Aedes albopictus (Diptera: Culicidae) Population Size Survey in the 2007 Chikungunya Outbreak Area in Italy. I. Characterization of Breeding Sites and Evaluation of Sampling Methodologies

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ABSTRACT   The population of *Aedes albopictus* (Skuse) (Diptera: Culicidae) was measured in four towns in northern Italy by means of *Stegomyia* indices (house index: percentage of houses with at least one active breeding site; container index: percentage of containers with larvae; and Breteau index: number of active breeding sites per premises), pupae per premises index (PPI), pupae per hectare index (PHI), and ovitrap monitoring. A detailed investigation on types and number of productive or potential larval habitats in the inspected premises was performed. Catch basins were the most productive breeding sites in both private and public areas. The influence of the premises maintenance and premises shading indices (PMI and PSI) on the population indices and the correlations between the latter and egg density were assessed. The highest PPI was found in badly maintained premises, most times combined with shady coverage. *Stegomyia* indices and PPI were well correlated with each other, but not with the PHI, which also included the pupae collected in public areas. We obtained a highly positive correlation ($R = 0.86$) between the PHI and weekly mean egg density estimated 7–14 d after inspection of the premises. The number of females per hectare, calculated using the Focks model, was correlated with the mean egg density of the week after sampling ($R = 0.79$), and we propose that ovitrap monitoring can be used to evaluate the efficiency of the control activities or to determine a mean egg density threshold for epidemic risk evaluation.

KEY WORDS   premises maintenance index, premises shading index, *Stegomyia* indices, ovitrap monitoring, surveillance

Aedes albopictus (Skuse) (Diptera: Culicidae) was accidentally introduced into Italy at the end of the 1980s (Sabatini et al. 1990) with the trade of used tires, probably coming from the United States (Dalla Pozza and Majori 1992, Urbanelli et al. 1999). The species is now widespread over the peninsula (Romi et al. 1999) and well established in several other European countries, including Albania (Adhami and Murati 1987); Serbia and Montenegro (Petric et al. 2001); Switzerland (Flacio et al. 2004); Belgium (Schaffner et al. 2004); Greece (Samanidou et al. 2005); Spain (Aranda et al. 2006); Croatia (Klobucar et al. 2006); and Slovenia, Bosnia-Herzegovina, and France (Scholte and Schaffner 2007).

In the Emilia-Romagna region, after first being detected in the town of Bologna in 1994 (our unpublished data), the species rapidly spread and colonized the entire regional territory, reaching 600 m in elevation (Albieri et al. 2010). In summer 2007, an epidemic of Chikungunya (CHIKV) occurred in the villages of Castiglione di Cervia and Castiglione di Ravenna, involving ≈250 cases, with secondary outbreaks in other Emilia-Romagna urban areas (Rezza et al. 2007, Bonilauri et al. 2008). The main objective was then to develop a control strategy to contain the vector below the critical population density threshold to prevent new epidemic events, considering both CHIKV and dengue fever.

The indices traditionally used to evaluate *Stegomyia* population densities and the efficiency of control campaigns, such as the house index (HI: percentage of houses with at least one active breeding site), container index (CI: percentage of containers with larvae), and Breteau index (BI: number of active breeding sites per 100 premises), are widely used as empirical standard parameters in developing countries (WHO 1972). Most previous studies based on these indices concerned *Aedes aegypti* (L.), and they are often of little usefulness in European urban areas because of the different socioeconomic and environmental conditions. In addition, the indices have disadvantages when adopted in epidemiological studies, due to the lack of information on the real productivity of containers and their relative contribution to the adult population size. As a new tool, Tun-Lin et al. (1995a,b) proposed the adult productivity index (API), which is based on the sum of positive contain-

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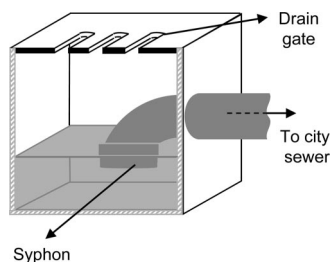[4] Corresponding author, e-mail: rbellini@caa.it.

Fig. 1. Section of a typical catch basin in Italy. Most have side dimensions of ≈40 by 40 cm.

