

ENTOMOLOGY

Monitoring of *Aedes albopictus* (Skuse) (Diptera, Culicidae) in the city of Catania (Italy): seasonal dynamics and habitat preferences

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Abstract

Estimated seasonal abundance of the Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894) (Diptera, Culicidae) in the municipality of Catania (eastern Sicily, Italy) is provided and discussed. A monitoring campaign was carried out in urban and suburban sectors of the city during the autumn of 2007 and all of 2008 and 2013. Populations of the mosquito were surveyed weekly with standard ovitraps as a tool for estimating its population density. Analysis of the distributional data, seasonal occurrence, habitat preferences, and egg-laying intensity of the mosquito is provided.

Introduction

Over the last 30 years, seven alien mosquitoes (Diptera, Culicidae) have been found in Europe (Kenis & Branco, 2010)

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and, among the genus *Aedes*, the following species have been intercepted: *Aedes aegypti* (Linnaeus, 1762), *A. albopictus* (Skuse, 1894), *A. atropalpus* (Coquillett, 1902), *A. japonicus* (Theobald, 1901), *A. koreicus* Edwards, 1917, and *A. triseriatus* (Say, 1823) (DAISIE, 2018; ECDC, 2018).

During this last decade, preeminent importance has been devoted to the Asian tiger mosquito, *Aedes albopictus* (Diptera, Culicidae), in relation of its threatened capability to human health. This species, native to tropical and subtropical forests in south-eastern Asia, has spread from its original areas to most countries in all continents through shipments of used tires (Romi, 2001; Severini *et al.*, 2009). Dispersal capabilities (passive transport) of adults of this species in cars and trucks have been recently confirmed and investigated by Eritja *et al.* (2017). *Aedes albopictus* is well adapted to a temperate climate and has already invaded a large part of southern Europe and is now spreading northwards (Cunze *et al.*, 2016). The diffusion of the Asian tiger mosquito in Europe has raised serious concerns due to its possible role in the transmission of viruses (Flaviviridae: Flavivirus) and filarial nematodes (Onchocercidae: Spirurida) (Masetti *et al.*, 2008; Romi *et al.*, 2009a). Nematodes *Dirofilaria immitis* (Leidy) and *D. repens* (Railliet & Henry) are the etiological agents of canine heartworm and dog subcutaneous granulomas, respectively; both species can cause parasitic zoonosis: accidental infections in humans may result in subcutaneous, conjunctival, and pulmonary nodules (Pampiglione *et al.*, 2001). This species is a vector of exotic disease agents introduced through the circulation of infected humans and animals, e.g. many arboviruses such as Dengue, Yellow fever, West Nile, and Chikungunya (Romi *et al.*, 2009b). From 2004, this mosquito has become the principal vector in a large epidemic of Chikungunya fever in tropical Asia and Africa (Enserink, 2007; Weeratunga *et al.*, 2017).

After nearly 20 years in Italy, *A. albopictus* was responsible for the first Chikungunya virus (Togaviridae, gen. *Alphavirus*) epidemic in Europe (Bonilauri *et al.*, 2008) in 2007 in the Emilia-Romagna region, causing more than 250 human cases of a febrile illness with benign outcome (Rezza *et al.*, 2007). More recently, in the autumn of 2017, other autochthonous infection cases of Chikungunya have been ascertained in Italy in the cities of Anzio and Rome in the Latium region and in the town of Guardavalle Marina in the Calabria region. In the European Union, in addition to Italy, only France has reported outbreaks of indigenous cases of Chikungunya in the past (*i.e.* in 2010, 2014, and 2017) (Fortuna, 2017). Infections of *D. immitis* have been reported for natural populations of *A. albopictus* in central and

northern Italy (Cancrini *et al.*, 2003a,b; Cancrini *et al.*, 2007), and confirmation of the role of *A. albopictus* in the circulation of dog heartworm in Italy (northern regions) was provided by Masetti *et al.* (2008). More recently, specimens of *A. albopictus* from different areas of Italy were found infected with the West Nile virus, the causative agent of a zoonosis that is also becoming common in the country (Rizzo *et al.*, 2009).

