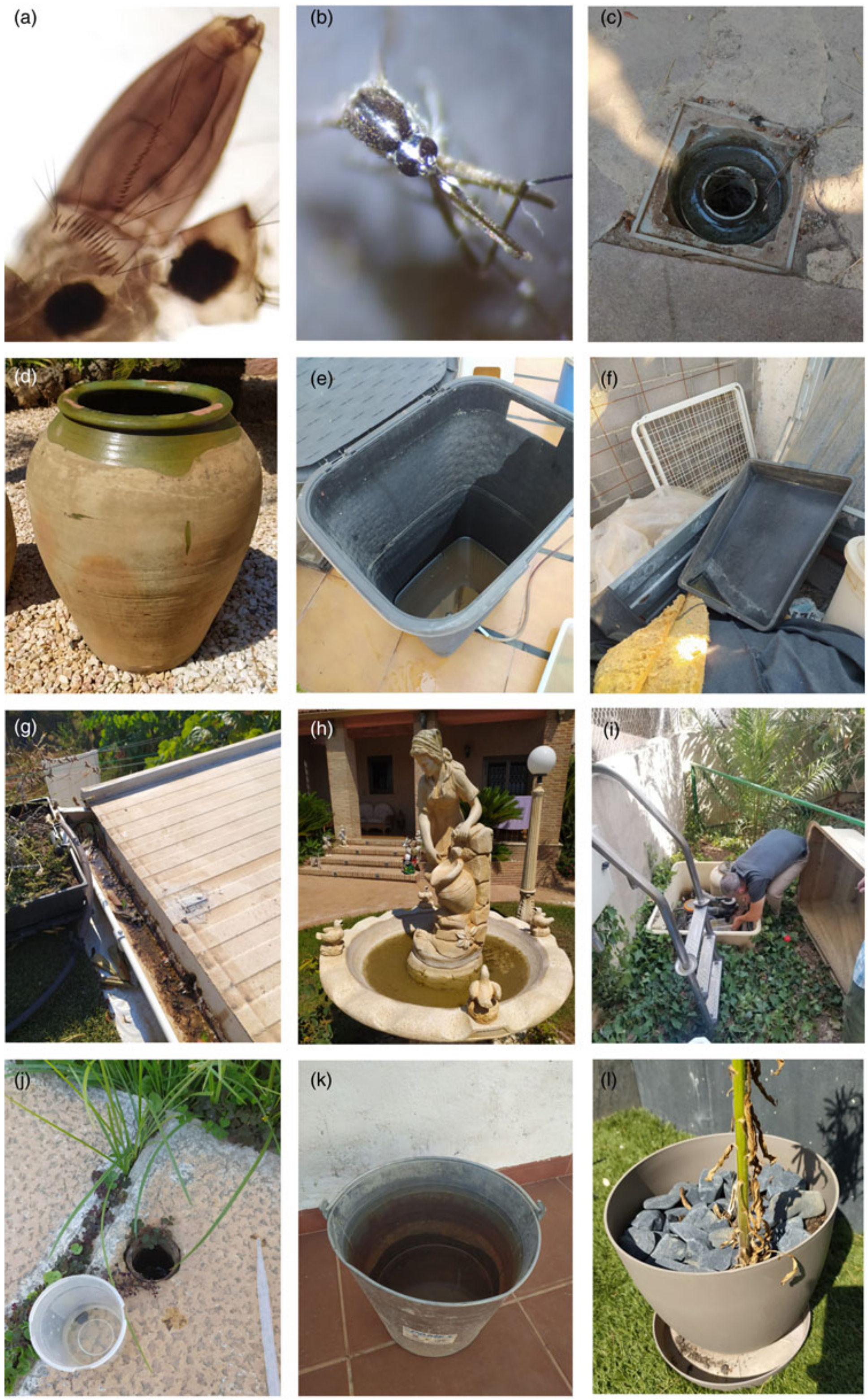

Figure 1. *Aedes albopictus* and its breeding sites in Torrent, Valencia, July–August 2022. (a) *Ae. albopictus* larva posterior end; (b) *Ae. albopictus* imago (dorsal view); (c) water drain; (d) plant pot; (e) water drainage system; (f) construction materials; (g) gutter; (h) ornamentation items (fountain); (i) water depuration system (major mosquito foci, *Ae. albopictus* larvae $n = 7715$; *Culex pipiens* larvae, $n = 2600$); (j) structural deficiency; (k) bucket; (l) plant pot plate.