Fig. 2. Location of the four study towns in the Emilia-Romagna region and subdivision into quadrants (sampling zones). Light gray and gray represent the residential areas, dark gray represent industrial and commercial areas, and black lines represents the road and train networks. The small gray dots indicate the ovitrap stations.

ers of different typology considering their specific relative density. The pupal demographic survey (PDS) seems to be the most appropriate index for epidemiological studies, because it exploits the strong correlation between the number of pupae and the number of adults in a definite area (Focks and Chadee 1997; Focks et al. 2000; Focks and Alexander 2006; Chadee et al. 2007a,b).

The cities of northern and central Italy are characterized by high urbanization with limited well-maintained green areas. The most diffused potential larval habitat, both in public and private properties, is catch basins for the drainage of rainwater (Fig. 1), which is part of a sewer system in which grids and a siphon prevent large objects and debris from falling into the sewer system; water from the top of the catch basin drains into the sewer proper. Their side measurements vary from 20 to 30 cm for catch basins placed in private properties and from 40 to 60 cm for those in public areas.

The most frequent containers' inhabiting mosquitoes are *Ae. albopictus* and *Culex pipiens* L. *Culiseta annulata* Schrank, *Culex hortensis* Ficalbi, *Anoheles maculipennis* Meigen, and *Anopheles plumbeus* Stephens also have been described (Carrieri et al. 2003). In the town of Bologna in 2010, *Cx. impudicus* Ficalbi and *Orthopodomyia pulcripalpis* Rondani were caught with BG-Sentinel traps (M.C., unpublished data). *Ae. aegypti* disappeared from southern Europe after the Second World War and was last recorded in Italy in Desenzano del Garda (Brescia province, northern Italy) in 1971 (Fontenille et al. 2007).

In this study, we examined the main characteristics of the urban areas where the CHIKV epidemic occurred in 2007 to highlight which control and monitoring strategies can be adopted to reduce the density of the vector and avoid new outbreaks. Toward this aim, vector surveillance is a crucial point, but because the virus is not endemic to Italy the surveillance systems must have sustainable costs. Measuring *Ae. albopictus* populations using a network of ovitraps is inexpensive, simple to execute, and commonly used in Italy in integrated control campaigns (Hawley 1988, Bellini et al. 1996, Carrieri et al. 2011). In a recent study conducted in Italy over an urban area measuring 1 km^2, a significant correlation between the CI and mean number of eggs per ovitrap was found (Carrieri et al. 2008). The current study was 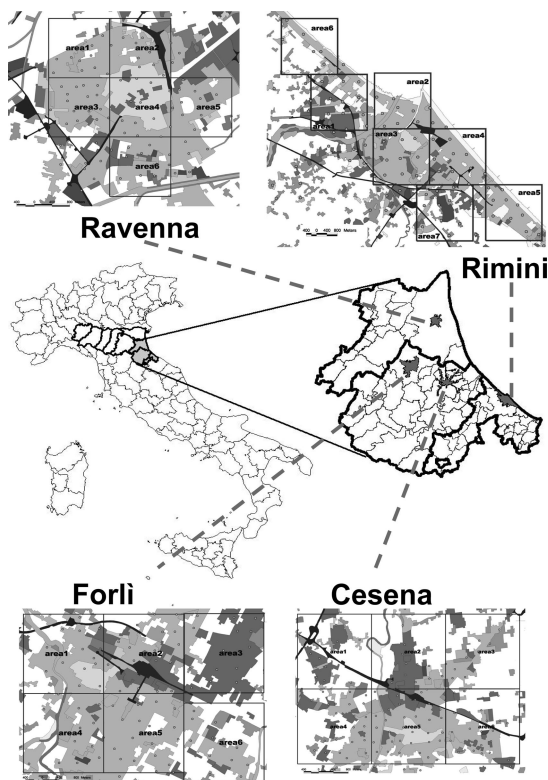designed to evaluate the reliability of some larval population estimation indices in the environmental and socioeconomic conditions of northern and central Italy and to investigate their relationships with egg density data obtained by means of an ovitrap network.

Materials and Methods

Study Period. In northern Italy, the *Ae. albopictus* development season spans from April to October, overwintering occurs by means of eggs only, and no adult activity has ever been observed during the winter. Therefore, three sampling sessions were performed to follow the expected increasing trend of population density: 1) June (weeks 24–26); 2) July (weeks 29–30); and 3) August (weeks 34–35), during which both private properties and public areas were inspected.