The aim of our study was to evaluate the seasonal abundance of the Asian tiger mosquito in the municipality of Catania (eastern coast of Sicily, Italy) through oviposition responses to standard ovitraps during all seasons of the year.

Diffusion of *Aedes albopictus* in Italy

The first record of *A. albopictus* in Italy was in the city of Genoa (Sabatini *et al.*, 1990), followed a year later in Padua (Dalla Pozza & Majori, 1992). The colonisation of Padua was caused by the arrival of one or more loads of infested tires from the United States of America, and in fact all the Italian populations have a high genetic affinity to those from the United States and Japan (Dalla Pozza *et al.*, 1994). The rapid spread of *A. albopictus* in Italy is probably the result of several successive introductions, each with large numbers of individuals (Romi *et al.*, 1999; Urbanelli *et al.*, 2000). The Asian tiger mosquito in Italy spread first in the Po Valley (especially in the northeastern sector); it then continued in the direction of Bologna and then Florence, to the centre of Italy, first colonising the Tyrrhenian coast (Knudsen *et al.*, 1996; Romi, 2001), then the Adriatic coast, and finally entering southern Italy and Sicily (Romi, 2005). Stable colonies of *A. albopictus* have been identified in Italy since the early 1990s (Sabatini *et al.*, 1990; Dalla Pozza & Majori, 1992; Romi 1994). The species is currently present in all regions of Italy (Severini *et al.*, 2009; Romi *et al.*, 2012). It was recorded in Sardinia for the first time in the city of Cagliari in 1994-95, (Romi, 1995; Nuvoli & Pantaleoni, 2003), but the prompt intervention of the local public-health agency eradicated the introduced population. In autumn 2006, the Asian tiger mosquito was detected again on islands in the two port cities, Cagliari and Olbia (Cristo *et al.*, 2006). *A. albopictus*

was recorded for the first time in Sicily in 2003, in the city of Palermo (Liotta & Matranga, 2004).

Materials and Methods

Field observations were conducted in the urban and suburban areas of Catania in the autumn of 2007 and subsequently for all of 2008 and 2013. In 2007, ovitraps were allocated in ten sectors of the municipality (Figure 1), while in 2008 and 2013, *A. albopictus* was monitored only in the sector with the highest peaks of the population in 2007 (Table 1). The monitoring used standard ovitraps (Celli *et al.*, 1994; Bellini *et al.*, 1996), consisting of a black conic plastic cup (~400 ml capacity, upper diameter 8 cm, lower diameter 6 cm) filled 2/3 with water (~300 ml), with a masonite strip (12.5×2.5 cm) fastened to the inner edge to provide a suitable surface for oviposition (Albieri *et al.*, 2010). A small hole above the water level (300 ml) prevented the cups from being completely filled with water after a rain, which would reduce the ovipositional surface to zero.

Three ovipositional traps were placed in each station investigated during 2007 (for a total of 30 ovitraps) separated by at least 100 m. Ovitrap were placed in shaded sites, at ground level, and with free space above them of at least 50 cm. Two ovitraps were placed in the monitored sector in 2008 and 2013, which remained in the same location all year, one in microhabitat conditions of *partial shade* at the base of a citrus tree, the other with the same microhabitat conditions but in contact with a building. The distance between the two ovitraps was 20 m. The masonite strips with *A. albopictus* eggs were collected weekly, individually protected in transparent plastic bags, labelled (date, trap, and sample number), and brought to the laboratory for counting the eggs under a stereomicroscope. After each check, the masonite strip and water were replaced, and the ovitraps were washed with water.

Data on weather conditions (average weekly temperature) were recorded at the Fontanarossa meteorological station (weather station 228 Catania, 37.263006 N, 15.4322 E).