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the lowest indices. A total of 380 pupae were identified in 14 breeding sites (average of 27.14 pupae/positive breeding site). A mean PI value of 633 was observed (table 2).

Container analysis

Plant pot plates were the most common potential breeding site in the study area, with a mean of 6.8 units/household, followed by flowerpots ($n = 2$ units/household), construction materials ($n = 1.3$ units/household), drains ($n = 1.2$ units/household) and buckets ($n = 1$ unit/household). While plant pot plates were the most abundant breeding site, only 12.8% contained water, of which only 17% had larvae presence. On the contrary, other less abundant containers were more commonly filled with water, being real potential breeding sites during the study period, such as bromeliad plants (80%), animal drinkers (53.6%) and even empty plant pots (50%) (table 3).

In general, flowerpots and plant pot plates constituted more than half of the real water-holding containers positive for *Ae. albopictus* larvae. From the potentially infested breeding sites (PBS), among the total evaluated (BS), those with the highest intrinsic larvae positivity rates (PBS/BS) were water irrigation tanks (33.3%), exotic tank bromeliads (20%) and water depuration systems (16.7%). However, when considering only the breeding sites containing water (BSw), the highest rates of larval presence (PBS/BSw) were observed in water irrigation tanks (66.7%), construction tools (carrycot) (66.7%), bromeliads (50%), flowerpots (45.5%) and structural deficiencies (40%). Nevertheless, several identified breeding sites showed larvae presence, even though no pupae were observed. Such containers were bromeliad plants (n larvae = 21; n pupae = 0), buckets (n larvae = 8; n pupae = 0) and structural deficiencies (n larvae = 46; n pupae = 0). Also, only one pupa was found in ornamental items, even though a total of 77 larvae were observed during the study in those container types.

In addition, a single disused swimming pool's water depuration system filled with water presented a great infestation level, being the most productive habitat in the study area (n larvae = 7,715, 73.6%; n pupae = 205, 53.3%). Other highly productive reservoirs were water irrigation tanks (n larvae = 911, 8.8%; n pupae = 39, 10.1%), flowerpots (n larvae= 895, 8.5%; n pupae= 73, 19.0%) and animal drinkers (n larvae= 386, 3.7%; n pupae = 43, 11.2%) (table 3, fig. 1).

Material type and total water effect

No association was found between the material type and the abundance of *Ae. albopictus* larvae (Kruskal Wallis test; $n = 35$, df = 6, p-value = 0.72). Based on a descriptive analysis, PVC plastic showed the highest median abundance value, although a single recipient was found positive in this case, with pottery apparently being the most productive breeding site.

Based on data from the positive containers, a linear correlation was observed between total water volume and larvae abundance, with an increase of 1 larvae/11.76 ml (lm; 0.085 ml larvae^{-1}; adjusted $R^2 = 0.78$, $p < 0.0001$).

Discussion

To our knowledge, this is the first study concerning aedic indices carried out in Europe, although some researchers have delved into the diversity of breeding sites of the Asian tiger mosquito in countries such as Italy (Baldacchino *et al.*, 2016). Nevertheless, this methodology is commonly employed in vector surveillance programmes in endemic areas in Asia, Africa and the Americas (Correia *et al.*, 2015; MSCBS, 2018; Aryaprema and Xue, 2019; Diéguez Fernández *et al.*, 2021).

