Monitoring of *Aedes albopictus* (Diptera, Cilicidae) in Calabria, Southern Italy

Teresa Bonacci[1], Antonio Mazzei[2], Vesna K. Hristova[3], M. Ayaz Ahmad[4]

Abstract — A control program currently active in Calabria (southern Italy) was carried out to population density estimation of *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894) (Diptera, Culicidae). For the first time we report on the spatial and temporal (seasonal) distribution of the mosquito in the urban area of Cosenza and Rende by ovitrap, from May, 2013 through the mid October, 2013. In spring and summer season 2013, ovitraps were activated according to standard and guidelines and were checked on average weekly. The peak of adult abundance occurs from July to first part of October. The aim was assess the mosquito population's aggregation degree, through the application of the Taylor's power law and to study the distribution and phenology of this mosquito.

Index Terms — population density, spatial and temporal (seasonal) distribution, *Aedes albopictus*.

1 Introduction

Aedes albopictus (Skuse) (Diptera Culicidae) is a mosquito which has spread from its original areas in Asia to the rest of the world through shipment of used tires. The species considered as the most invasive species of mosquito in the world, was introduced in Italy in the 1990.

Ae. albopictus disperse at a maximum distance comprised in the range of 600-800 m [1, 2] and its biology is related to water-filled containers distributed throughout urban and suburban landscapes [3] -. Eggs hatch, within few days after immersion during the warm months. The duration of life cycle is temperature dependent and the adult may take from 6 to 10 days. Its distribution was observed in urban, suburban and natural areas [4].

The data about the distribution of *Ae. albopictus*, the primary vector of CHIKV in Italy [5], were obtained from control programs carried out in many Italian regions [6-13] and in these investigations GIS (Geographical Informatics System) provided important contribution on spatial distribution of vector-borne diseases [14, 15]. Any data were reported for the Calabria region.

In fact, the lack of data on the distribution and abundance of the vector in this region was the reason to:

(*i*) to study the spatial and temporal (seasonal) distributions *A. albopictus* in two urban areas of Calabria based on the egg density and;

(*ii*) to evaluate the habitat preference and phenology of the species in the sampling areas.

1. Dipartimento di Biologia, Ecologia e Science Delta Terra, Universita Delta Calabria, Via Pietro Bucci, 87036 Rende, CS, Italy.
E-mail: teresa.bonacci@unical.it
2. Dipartimento di Biologia, Ecologia e Science Delta Terra, Universita Delta Calabria, Via Pietro Bucci, 87036 Rende, CS, Italy.
E-mail: mazzeiant@gmail.com
3. Faculty of Biotechnical Sciences, University of St. Clement Ohridski, Partizanska, bb 7000 Bitola, Macedonia. E-mail: v.primavera79@gmail.com
4. Physics Department, Faculty of Science, P.O. Box 741,
University of Tabuk - 71491, Saudi Arabia. E-mail: mayaz.alig@gmail.com

This investigation is part of a project financed by Calabria Region and developed in collaboration with the Department of prevention of Provincial Health Authority of Cosenza (ASP). The aim of this project was to develop Aedes albopictus monitoring, at low cost in Southern Italy.

2 Materials and Methods Used

2.1 Sample Collections and Analysis

Calabria Region is situated in the South of Italy and is characterized by Mediterranean climate with warm summer and low rainfall. This study was carried out in 2013 in urban and peri-urban areas of two towns; in Cosenza (39°19′28.80′′ N, 16°14′45, 40′′E) with a population of 70.068 residents and a total urban area of ca. 3.724 ha of surface and Rende (39°20′29,78′′N, 16°11′ 24,29′′E) with a population of 35.488 residents and a total urban area of ca. 5.479 ha of surface. Both cities are situated at an altitude of 180-200 m asl. The ovitrap used in this investigation consist in a black plastic container (800 ml capacity) equipped with a masonite strip (2.5 x 20 cm). The traps were used and positioned vertically to provide at gravid females a surface for oviposition. In order to standardize the environmental parameters and to avoid differences in the attractiveness, the ovitraps were placed in the suitable habitats for the presence of mosquito. In the field, 500 cc of dechlorinated water was used to activate the traps. Every week the strip was replaced and the cup carefully cleaned. The strips were examined by stereomicroscope at laboratory of Entomology at University of Calabria for eggs counting. In the two sampled areas, the ovitraps were distributed inside 27 stations (Table 1). Ovitraps were georeferenced in the field by using Gps-Garmin and identified with a code. The ovitrap density was decided upon according to the Emilia Romagna guidelines and without previous monitoring data [16] . Two traps were positioned in each site at a distance of 150-200 m from each other. Some detail of sample collections have been depicted in Table 1 in terms of environment effect.