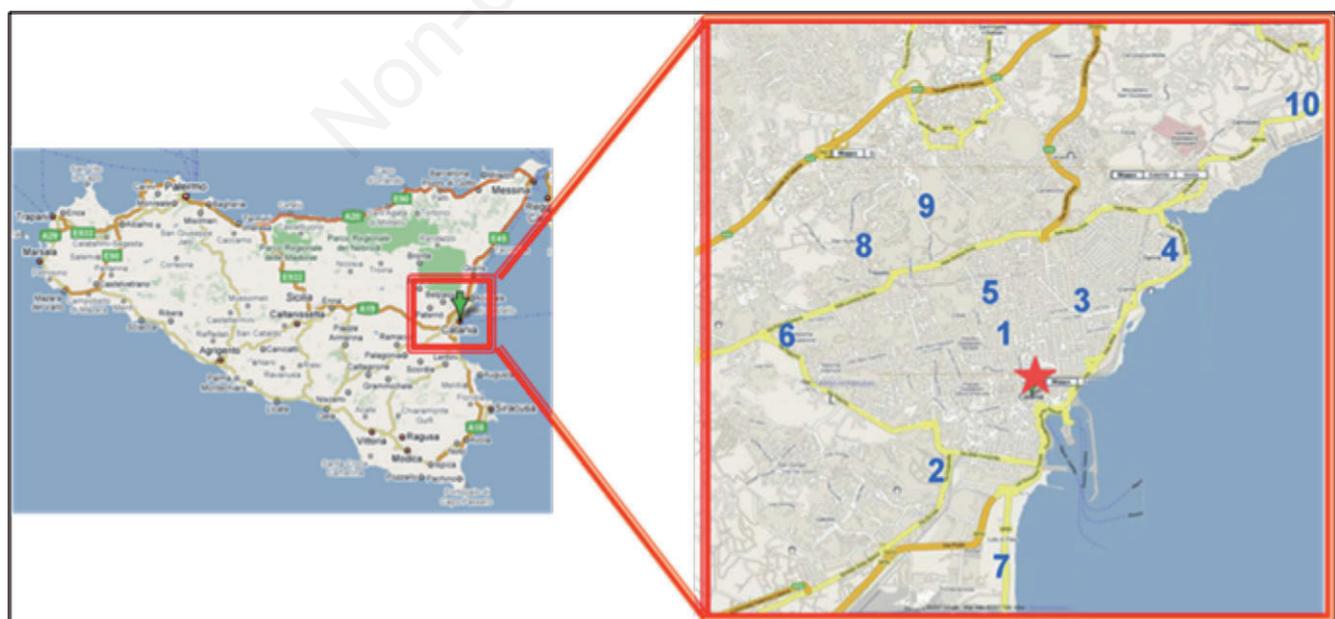


Figure 1. City map of Catania showing the ten sites investigated (ovitrappositions) in 2007.

Sampling sites

In the autumn of 2007, ovitraps were allocated in ten sectors of the municipality in urban areas with high population concentrations (sites I, III, and V), suburban areas (sites IV, VI, VIII, and IX), and semirural areas (sites II, VII, and X) (Figure 1). The data for egg density collected by the ovitraps in the 2007 season were used to assess the mosquito populations and to identify the area of the city with an increased presence of the mosquito for monitoring in subsequent years. Some of the sites were chosen because they represented preferred habitats in human environments, as reported in previous studies (Bartlett-Healy *et al.*, 2012; Bonacci *et al.*, 2014), such as nurseries (site IV), cemeteries (site II), public and private parks, and gardens (sites I, V, VII, and VIII) (Table 1). The ovitraps were placed in an altitudinal range varying from sea level to 110 m a.s.l. In 2011 and 2013, ovitraps were positioned in optimal microhabitat conditions: green, semi-shaded positions, as defined in Bartlett-Healy *et al.* (2012), and Crepeau *et al.* (2013), easily accessible on the ground, with an upper free space of about 50 cm, and were maintained unchanged until the end of the flight season and throughout the two years of investigation.