Aedes albopictus was first detected in Spain in 2004 (Aranda *et al.*, 2006), and since then, it has quickly dispersed countrywide, having been reported for the first time in the Valencia province in September 2013 (Alarcón-Elbal *et al.*, 2013; Bueno *et al.*, 2013). In July 2015, the species was first detected in the city of Valencia (Bueno and Quero de Lera, 2015). In September of that same year, the increase in insect bites and the consequent public alarm led to suspicion that this species might had arrived in the municipality of Torrent, although this was not officially confirmed until 2016 (GVA, 2016). This invasive exotic species causes significant discomfort, a fact confirmed by the vast majority of residents during informal conversations while households were inspected. Nonetheless, the main concern resides in its capacity for disease transmission, as exemplified by its role as a vector in several autochthonous dengue outbreaks in Spain over recent years (ASP, 2013; ECDC, 2017; MSCBS, 2018; Monge *et al.*, 2020; Campelli *et al.*, 2023).

Aedic indices

Among the different evaluated indicators, the BI is considered the most noteworthy since it has been employed as an early indicator of adult mosquito density (Parra *et al.*, 2022) and for dengue transmission risk (Aryaprema and Xue, 2019; Liyanage *et al.*, 2022). However, some authors question the validity of those relations (Bowman *et al.*, 2014; Parra-Amaya *et al.*, 2016), and the World Health Organization (WHO) does not recommend the use of aedic indices as a primary entomological indicator (WHO, 2016). While these indices do not quantify population size or density, this method, proposed a century ago (Connor and Monroe, 1923), is the only entomological surveillance tool used in most programmes.

According to the Pan American Health Organization's (PAHO) recommendation (PAHO, 1994), an area with endemic disease potential is at a high risk of outbreaks when BI > 5% and CI > 3%. In the case of El Vedat de Torrent, the aedic indices are approaching these criteria (HI = 40%; CI = 2.89%; BI = 55%), even though Eastern Spain is not considered for the time being a high-risk area for the transmission of arboviruses. Nevertheless, it should be taken into consideration that such risk thresholds are established for *Ae. aegypti* populations, while the present study delves into aedic indices gathered for *Ae. albopictus*. Similar values for the HI and BI were obtained in surveys conducted in the Congo (HI = 33.3%; CI = 49.6%; BI = 26.6%) (Wilson-Bahun *et al.*, 2020), in Thailand (HI = 39%; CI = 2.5%; BI = 47%) (Preechaporn *et al.*, 2006) and an extremely high BI in Mexico (>200) (Winch *et al.*, 1992), just to mention three examples of tropical countries.

The identification of 33 positive reservoirs for *Ae. albopictus* (0.55 foci per household) and a mean PI value of 633 pupae per 100 houses (table 3) underscore the significance of residential settings in the proliferation of this mosquito species within urban areas. It is worth noting that this study took place during a major drought period, characterised as the hottest summer recorded in Spain (AEMET, 2022). Consequently, a substantial rise in active foci per household can be anticipated following a rainy period.

Container analysis

Numerous studies have pointed out the preferred breeding sites of synanthropic *Aedes*, although cultural practices concerning water

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Table 3. *Aedes albopictus* larvae breeding sites identification in El Vedat de Torrent, Valencia, July–August 2022