2.2 Statistical Approach

The aggregation degree and the statistically significant

sample size of *Ae. albopictus* in the sampled areas, was calculated first according to the Taylor's equation [17-19].

We used the variance (s^2) that is related to the mean density

TABLE 1

DETAILS OF SAMPLED AREAS WITH TYPE OF ENVIRONMENT INDICATED AS CLASSES

Regions / Sample areas	Type of environment	Classes
Contrada Molicelle / Ponte Bucci	Campus universitario	5
C.da Cutura	Torrente Emoli, Vivaio	6
C.da Maio	Area rurale con Allevamento bovini	6
C.da Serra d'Ulivo / Via Campania	Area Rurale	6
C.da Surdo	Torrente Surdo - Giardino comunale Ortomatera	3
Viale Busento - Stazione FS	Verde urbano	2
Piazza G. Mancini	Centro urbano/stazione FC centrale	2
Viale Trieste/villa comunale	Verde urbano	3
Via Rodi	Area fluviale Fiume Busento	7
P.zza 15 marzo/villa comunale	Verde urbano	3
Via Dante Alighieri/lungo Crati	Area fluviale Fiume Crati	7
Via Riccardo Misasi	Centro urbano/verde urbano	3
Via Roma	Centro urbano/scuola Zumbini	2
Via Padre Giglio / villa comunale	Villa comunale	3
Viale Sergio Cosmai	Parco urbano Robinson	4
Viale Giacomo Mancini	Parco urbano Nicolas Green	4
Via Verdi	Verde urbano nei pressi torrente Surdo	2
Via G. Donizetti	Parco Fluviale Torrente Emoli	7
Quattromiglia/ Via A. Volta	Centro urbano/stazione di Castiglione	1
Quattromiglia/Via L. Da Vinci	Centro urbano/verde urbano	3
C.da Rocchi/via F. Pizarro	Area rurale / Ist. Agrario "Todaro"	6
Arcavacata/c.da Vermicelli	Area rurale	6
Campus Universitario / via Savino	Orto Botanico Unical	4
Ospedale Civile "Anunziata"	Centro sanitario	1
Viale F. Falcone	Villa comunale	3
Via Repaci	Area residenziale	5

(m) and which has been largely used to evaluate aggregation degree of insect population. The equation used is:

$$s^2 = a * m\ b \quad (1)$$

where, "*b*" is a constant for the species and measures data aggregation similar to VMR (Variance Mean Ratio); "*a*" is a constant depending on environmental conditions; *m* is the mean eggs density value.

The ratio between the variance and the mean of the number of eggs laid is indicated as VMR (Variance Mean Ratio). This value is often used to provide the information on the dispersal pattern of the species. If the VMR value is equal to 1 the species is randomly dispersed; lower than 1 when the species dispersal is uniform; higher than 1 when the species distribution is aggregated or clustered [20,21]. Than, the number of eggs was subjected to analysis using the I_U index (Eggs Intensity) [22];

$$I_U = n_i / us$$

where, "n_i" = eggs number; "**us** " = Effort of sampling.
us = Ntrap * (Ngg /7); **Ntrap** = Traps active for each station;
Ngg = exposure days of the traps.
The I_U consists in a statistical analysis of data which consider the "Effort of sampling" in each study area.

3 RESULTS AND DISCUSSIONS

In this year of monitoring the seasonal analysis showed three peaks of egg density; the first in the weeks 5 and 6, in the month of June; the second for weeks 10, 11 and 12 in the month of July and first part of the August; and the third from the week 14 to week 20, in end-August, September and the first part of October (Fig. 1). In Fig. 1, a statistical study of the the dynamics of seasonal population of the *Ae. albopictus* has been shown, based on the egg density in the years 2013. For this tasks, a total of 191.899 eggs were collected by 1.134 masonite strips that were examined within 21 weeks of sampling. The Variance Mean Ratio (VMR) of the mean number of eggs/ovitrap/week in 2013 was always found higher than 1. In fact, the data showed in figure 1, indicate an aggregated distribution of the mosquito population.