Study Area. The study was conducted during the 2008 season in three towns where the CHIKV epidemic occurred in 2007: Ravenna (urban area, 13 km^2; population, 5,704 people per km^2), Cesena (urban area, 20 km^2; population, 3,752 people per km^2), and Rimini (urban area, 27 km^2; population, 8,679 people per km^2 in winter and 9,592 people per km^2 in the

tourist season). The study also was extended to the town of Forlì (urban area, 21.5 km^2; population, 4,252 people per km^2), where CHIKV cases did not occur (Fig. 2). These locations are representative of small- and medium-sized towns as well as residential areas of large cities in northern and central Italy.

Vector Control Campaigns. In all of the towns, a vector control program was carried out and included larval control in public catch basins according to a monthly program, using pyriproxyfen-based products in Cesena and diflubenzuron-based products in the other three towns. Their efficacy was monitored as follows: in pyriproxyfen-treated catch basins, pupae were sampled to assess its efficacy in preventing adult emergence; and in the diflubenzuron-treated catch basins, pupae were counted to evaluate its efficacy in killing immature stages.

In the towns where the CHIKV outbreaks occurred in 2007 (Ravenna, Rimini, and Cesena, within a radius of 300 m from the confirmed cases) a door-to-door control campaign was conducted in spring 2008. In addition, in Rimini four door-to-door campaigns were conducted during the summer, whereas in Ravenna a *Bacillus thuringiensis israelensis*-based product was offered free of charge to the citizens for the treatment of the private areas (www.zanzaratigreonline.it/CosafalaRegione/IlProgettodellaRegioneEmiliaRomagna).

Premises Survey Organization and Data Collection. Each urban area was divided into six zones (Rimini into seven zones; Fig. 2), and 40 premises per zone and per session were randomly selected from a general list for inspection to detect active breeding sites. In case of an inaccessible private property, the inspectors moved to the next premises situated on the same side of the road. At the end of the survey session, the inspected premises were removed from the list, and new premises were randomly selected for the next one. The inspections were conducted by three teams, each composed of two skilled technicians equipped with Palmtop and global positioning system (GPS) for georeferencing of the surveyed sites and an aquatic net, plastic tray, and pipettes to sample the water bodies. When possible, the active breeding sites were removed; otherwise they were treated.

During the survey, the owner was asked how many people lived in the premises, to calculate the number of pupae per person. Each survey session was concluded within 2 wk.

Premises Condition Indices. The premises were assessed according to the levels of two main parameters: the premises maintenance (PMI) and the premises shading (PSI).

The PMI evaluated the state of maintenance of the yard, according to the regularity of the gardening operations, such as cutting the grass, maintenance of the trees in good conditions, and the presence of abandoned potential water containers. Four levels were considered: 0, no green area or open courtyard was present; 1, the yard was well maintained with regular gardening operations and absence of abandoned potential water containers; 2, the yard was at an intermediate level of maintenance; and 3, the yard was badly maintained or abandoned.

The PSI provided information about degree of shading of the yard: 0, no garden or open courtyard was present; 1, the shaded area was up to 25%; 2, the shaded area was the 25–50% of the yard; and 3, >50% of the area was shaded.

Premises Container Typology. For each inspected property data were collected concerning the house characteristics, the typology and number of artificial containers potentially acting as breeding sites, and the presence of water and of *Ae. albopictus* larvae and pupae. The containers were subdivided into 10 types: catch basins, plant saucers, drums (metal and plastic), buckets, tarpaulins, tires, bathtubs, generic containers with a volume <1 liter, generic containers with a volume of 1–10 liters, generic containers with a volume >10 liters.

Public Areas and Catch Basins. During the survey sessions, teams also inspected public areas, where catch basins constitute the unique *Ae. albopictus* larval habitat in northern Italy. In public areas, the average spatial density was 7–19 catch basins per hectare, whereas in private properties it was 30–40 catch basins per hectare. Surveys were performed on a total of 3,317 catch basins (822 in Ravenna, 502 in Forlì, 663 in Cesena, and 1,330 in Rimini) by one team (two skilled technicians).

Sampling Technique. During the inspections, both larvae and pupae were counted and classified for the calculation of the *Stegomyia* indices, whereas only the pupae were considered for PDS. Classification was performed in the field; when the result was doubtful, samples were examined by means of a stereomicroscope in the laboratory.