Classification	BS	BS/H	BSw	PBS	PBS/ BS	PBS/ BSw	TOT	TOT/ PBS	L	L/ PBS	P	P/ PBS
	n (%)	*n*/ household	*n*(%)	*n*(%)	%	%	*n* (%)	*n*	*n* (%)	*n*	*n* (%)	*n*
Animal drinkers	28 (2.45)	0.47	15 (53.57)	1 (4.76)	3.57	6.67	429 (3.95)	429	386 (3.68)	386	43 (11.17)	43
Bottles, cans, cups and similar	56 (4.90)	0.93	15 (26.79)	1 (4.76)	1.79	6.67	48 (0.44)	48	44 (0.42)	44	4 (1.04)	4
Bromeliad plants	5 (0.44)	0.08	4 (80.00)	1 (4.76)	20.00	25.00	21 (0.19)	21	21 (0.20)	11	0 (0)	NA
Buckets	60 (5.24)	1.00	21 (35.00)	3 (14.3%)	5.00	14.29	8 (0.07)	3	8 (0.08)	4	0 (0)	NA
Childs' toys	33 (2.88)	0.55	0 (0.00)	0 (0.00%)	0.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Construction materials and tools	76 (6.64)	1.27	3 (3.95)	1 (4.76)	1.32	33.33	31 (0.29)	31	24 (0.23)	12	7 (1.82)	7
Fishbowls	1 (0.09)	0.02	1 (100)	0 (0.00)	100.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Flowerpots	119 (10.40)	1.98	22 (18.49)	6 (28.6)	5.04	27.27	968 (8.91)	161	895 (8.54)	90	73 (18.96)	15
Garbage bins	35 (3.06)	0.58	0 (0.00)	0 (0.00)	0.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Gutters	29 (2.53)	0.48	0 (0.00)	0 (0.00)	0.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Kitchen utensils	13 (1.14)	0.22	0 (0.00)	0 (0.00)	0.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Moviliary	8 (0.70)	0.13	2 (25.00)	0 (0.00)	0.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Non-bromeliad plants	29 (2.53)	0.48	4 (13.79)	0 (0.00)	0.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Ornamentation items	58 (5.07)	0.97	7 (12.07)	1 (4.76)	1.72	28.57	78 (0.72)	78	77 (0.74)	77	1 (0.26)	1
Pot plates	389 (34.00)	6.48	47 (12.08)	8 (38.10)	2.06	17.02	249 (2.29)	31	244 (2.33)	31	5 (1.30)	3
Pots	6 (0.52)	0.10	3 (50.00)	0 (0.00)	0.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Structural deficiencies	17 (1.49)	0.28	5 (29.41)	2 (9.52)	11.76	40.00	46 (0.42)	23	46 (0.44)	23	0 (0)	NA
Sun umbrellas, canvas and canopies	42 (3.67)	0.70	4 (9.52)	0 (0.00)	0.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Swimming pools	9 (0.79)	0.15	5 (55.56)	0 (0.00)	0.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Tires	3 (0.26)	0.05	1 (33.33)	0 (0.00)	0.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Water depuration systems	6 (0.52)	0.10	1 (16.67)	1 (4.76)	16.67	100.00	7920 (72.92)	7920	7715 (73.64)	7715	205 (53.25)	205
Water drains	69 (6.03)	1.15	18 (26.09)	1 (4.76)	1.45	5.56	113 (1.04)	113	105 (1.00)	105	8 (2.08)	8
Water irrigation tanks	6 (0.52)	0.10	3 (50.00)	2 (9.52)	33.33	66.67	950 (8.75)	475	911 (8.70)	456	39 (10.13)	39
Watering cans	28 (2.45)	0.47	1 (3.57)	0 (0.00)	0.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Other sites	19 (1.66)	0.32	0 (0.00)	0 (0.00)	0.00	0.00	0 (0.00)	NA	0 (0.00)	NA	0 (0)	NA
Mean	–	0.76	182 (15.91)	–	1.84	11.54	433	776	418	746	15	36
Total	1144	19.07	–	28	–	–	10,861	–	10,476	–	385	–

BS: breeding site, BSw: breeding site with water, PBS: positive breeding sites, H: inspected houses (60), TOT: total preimaginal stages (larvae and pupae), L: total number of larvae, P: total number of pupae.

management have a strong influence over the results. Water gathering and/or collection systems play a key role as mosquito breeding sites, with water tanks, barrels or drums consistently identified as the primary *Aedes* sources in numerous prior studies (Baldacchino *et al.*, 2016; Diéguez *et al.*, 2016; Morales-Perez *et al.*, 2017; Vannavong *et al.*, 2017; Abilio *et al.*, 2018; Rodríguez-Sosa *et al.*, 2019; Leal *et al.*, 2020; Alarcón-Elbal *et al.*, 2021). The studied area counts with constant public water supply, making water gathering and collection unnecessary and eliminating the risk that breeding sites such as water drums may imply in other epidemiological scenarios. Nevertheless, other big water-holding capacity sites such as water depuration tanks seem to be major *Ae. albopictus* focus in the study area. In this sense, bigger water-holding capacity containers have been positively correlated with *Ae. aegypti* egg density (Harrington *et al.*, 2008), as was observed for *Ae. albopictus* larvae in our survey. Other major *Aedes* breeding site identified in the literature are discarded tires (Abilio *et al.*, 2018; González *et al.*, 2020). Nevertheless, in the researched area, only three dry tires were identified, and they were not perceived as relevant mosquito sources during the studied period. Finally, other key breeding sites in residential areas identified in the literature are flowerpots and several plastic-type containers (Diéguez-Fernández *et al.*, 2019; Leal *et al.*, 2020; Alarcón-Elbal *et al.*, 2021; Yuliani *et al.*, 2021). Even though no statistical preference was observed for either plastic or any other specific material type, the most frequently infested breeding sites found were pot plates (38.10%) and flowerpots (28.60%). The high positivity of these larval habitats is explained by the regular watering of the plants by the inhabitants, in addition to the lack of cultural control measures.