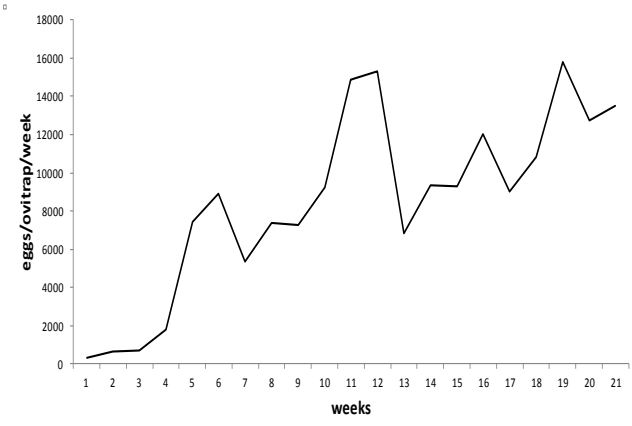

Fig.1. A statistical study of seasonal population dynamics of *Ae. albopictus* based on egg density for the years 2013.

Further, we calculated the Taylor's coefficients *a* and *b*, by the functional relationship between the variance (s^2) and the mean (*m*) of samples (on a logarithmic scale).
In Fi.g 2, the Taylor's power law regression line for the number

of eggs/ovitrap/week of *Ae. albopictus* collected by means of 1.134 masonite strips in 21 weeks in 2013 has been shown.The mean egg density inside the sample areas was also reported in the same Fig.2. All ovitraps are resulted positive on the presence of *Ae. albopictus*. The egg density calculated and also reported for each station of all collected samples in accordance to GIS map (Fig. 3). The mean egg number calculated over all traps placed in two urban areas for entire sampled period was 8506.6 ± 4770.4 eggs/ovitraps/all weeks. The highest mean egg density (± SD) in the weeks 1-21 was recorded in stations: 22 (553.1 ± 448.4); 23 (530.0 ± 377.7); 18 (502.9 ± 408.3); 25 (490.7 ± 354.0); 16 (488.7 ± 360.0), 17 (445.1 ± 363.4). The lowest value was registered in station 03 (192.0 ± 144.8). The peaks in the eggs laid was observed in July (weeks 10 and 11), in August (week 12), at end-September (week 19) and in early-mid- October (weeks 20 and 21). The Iu index considering the effective Effort of sampling show a different level of aggregation of the vector. The stations with a highest IU are: 17 (290,8); 13 (274,6); 28 (271,5); 23 (265,3); 18 (256,7), following by others that showed lower value of Iu.

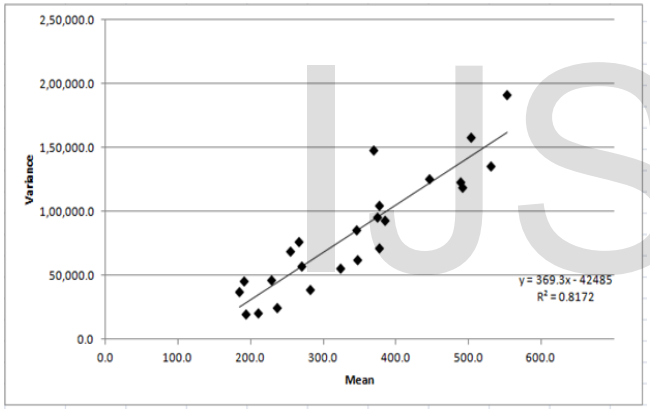

Fig.2. Taylor's power law regression line for the number of eggs/ovitrap/week of *Ae. albopictus* collected by means of 1.134 masonite strips in 21 weeks in 2013.

4 Conclusions

The rapid increase of population of *Ae. albopictus* in many countries has driven considerable researches that effort to develop effective surveillance tools for early detection of this invasive mosquito. The mosquito is a potential health threat, in fact it's a potential vector of multiple arbovirus and nematodes also in Italy [23- 25], even to countries that normally do not have endemic disease. As indicated above, all ovitraps are resulted positive to *Ae. albopictus* in the sampling period. The number of eggs remained higher for the entire study, with a lower level in the first part of July and mid-August. Moreover, the Iu index (Eggs Intensity) allows to overcome the inconveniences to different units of effort, as the number of ovitraps found active and, especially, the number of days of activity of each trap.