Results

Our field data showed that the presence of *A. albopictus* in the city of Catania remained at worrisome levels for most of the year. This species, due to favourable climatic conditions in Sicily, not only in the hottest months of the year, was also abundant in late autumn and, although with lower density, also in spring. In fact, the mosquito was absent only from December to March. At the beginning of the investigation in 2007, the mosquitoes were absent from only three of the ten sectors in the cities affected by the census (III, VI, and VIII) (Figure 2).

In the 2007 season, the largest number of eggs was detected at site V (old citrus orchard) (Figure 2). It was located in a dense residential district in the north of the city and featured large gardens. This confirmed the findings of other authors of the preference of this species for green areas, mostly with the presence of trees for increased populations (Crepeau *et al.*, 2013). At site V, judging by the number of eggs, they followed the ovitraps allocated to sites VII (Boschetto Playa) and X (Nursery), about 100 and 300 m from the coast, respectively. Both areas were characterised by extensive plant cover, trees at site VII and shrubs at site X (Figures 3 and 4).

In the following two years of monitoring, in 2008 and 2013,

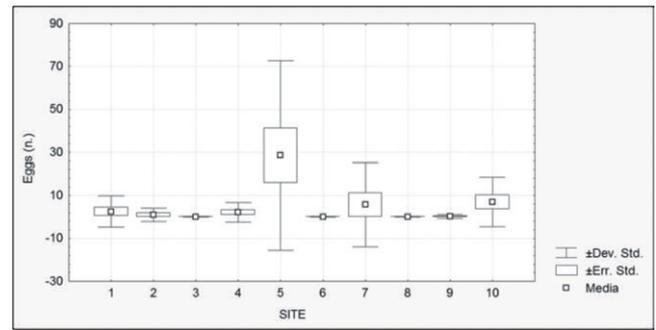


Figure 2. Mean number of eggs of *A. albopictus*, layed in the selected sites during 2007.

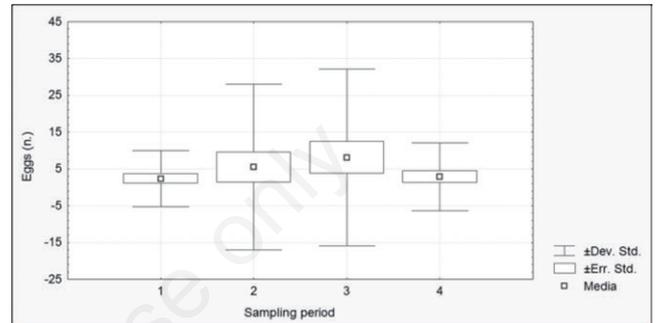


Figure 3. Mean number of eggs of *A. albopictus* layed during the four sampling period carried out in 2007.

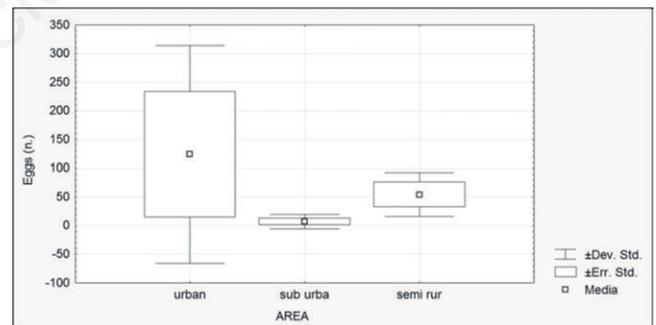


Figure 4. Mean number of eggs of *A. albopictus*, layed in the ovi-traps in the three different areas considered in 2007.

Table 1. Code traps, area where the traps were positioned and number of traps allocated per site.