Aedes albopictus *control in residential areas*

While public areas are subject to control by municipal services, private areas are inaccessible to public vector control practitioners, presenting a constraint in public control programmes (Stefopoulou *et al.*, 2018). In our research, public water-catch basins were previously treated in the scope of the NESCOTIGER project with insecticide paint (Inesfly 5A IGR NG; Alphacypermethrin 0.7%; D-Alletrin 1.0%; Pyriproxyfen 0.063%) (table 1) and were shown to be non-significant foci for *Ae. albopictus* mosquitoes (unpublished data). As such, domestic larval habitats were the main ones during the field inspection.

In this context, the role of citizens in the control of the Asian tiger mosquito is considered key, given the importance of the elimination of breeding sites in private areas (Gratz, 1994; ECDC, 2017). In this sense, prioritising the identification of potential breeding sites by residents and understanding the mosquito's biology becomes essential (Caputo *et al.*, 2020). Nevertheless, it has been shown that both children and adults in the study area have limited knowledge on this topic, with a general tendency to misidentify swimming pools as breeding sites (Alarcón-Elbal *et al.*, 2024). In fact, swimming pools without proper maintenance are not suitable places for the development of this species, as they contain a large amount of water, although unmaintained children's swimming pools do constitute an ideal habitat in urban environments (Rust, 2009). For this reason, pools (both maintained and unmaintained) were not considered as potential breeding sites during the development of this study, although they were also prospected, always with negative results. Nevertheless, certain elements tightly associated with pools were found infested by *Ae. albopictus*, as was the case of the most productive breeding site found in the study, a disused pool's water depuration system (fig. 1i), or structural deficiencies filled with splashed water (fig. 1j). As such, the identification and management of the main larval habitats becomes a priority in the implementation of any *Aedes* spp. control programme.

It should be noted that sectors 3 and 4, where the larvicide Inesfly Larva IGR was deployed independently or in combination with other control tools respectively (table 1), presented the lowest BI values (table 2). This spraying larvicide was employed by citizens in sectors 3 and 4 to coat potential *Aedes* mosquitoes breeding sites in their homesteads. Even though adults in Torrent showed during previous research a limited capacity to identify potential *Ae. albopictus*' breeding sites (Alarcón-Elbal *et al.*, 2024), it could be argued that the employment of this tool could have contributed in some way to the observed reduction of mosquitoes' preimaginal population densities in comparison with other studied areas. Nevertheless, due to the limited household sample size ($n = 60$), further research would be needed to assess the effect of the Inesfly Larvae IGR over the BI in any residential area.

In any case, it must be considered that a single highly infested house may affect a whole neighbourhood (Unlu *et al.*, 2011). In our study, a single heavily infested disused water depuration system contributed to over 73% of the total identified *Ae. albopictus* larvae and 53% of pupae within the study area while harbouring an estimate of 2600 *Cx. pipiens* larvae. This depuration system had an estimate of 60 l of water, with a potential total capacity of 300 l. While this focus cannot be regarded as a typical breeding site of *Ae. albopictus* based on prior literature, it underscores the challenges associated with source reduction in expansive residential areas. Nevertheless, recognising the significance of such highly productive breeding sites is crucial in the planning of future control campaigns in the Mediterranean area of either *Ae. albopictus* or *Cx. pipiens*.

