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# *Aedes (Stegomyia) albopictus* (Skuse) (Diptera: Culicidae) in Malta – the first winter

Paul Gatt[1], Francis Schaffner[2] and Louis F. Cassar[3]

[1]Research Associate, Institute of Earth Systems, Chemistry Building 3rd Floor, University of Malta, Msida MSD 2080, Malta. Email: paulgatt@onvol.net
[2]Avia-GIS, Risschotlei 33, 2980 Zoersel, Belgium & Institute of Parasitology, University of Zurich, Zurich, Switzerland. Email: fschaffner@avia-gis.be
[3]Institute of Earth Systems, Chemistry Building 3rd Floor, University of Malta, Msida MSD 2080, Malta. Email: louis.f.cassar@um.edu.mt

## Abstract

Subsequent to the discovery of *Aedes albopictus* (Skuse) in Malta in September 2009, an attempt was made to investigate the mosquito's presence and behaviour during the winter following its discovery. The objective was to determine if the species had survived its first winter, and, if so, whether adults and/or immature stages were active during that period. Oviposition was last observed at the end of October. Larvae and pupae developed in the ovitraps throughout the winter and adult emergence of *Ae. albopictus* continued despite the apparent absence of adults in field surveys during this period. Control measures may therefore be applied continuously throughout the year. Adult biting activity in winter was not observed in this study. Should this be documented in future, however, then potential transmission of arboviruses like chikungunya and dengue must be considered possible throughout the whole year.

## Keywords

Mosquito, *Aedes albopictus,* Malta, first winter, survival, development.

## Introduction

The invasive species *Aedes albopictus* (Skuse, 1894) (*Stegomyia albopicta* sensu Reinert & Harbach, 2005) was first discovered in Malta in September 2009 in an isolated focus in Mellieha in the north of the island (Gatt *et al.,* 2009) and was soon after also reported from Marsascala in the east of Malta (Buhagiar, 2009). Its origin and the mode and time of its introduction are not known. Previous investigations before September 2009 (Gatt *et al.*, 2009; Schaffner *et al.,* 2010 *in press*), including the use of specific ovitraps as well as fieldwork in the precise area where the mosquito was eventually first reported had not provided any evidence of its occurrence. There is some indication, however, suggesting that the species may have been present earlier in the eastern region of the main island, possibly since June 2009 (A. Taliana, *pers. comm.*). Whether it was introduced separately to both geographical areas almost simultaneously or whether it was transported by vehicles from the east to the northern locality remains unknown.

Following the discovery of a few adult specimens in September 2009, an attempt was made to gather data on the abundance and breeding activities of the mosquito during the winter months, in order to determine whether it would establish and survive its first Maltese winter and, if so, in what stage or stages it would survive the cold, wet season. The objective was to provide information necessary to assess the risk of establishment and further spread of the species. Maltese winters are mild, with mean monthly temperatures of around 12°C, although short cold winter snaps with a minimum night time temperature of 1-3°C can occur in December and January (Cutajar *et al.,* 1992). A winter isotherm of between -3° and 0°C is

considered a limiting factor for an establishment of the species (Nawrocki & Hawley, 1987), a condition not known to occur during Maltese winters. Also the climate in Malta appears to be favourable for the species' establishment according to both a statistical random forest model and a Multi Criteria Decision Analysis based on expert advice recently published by the European Centre for Disease Prevention and Control (ECDC, 2009). Besides this, the observation of winter activity of larvae and/or adult females is important not only for the risk assessment of arbovirus transmission but also for the planning of mosquito control activities. This becomes even more crucial considering the recent occurrences of chikungunya and dengue transmissions by *Aedes albopictus* in regions of southern Europe (Italy, 2007 (Rezza *et al.* 2007); France and Croatia, 2010 (www.promedmail.org)).

## Materials and Methods

Oviposition traps were set up at a site in Ghajn Zejtuna, Mellieha in the the north of Malta, where adult mosquitoes were first discovered in September 2009 (Gatt *et al.*, 2009) and monitored between 21st of October 2009 and 25th of April 2010, i.e. for a total duration of 27 weeks. The research site covers approximately $20m^2$ in area and is semi-permanently shaded by surrounding overgrown gardens and some derelict land on which dense thickets of semi-natural vegetation are present. Immature stages of the mosquito had previously been found to develop in low numbers in a small cistern located approximately within the central segment of the site. Five plastic ovitraps each measuring 20 x 20 x 20 centimetres and containing approximately two litres of water were placed at ground level amidst shaded vegetation in a circle around the cistern. A piece of polystyrene measuring 2 x 5 x 10 centimetres was left floating on the surface of each ovitrap to serve as oviposition support. The support was subject to repeated natural inundation by rainfall during winter.

In addition, two BG-sentinel™ traps baited with BG-lure™ (one with additional UV-light) and two CDC light traps (without carbon dioxide) were operated continuously on site for ten weeks between the 17th of February and the 25th of April 2010. The site was visited at one to three weekly intervals during which adult mosquitoes were searched for in the cistern and surrounding undergrowth both on site and the neighbouring area, hand-netted and counted. The oviposition support and water in the ovitraps were inspected for immature stages and the adult traps emptied. Developing pupae were removed from the ovitraps to prevent egress of emerging adults. Some larvae were also moved to the laboratory and reared to maturity under similar conditions in order to provide precise data on development times.

Climate data were obtained from the following online sources:
http://www.maltaweather.com/archives.shtml; http://www.tutiempo.net/en/Climate/Luqa/165970.htm

## Results

Adult females laid eggs in all five ovitraps as evidenced by the appearance of larvae. In one of the ovitraps, one batch of eggs was laid directly on the oviposition support, allowing for more detailed observations on egg hatching and larval development of this cohort.