Our results indicated that the mosquito shows aggregated distribution with high level of abundance (Iu) inside the parks and in the urban green areas (17, 13 stations) where it finds refuge and sources of moisture constant and breeding-sites.

The *Ae. albopictus* population increasing during summer months with hight level of abundance until October. This data show that the epidemiological risk in Calabria exist and is very hight, because of the various habitats that characterize the urban and peri-urban areas, rich in humidity sources and artificial shelters . Finally, the use of IU index will overcome differences in time of exposure of the traps and enables to get a correct interpretation of the aggregation level of *Ae. albopictus*.

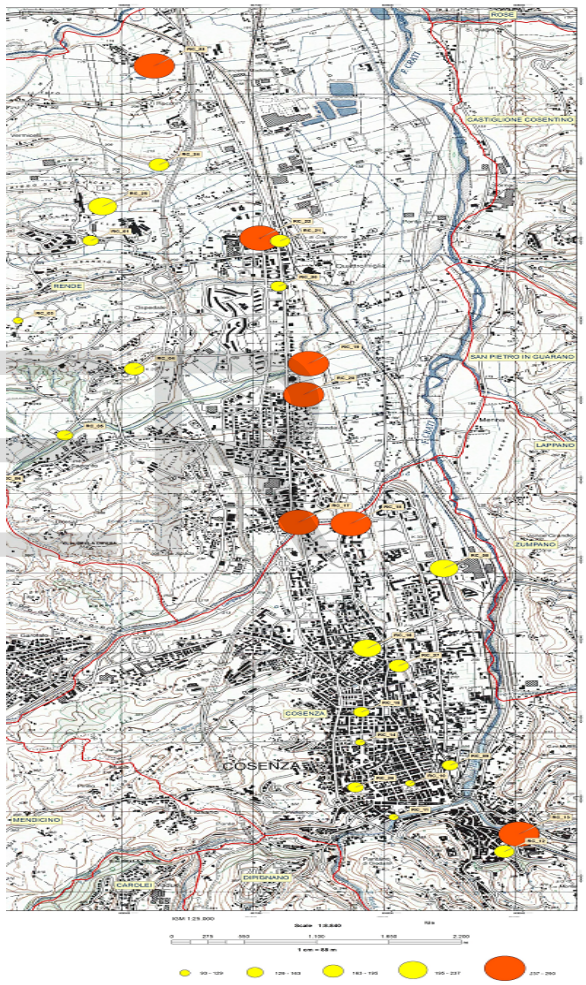

Fig.3. IGM map 1:25.000 realized with GIS system reporting the eggs density of *Ae. albopictus* for each sampled station.

References

[1] Honório N. A., W Da Costa Silva., Leite P. J., Gonçalves J. M., Lounibos L. P., Lourenço-De-Liveira R., - Dispersal of Aedes aegypti and Aedes albopictus (Diptera: Culicidae) in an urban endemic dengue area in the state of Rio de Janeiro, Brazil.- Memórias do Instituto Oswaldo Cruz, 98, pp. 191-198 (2003).

[2] Liew C., Curtis C. F.,.- Horizontal and vertical dispersal of dengue

vector mosquitoes, Aedes aegypti and Aedes albopictus, in Singapore.- Medical and Veterinary Entomology, 18, pp. 351-360 (2004).

[3] Boyer S., Foray C., Dehecq J.S., "Spatial and temporal heterogeneities of Aedes albopictus density in La Reunion Island: rise and weakness of entomological indices" PLoS ONE 9(3), pp.1-12 (2014).

[4] Hamon, J.) "Une nouvelle espèce d'Aedes de l'île de La Réunion (Diptera: Culicidae)". Le Naturaliste Malgache 5: 35-41 (1953).

[5] Bonilauri P., R. Bellini, M. Calzolari, R. Angelini, L. Venturi, F. Fallacara, P. Cordioli, P. Angelini, C. Venturelli, G. Merialdi, M. Dottori,."Chikungunya virus in Aedes albopictus, Italy".- Emerging Infectious Diseases, 14, 852-853 (2008).