In general, excluding flowerpots, the most abundant breeding sites exhibited low larval production. For instance, out of the 388 plant pot plates identified, only eight were positive, accounting for a total of 244 larvae (2.33% of total larvae) and five pupae (1.30% of total pupae). A general density reduction (individuals/breeding site) from larvae to pupae was noted, with only 385 pupae identified out of 10,476 counted larvae, showing a calculated ratio of 0.037 pupae per larva. Based on this data, pupae density per breeding site type should serve as the primary indicator, as pupae offer a more reliable gauge of adult mosquito populations compared to larvae (Focks, 2003). Among the various breeding sites identified in our study, tank bromeliads, structural deficiencies and buckets exhibited the presence of larvae but lacked pupae. Consequently, they did not seem to play a significant role as sources of adult *Ae. albopictus* in El Vedat de Torrent during the study period despite the species being known to breed in natural phytotelmata environments (Paupy *et al.*, 2009), a behaviour previously observed in the city of Valencia (Bueno *et al.*, 2016). Similar findings were previously reported by Mocellin *et al.* (2009) in an urban area of Rio de Janeiro, Brazil. However, it is important to exercise caution as different outcomes may arise in various research areas or under distinct climatic conditions.

In this sense, among the study's limitations, we adopted a descriptive cross-sectional design for this research, i.e. each container was sampled just once. Furthermore, the field research took place during a time of the year when populations of this species were expected to be not very abundant because of high

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temperatures and low rainfall, typical in the mainland Spanish Levante (Collantes *et al.*, 2015). Undoubtedly, conducting such studies in different climatic periods provides a more comprehensive perspective on the entomological situation (Sánchez *et al.*, 2006; González *et al.*, 2019; Monzón *et al.*, 2019). Therefore, it would be advisable in the future to collect these types of observations over a more extended period, while gathering data from different municipalities with variable climatic conditions. Another significant limitation is that aedic indices rely on visually locating containers, which may not accurately reflect the true prevalence of synanthropic mosquitoes given the presence of cryptic and/or inaccessible containers such as roof gutters, catch basins and septic tanks (Arana-Guardia *et al.*, 2014), although these sites were considered (when possible) during the development of the field research. Lastly, it is also important to note the limited number of households inspected ($n = 60$). A larger data sample would be of interest for a better understanding *Ae. albopictus*' breeding sites in residential areas of similar or greater size.

To conclude, gaining knowledge about the breeding sites of *Ae. albopictus* in residential areas of the Mediterranean Basin, and effectively communicating this information to the citizens, is of utmost importance. This is especially the case in areas where both adults and students are proven to lack profuse knowledge concerning the biology and ecology of the vector species, as was the case of El Vedat de Torrent (Alarcón-Elbal *et al.*, 2024). This information enhances our comprehension of the biology of this vector and, more importantly, contributes to the formulation of targeted control programmes, for which community-based strategies are deemed indispensable.

Conclusions

To our knowledge, no aedic index research had previously been published in Spain or even Europe. The identification of the most productive breeding sites is considered key for the development of environmental education and awareness campaigns. In this study, we found that an isolated breeding site accounted for more than half of the total identified *Ae. albopictus* larvae in this residential area, and should be taken into consideration during the design phase of any control programme in the area. Irrigation tanks, flowerpots and animal drinkers were found as additional important productive sites. Consequently, the implementation of routine aedic index studies that comprise the identification of domestic breeding sites is highly recommended in infested areas of Southern Europe. Twenty years after its first detection in the country, the control of this species is still extremely challenging and requires strategies that necessarily involve the community.

Author contributions. Pedro María Alarcón-Elbal: conceptualisation, data curation, investigation, methodology, supervision, project administration and writing – original draft preparation, review and editing. Marcos López-de-Felipe: conceptualisation, data curation, investigation, methodology, formal analysis and writing – original draft preparation, review and editing. Ignacio Gil-Torró: conceptualisation, funding acquisition, methodology, project administration, supervision and writing – original draft preparation, review and editing. Isaac García-Masiá: funding acquisition, project administration and writing – review and editing. Pilar Mateo-Herrero: funding acquisition, project administration and writing – review and editing. Rubén Bueno-Marí: funding acquisition, project administration and writing – review and editing.

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