In small containers, the counting was performed by direct visual inspection. In the larger containers (catch basins, 1–50 liters; drums, 50–100 liters), immature stages were collected using an aquatic net (10 by 10 cm). In the catch basins, after the removal of floating materials, the water body was carefully explored using a net and all pupae were collected and put in a white pan with clear water for counting. In the catch basins with little water and/or lots of suspended material, a dipper (0.5-liter capacity) was used to sample the water, and the number of pupae was then multiplied by the estimated water volume contained in the catch basin. Colonized drums containing clear water for domestic garden irrigation were gradually emptied while filtering the water by using an aquatic net to collect all the larvae and pupae.

Number of Females. An estimate of the female density was based on the number of sampled pupae using the stochastic model developed for *Ae. aegypti* (Focks et al. 1993, Otero et al. 2006) and adapted to *Ae. albopictus* by Vallorani et al. (2010). The number of females was calculated considering half the number of pupae and the mortality of pupae and adults, then applying the Focks model. The mortality of the pupae (mp) was calculated as follows (Otero et al. 2006):

$$mp = 0.01 + 0.97 \exp[-T - 278)/2.70]$$

$$[1]$$

where T is the temperature expressed as Kelvin.

The adult mortality (ma) rate was calculated as follows (Otero et al. 2006):

$$ma = 0.091/d \qquad [2]$$

Monitoring Egg Density With Ovitraps. Based on knowledge of the mean egg density in the study areas during the 2007 season, an ovitrap monitoring network was set up. The minimum necessary number of ovitraps was calculated using the Taylor equation (Taylor 1961):

$$N = m^{b-2} \times a \times [Z_{\alpha/2}/D]^2 \qquad [3]$$

where m is the known average egg density value; a and b are coefficients defined by Taylor's power law (Taylor 1961, 1984; Kuno 1991) that quantify the aggregation degree of ovitrap data: b measures data aggregation and, when >1, indicates a relationship between mean and variance (i.e., ovitrap data are aggregated), and a is a constant depending on the size of the monitoring network (number of ovitraps) and environmental conditions; Z is the standard normal distribution value for a given probability (Buntin 1994); and D is the degree of accuracy expected by the monitoring, which can be set as a percentage of the mean ($D = 0.2$ in our study) (Southwood and Henderson 2000).

According to the Taylor equation, the number of ovitraps to be set in the four towns was 67 in Rimini, 87 in Forlì, 114 in Cesena, and 69 in Ravenna. The location of each ovitrap was set to ensure homogeneity in spatial distribution and standardization of the environmental conditions that influence the efficacy of the ovitrap itself (Williams et al. 2006); therefore, this task was performed by skilled technicians. By using Geographic Information Systems (ArcView, ESRI, Redlands, CA), each town was divided into a number of quadrants equal to the number of ovitraps needed. In each quadrant, one ovitrap was placed in an easily accessible shaded location, positioned on the ground with at least 1 m of free space above the trap. The ovitrap stations were georeferenced by GPS-equipped Palmtops and kept fixed throughout the entire season.

Each ovitrap consisted of a black plastic 400-ml pot with an upper diameter of 8 cm, filled to about two-thirds its height with ≈285 ml of dechlorinated water, including a substrate for egg deposition (strips of masonite, 12.5 by 2.5 cm) fixed to the trap with a metal clip. Each ovitrap was labeled with an identification code. Ovitraps were checked every 7 d, and the deposition substrate and water were replaced after cleaning the ovitrap's inside wall to remove any eggs. Masonite strips were then delivered to the provincial section of the Regional Agency for the Protection of the Environment for egg classification and counting.

For each town the mean egg density provided by the ovitraps was calculated. To assess the efficiency of the monitoring method, the relative variation (RV), the rate between the mean and the SE of the weekly egg density, also was provided.

Population Indices. Five indices were calculated to provide synthetic information on the infestation level of *Ae. albopictus* at a single premises: HI (house index, percentage of houses with at least one infested container); CI (container index, percentage of infested containers); BI (Breteau index, number of infested containers per premises); PPI (number of pupae per premises); and PHI (number of pupae per hectare), which reflects the mosquito density per unit area, for both public and private domains.

Meteorological Data. In all the four towns a meteorological station of the Emilia-Romagna region was present (CLINUR net, www.arpa.emr.it) and the following weather parameters were registered: wind speed at 10 m from ground level, relative humidity (minimum, maximum, and average daily mean), air temperature at 2 m from ground level (minimum, maximum, and average daily mean), and daily rainfall (millimeters per day).

Statistical Analyses. The total number of catch basins, the number of infested catch basins, and the number of larvae, pupae, or both found in the four towns were analyzed by means of one-way analysis of variance (ANOVA).