Code trap	Sites	Localities	Zone of city	No. traps
I	Bellini garden	via Etna	Urban area/Central sector	3
II	Cemetery	via Acquicella	Urban area/South-West sector	3
III	Primary school	corso Italia	Urban area/Central sector	3
IV	Ognina	via Messina	Suburban/North-East sector	3
V	Old Citrus orchard	Cibali	Urban area/North sector	3
VI	Nesima superiore	via Palermo	Suburban/West sector	3
VII	Boschetto Playa	viale Kennedy	Suburban/South sector	3
VIII	Parco degli ulivi	San Nullo	Suburban/North-West sector	3
IX	Agrarian Faculty	via Santa Sofia	North sector	3
X	Nursery	Cannizzaro	Suburban/North-East sector	3

surveys continued only at site V, which had the largest number of eggs in 2007. The difference in preference in microhabitat by *A. albopictus* for oviposition was evident in fact, in both years of the investigation, the preference was for the ovitrap positioned at the base of the tree compared to the other with the same microhabitat but in contact with a building (Figures 5 and 6).

As Figures 5 and 6 show, the start and end of the life cycle of *A. albopictus* coincided both in 2008 and 2013, and oviposition began from the third week of April, with weekly temperatures of 17-18°C in both years; in the cold season, however, the end of the life cycle was in the third week of November in 2008 and in the second week of November in 2013, with average weekly temperatures of 14°C in both years.

In the two years of investigation, the main peak of oviposition was recorded at different times of the year (Figures 5 and 6), between the third week of June and the second of July (average weekly temperatures of 23-30°C) and between the second week of September and the second of October (average weekly temperatures of 19-22°C).

The total number of eggs deposited over the two years was noticeably different (Figure 7). In fact, the number of eggs deposited in 2008 was 3947 in the *tree ovitrap* and 1828 in the *building trap*; in 2013, there was a net increase, 7365 in the *tree ovitrap* and 1702 in the *building trap*.

Discussion and Conclusions

Good surveillance is the foundation of informative field epidemiology and effective programmes to control vectors or nuisance mosquitoes (Romi *et al.*, 2009a). About 25 years after the first finding of *A. albopictus* in Italy, the phenology of the species was well-known (Romi *et al.*, 2012); in fact, the flight time runs from February-March to October-November in the various regions depending on latitude and seasonal climate change.

Mosquito control is mainly directed against larvae and, only if necessary, against adults. *Bacillus thuringiensis israelensis* (Bti) has good larvicidal activity (Weeratunga *et al.*, 2017) and is often used because it is considered to be harmless to humans and other non-dipteran insects. Unfortunately, the insecticidal activity of Bti is lost after 24 hours, and it is completely ineffective against eggs and pupal stages of *A. albopictus* (Macchioni *et al.*, 2008). There is a need to develop efficient and eco-sustainable control, that must take into account the possible occurrence of insecticide resistance tools (Medlock *et al.*, 2015). Eritja *et al.* (2005) reported that the suitable areas for *A. albopictus* must have: i) mean rainfall more than 500 mm per year, ii) more than 60 rainy days (0.1 mm rainfall minimum each) per year, and iii) mean yearly temperatures higher than 11°C. Catania's climate satisfies the requirements of temperature and mean rainfall per year but is at the limit for the number of rainy days per year (on average 56 days).

In the field trials, the ovitraps were effective in attracting ovipositing *A. albopictus*. The data from the ten sites investigated were useful for the study of abundance, distribution, ecological preferences, and fluctuations in *A. albopictus* infestations in different areas of the city of Catania and in all seasons of the year. Data on changes in infestation in the urban and suburban areas of the city were in line with those for other Italian cities (Celli *et al.*, 1994; Romi, 1995; Nuvoli & Pantaleoni, 2003; Toma *et al.*, 2003; Brianti *et al.*, 2008; Severini *et al.*, 2008; Bonacci *et al.*, 2014) and were related to the presence of optimal environmental conditions such as temperature, air humidity, large green and shady spaces, and availability of water of anthropic origin.