Field and laboratory observations are pooled together and summarised in Table 1.

Adults

Adults of both sexes were noted flying and feeding in the area in considerable numbers until the end of October 2009 with 35 specimens being hand-netted during two walkover surveys in the last week of October. A sharp decrease in numbers was observed in mid-November, and no further adults were observed in the field until the end of the study period. One specimen was collected in a BG-sentinel™ trap at the end of April 2010. Despite the apparent absence of adult mosquitoes during winter it is evident that adult emergence continues in small numbers throughout winter and spring (see discussion below). Peak adult emergence from diapausing autumn eggs occurred between 15 and 25 April 2010.

Eggs

The last (autumn) eggs (c. 100) were laid at the end of October 2009 (mean air temperature 21$^{o}$C, 11 daylight hours). Hatching of these eggs occurred in installments. A few of the eggs did not enter diapause, but hatched shortly after oviposition to produce early winter adults. Most of the eggs, however, entered diapause and hatched in early February 2010 (mean temperature 14.5$^{o}$C, 10.5 daylight hours). No unhatched eggs remained by the end of March 2010. The first (spring) eggs appeared at the end of April 2010 (mean temperature 17.3$^{o}$C, 13.5 daylight hours).

Larvae

Larvae (and pupae) were present in the ovitraps throughout the winter. Only a very few older (3$^{rd}$ and 4$^{th}$ instar) larvae from hatching of non-diapausing eggs were observed between mid-December to early January. First instar larvae from hatching of diapausing autumn eggs first appeared in early February, increasing in number from mid-February onwards. Some 4$^{th}$ instar larvae collected in late February pupated later than other 4$^{th}$ instar larvae in the same cohort collected earlier in the month. The last 4$^{th}$ instar larva from the autumn cohort completed its development at the end of April 2010.

**Discussion**

The main conclusion to be drawn from these preliminary observations is that local climatic conditions prevalent during the wet season (colder winter months) do not prevent larval development and adult emergence of *Ae. albopictus* in Malta. It is expected that the mosquito will continue to breed throughout the year although possibly in markedly lower density and with little (or no) adult activity during winter.

Oviposition was not observed to occur after the end of October, probably because the number of gravid females in the study area was too small to detect eggs in the traps. In Italy (Rome), egg laying has been demonstrated to continue throughout winter (Romi *et al.,* 2006) although no eggs were reportedly hatched after mid-December and larvae do not seem to develop in winter. The present study has shown that diapausing eggs hatched when the mean temperature was 14.5$^{o}$C and the length of daylight hours was 10.5 hrs.

Larval development clearly slowed down during winter (February 2010, mean temperature 14.5$^{o}$C) in some 4$^{th}$ instar larvae, suggesting a climatic induction of larval diapause. Larval development in *Ae. albopictus* has been recorded to cease at temperatures below 11$^{o}$C (Udaka, 1959, in Hawley, 1988).

These observations indicate that if control measures are to be implemented in the future, they may be applied throughout the whole year. Larvicide sprayings could be applied at longer intervals during the winter period as larval development takes longer. Given that larvae in diapause will feed less, however, it may be expected that the efficiency of larvicides that require ingestion, like *Bacillus thuringiensis israelensis* might be reduced. Adult biting activity in winter was not observed in this study possibly because the number of circulating gravid females was too low. Should this be documented in the future, however, then potential transmission of arboviruses like chikungunya and dengue must be considered possible throughout the whole year.

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TABLE 1. Pooled field and laboratory observations on a breeding population of *Ae. albopictus* in Malta between 21st October 2009 and 25th April 2010.

| Date of visit | Adults on site | Adults in traps | Eggs on support | Larvae in ovitraps/Lab |
|---|---|---|---|---|
| 21st Oct 2009 | 13 ♀♀, 7 ♂♂ | - | - | - |
| 27th Oct 2009 | 10 ♀♀, 5 ♂♂ | - | Eggs noted (c. 100) | - |
| 15th Nov 2009 | 2 ♀♀ | - | - | - |
| 24th Nov 2009 | - | - | - | - |
| 10th Dec 2009 | - | - | - | one 3rd instar<br>two 4th instar<br>one pupated 17th Dec<br>1 ♀ 21st Dec 2009 |
| 22nd Dec 2009 | - | - | - | one 4th instar<br>pupa 28th Dec<br>1 ♂ 2nd Jan 2010 |
| 07th Jan 2010 | - | - | - | three 3rd instar<br>two 4th instar<br>pupae 25th January<br>1 ♂ and 1 ♀ 2nd Feb 2010 |
| 02nd Feb 2010 | - | - | 80% of diapausing eggs hatched | two 1st and 2nd instar<br>two 4th instar<br>one pupated 9th Feb<br>1 ♂ 14th Feb 2010 |
| 17th Feb 2010 | - | - | Further egg hatching noted | numerous larvae, all instars<br>two 4th instar<br>one pupated 23rd Feb<br>1 ♂ 2nd March 2010 |
| 04th Mar 2010 | - | - | Almost all eggs hatched | numerous larvae, all instars<br>two 4th instar<br>pupa 12th March<br>1 ♂ 18th March 2010<br>pupa 21st March<br>1 ♀ 27th March 2010 |
| 27th Mar 2010 | - | - | No unhatched diapausing eggs remain | larvae, 2nd and 4th instar |
| 25th April 2010 | - | 1 ♂ in BG-sentinel™ | First (spring) eggs noted | last 4th instar larva<br>pupa 25th April<br>1 ♀ 30th April 2010 |

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