The effect of PMI and PSI on HI (considering the average HI of each zone), BI, and PPI were analyzed by one-way ANOVA. The differences in the PMI and PSI as a function of the town were analyzed by one-way ANOVA.

Two-way ANOVA was used to analyze the PPI depending on the town and container capacity, and in relation to the interaction between PMI and PSI, for which we considered only the data collected in houses with open areas.

Percentage data were submitted to angular transformation before analysis. The Newman–Keuls multiple comparison test was used for mean comparison. Data also are presented as mean ± SD; different letters are used in text and tables to indicate statistically significant differences among the means.

For each town and sampling data, the *Stegomya* indices, PPI and PHI were calculated and correlations were analyzed by means of the Pearson product number: 1) among all population indices considered in the study; 2) between the population indices and the mean egg density per ovitrap per week obtained during the week before, the same week, and the week after the inspection; 3) between the number of females (calculated from the number of pupae using the Focks model) and mean egg density in the ovitraps; and 4) between the female per egg rate and the weather factors during the week after the inspection.

Results

Cumulatively in the four towns, 2,919 premises (97.3% of the premises randomly selected from the initial list) were inspected (Table 1), and the colonized containers as well as all those potentially serving as larval habitat (all containers with water or empty but exposed to the rain) were classified. In total, 27,836 containers were counted, 8931 of which (32.1%)

Table 1. Descriptive data of the four towns

Town	Total surface (ha)	No. inhabitants/km^2	Study surface (ha)	% total surface	No. premises	No. premises in the study area	No. inspected premises	% sampled in the study area
Ravenna	1274	5704	821	64.4	23,793	15,468	706	4.56
Cesena	2000	3752	1,111	55.6	25,671	14,119	695	4.92
Rimini	2707	8,679–9,593	1,699	62.8	35,773	26,699	799	2.99
Forlì	2152	4,252	1,367	63.5	35,436	21,150	719	3.40

contained water. Mosquito larvae and pupae were found in 1583 containers, equal to 17.7% of those with water or 5.7% of the total number of potential habitats (Table 2).

According to interviews with the owners during surveys of the premises, the average number of person living in each inspected premises was 9.11 ± 21.96. The average inspection time for one premises was 1.35 h, whereas the average time for the inspection of a public catch basin was 0.5 h.

During the period of study (weeks 23–36) in the four urban areas, significant differences were found in the average daily wind speed measured at 10 m height ($F_{3, 388} = 96.93; P < 0.0001$) and relative humidity ($F_{3, 388} = 12.23; P < 0.0001$). Cesena had the highest wind speed (3.15 ± 0.55 m/s) and the lowest relative humidity (51.38 ± 10.95%), whereas Rimini had the lowest wind speed (2.07 ± 0.39 m/s) and the highest relative humidity (58.90 ± 9.88%). No significant differences were observed in the mean temperature (24.57 ± 2.92°C) and daily rainfall (0.82 ± 3.20 mm/d) among the four towns. The trends of the main meteorological data for each town are shown in Fig. 3.

There were green areas or yards in a majority of surveyed premises ($N = 2521$; 86.4% of the total inspected premises), and many of them ($N = 689$; 23.6%) hosted at least one active *Ae. albopictus* breeding site.

The most common container types found were plant saucers (60.7%) and private catch basins (24.7%), followed by buckets (3.3%), other medium-capacity (1–10 liters) containers (3.1%), low-capacity (<1 liter) containers (2.9%), abandoned tires (2.4%), drums (1.0%), tarpaulins and high-capacity (>10 liters) containers (0.9%) and bathtubs (0.3%). Their contribution in the premises' infestation is reported in Table 3.

Private Catch Basins. In total, 6,874 private catch basins were inspected in the four towns; 70.9% of them contained water, and 14.1% hosted larvae and/or pupae (15.14 ± 123.14, on average). Significant differences were found among the towns in the mean num-

ber of infested catch basins per premises ($F_{3, 2,915} = 30.78; P < 0.0001$) (Table 4) and in their relative pupae productivity ($F_{3, 2,915} = 2.78; P < 0.04$).