In the areas of the planet where *A. albopictus* has arrived, numerous biotic and abiotic factors have been investigated in an attempt to identify variables associated with the productivity of the species (Bueno-Marí & Jiménez-Peydró, 2015). In the northeastern United States, Bartlett-Healy *et al.* (2012), based on pupal abundance and container types, showed that tires, trash cans, and planter dishes were the most important containers for *A. albopictus* oviposition, and black and grey were the container colours that played a role in the selection of sites by females. Significant differ-

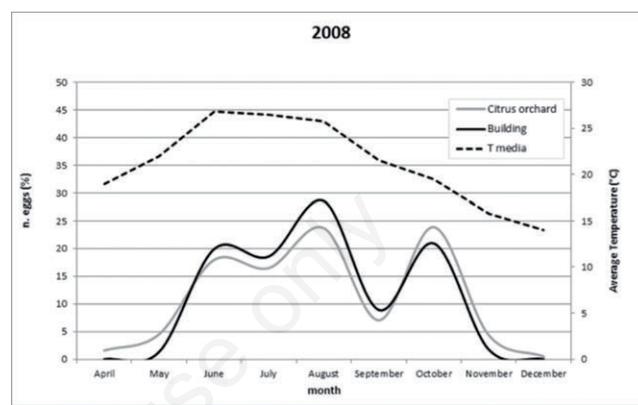


Figure 5. Oviposition trend of *A. albopictus* during 2008.

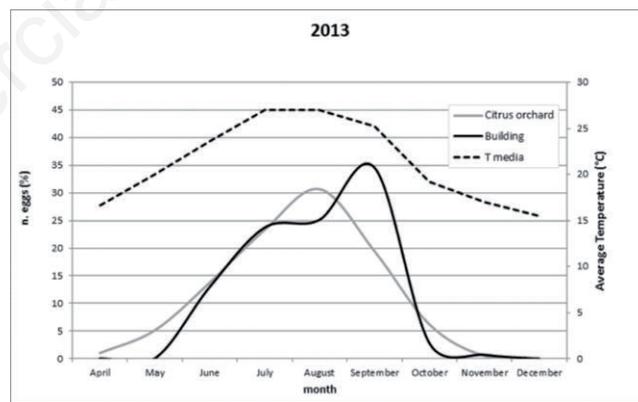


Figure 6. Oviposition trend of *A. albopictus* during 2013.

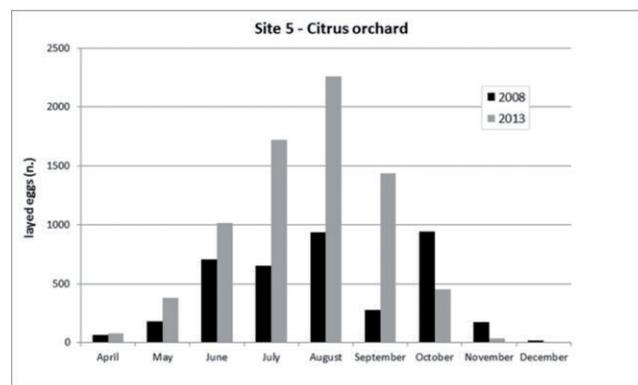


Figure 7. Oviposition trend of *A. albopictus* throughout the two years of investigation.

ences in abundance of the species were also detected between urban, suburban, and rural areas: *A. albopictus* was much more abundant in urban and suburban habitats.

The control programs for invasive species need to have standards in data collecting and data analysis in order to compare results for different ecological and geographical sites (Suma *et al.*, 2014; Nejati *et al.*, 2017). The new statistical analysis of monitoring data of *A. albopictus* proposed by Mazzei *et al.* (2014) that considered the *effort of sampling* to estimate seasonal abundance to the mosquito is thus interesting. Considering the primary role in recent Dengue and Chikungunya outbreaks that makes *A. albopictus* a very important public-health threat throughout the world, and in function of its particular ecological plasticity (*e.g.* in terms of larval breeding sites, feeding behavior, and climatic adaptation), it is easy to comprehend the importance of a better understanding and of monitoring the spread of this species throughout our country in order to limit the expansion of its range and impact on human health.

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