[6] Bellini R. M. Carrieri, G. Burgio, M. Bacchi "Efficacy of different ovitraps and binomial sampling in Aedes albopictus surveillance activity" J. American Mosuito control Association 12(4), pp. 632-636 (1996).

[7] Sabatini A. V.Rainieri,, G.Trovato, M. Coluzzi, "Aedes albopictus in Italia e possibile diffusione della specie nell'area mediterranea", Parassitologia, 32 (3), pp. 301-304 (1990).

[8] Toma L. F. Severini,, M. Di Luca, A. Bella, R. Romi . "Seasonal patterns of oviposition and egg hatching rate of *Aedes albopictus* in Rome", Journal of the American Mosquito Control Association, 19(1), pp. 19-22 (2003).

[9] Romi R. F. Severini, L. Toma . "Cold acclimation and overwintering of female Aedes albopictus in Rome", Journal of the American Mosquito Control Association, 22(1), pp. 149-151 (2006).

[10] Knudsen A.B. R. Romi, G. Majori . "Occurrence and spread in Italy of Aedes albopictus, with implications for its introduction into other parts of Europe", Journal of the American Mosquito Control Association, 12(2), 177-183 (1996).

[11] Romi R. "*Aedes albopictus* in Italia: un problema sanitario sottovalutato", Annali dell'Istituto Superiore di Sanità, 37(2), pp. 241-247 (2001).

[12] Liotta G. G. Matranga "La zanzara tigre è arrivata in Sicilia. Igiene Alimenti, Disinfestazione & Igiene Ambientale", 21(4), pp. 49-50 (2004)..

[13] Romi R. "History and updating on the stread of *Aedes albopictus* in Italy", Parassitologia, 37, pp. 99-103 (1995).

[14] Kitron U..- L"andscape ecology and epidemiology of vector-borne diseases: tools for spatial analysis", Journal of Medical Entomology, 35, pp. 435-445 (1998).

[15] Brooker S. S. I. Hay, D. A Bundy.- "Tools from ecology: useful for evaluating infection risk models?", Trends in Parasitology 18, pp. 70-74 (2002).

[16] Regione Emilia Romagna Linee guida per gli operatori (www.zanzaratigreonline.it/Lineeguidaperglioperatori/tabid/582/Default.aspx accessed on 21.05.2012) (.2008)..

[17] Taylor L. R..- "Aggregation variance and the mean"-Nature, 189, pp. 732-735 (1961).

[18] Taylor L. R. "Assessing and interpreting the spatial distributions of insect populations", Annual Review of Entomology, 29, pp. 321-357 (1984).

[19] Kuno E. "Sampling and analysis of insect population", Annual Review of Entomology, 36, pp. 285-304 (1991).

[20] Service M. V., "Mosquito ecology: field sampling methods" Chapman & Hall, London, UK, (1993).

[21] Carrieri M., A. Albieri, P. Angelini, F. Baldacchini, C. Venturelli, S. Mascali Zeo, R. Bellini "Surveillance of the chikungunya vector Aedes albopictus (Skuse) in Emilia-Romagna (northern Italy): organizational and technical aspects of a large scale monitoring system", J. Vector Ecol. 36(1), pp. 108-116 (2011).

[22] Mazzei, A. P. Brandmayr, S. Scalercio, M.G. Novello, Bonacci T. "Nuova metodologia di analisi dei dati di monitoraggio di Aedes albopictus (Skuse, 1894) (Diptera Culicidae)" Naturalista sicil., S. IV, XXXVIII (1), pp. 103-111 (2014).

[23] Mitchell, C.J. "Geographic spread of *Aedes albopictus* and potential for involvement in arbovirus cycles in the Mediterranean basin" Journal of Vector Ecology 20, pp. 44-58 (1995).

[24] Gratz, N.G. "Critical review of the vector status of Aedes albopictus", Medical and Veterinary Entomology 18, pp. 215-227 (2004).

[25] Rezza, G., L. Nicoletti, R. Angelini, R. Romi, A. C. Finarelli, M. Panning, P. Cordioli, C. Fortuna, S. Boros, F. Magurano, G. Silvi, P. Angelini, M. Dottori, M. G. Ciufolini, A. Cassone, and CHIKV Study Group." Infection with chikungunya virus in Italy: an outbreak in a temperate region", Lancet 370, pp. 1840 Ð1846 (2007).