Other Containers. For container types other than catch basins, the average number of infested containers per premises was 0.21 ± 0.99 and the total number of pupae per premises was 0.94 ± 14.62. If all the containers types are grouped according to capacity categories, 85.7% had a capacity <1 liter, 11.5% a capacity of 1–10 liters, and 2.8% >10 liters. The two-way ANOVAs considering the towns and container capacity as main factors showed a significant difference in the number of pupae per premises among the four towns when considering medium-capacity water containers (1–10 liters), whose contribution to the number of pupae per premises was lower in the two tourist towns, Rimini and Ravenna, with respect to the others ($F_{3, 2,915} = 2.92; P < 0.03$) (Table 4).

Public Catch Basins. The density of public catch basins was lower in Cesena than in the other three towns, and the number of catch basins with water per hectare was lower in Cesena than in Forlì and Rimini ($F_{2, 6} = 9.60; P < 0.02$) (Table 5). The percentage of infested catch basins was very low in Ravenna, Cesena, and Forlì (0.4, 0.6, and 1.2%, respectively), whereas this value was 10.5% in Rimini. In fact, the number of infested catch basins per hectare and the number of pupae per hectare were significantly higher in Rimini than in all the other three towns ($F_{3, 9} = 65.02; P < 0.0001$ and $F_{3, 9} = 6.78; P < 0.02$, respectively) (Table 5).

Effect of Premises Maintenance and Shading. *PMI.* Only 13.6% of the properties had no green area or an open courtyard (PMI = 0), 66.3% had well-maintained green areas (PMI = 1), 15.9% presented a medium level of maintenance (PMI = 2), and only 4.2% of the green areas were in bad condition or abandoned (PMI = 3). Differences emerged in the mean level of maintenance as a function of the town ($F_{3, 2,915} = 12.13; P < 0.0001$), with a significantly higher PMI (i.e., higher percentage of badly maintained green areas) in

Table 2. Data collected during the survey

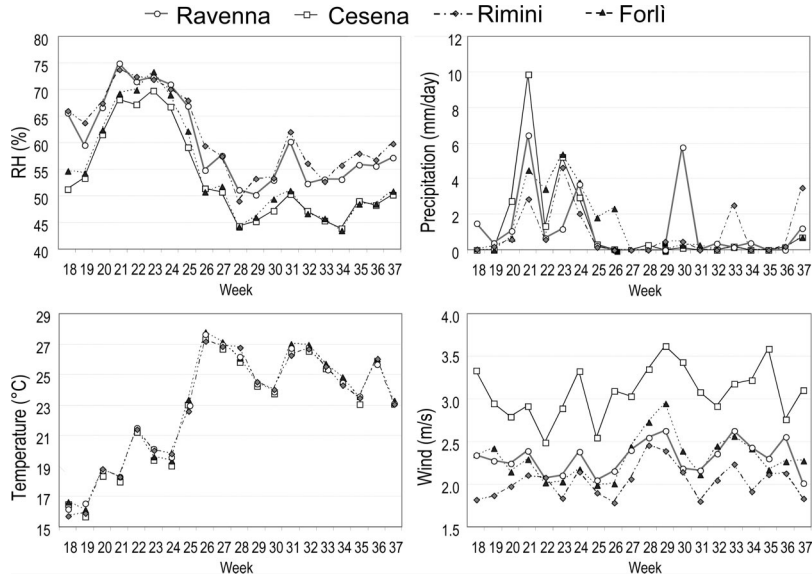| Town	No. inspected premises	No. premises with larvae, pupae, or both	No. containers	No. containers with water	No. containers with larvae, pupae, or both	No. pupae
Ravenna	706	139	5,945	2,063	303	8,912
Cesena	695	200	7,655	2,120	444	13,000
Rimini	799	99	6,747	2,042	182	5,192
Forlì	719	251	7,489	2,706	654	17,080
Total	2,919	689	27,836	8,931	1,583	44,184

Fig. 3. Trends of the main meteorological parameters registered in the four towns during the study period.

Forlì (1.23 ± 0.74a) with respect to the other study towns (Ravenna, 1.04 ± 0.64b; Rimini, 1.07 ± 0.66b; and Cesena, 1.08 ± 0.63b).

The HI in premises without a yard (PMI0, 2.30 ± 8.87a) was significantly lower with respect to all the three categories of maintenance. Between the optimal and the medium level of green area maintenance, no significant difference was found in the HI (PMI1 = 25.04 ± 15.82b and PMI2 = 31.60 ± 28.62b, respectively), whereas the badly maintained or abandoned ones showed a significantly higher HI (PMI3 = 47.48 ± 37.23c) ($F_{3, 262} = 36.96$; $P < 0.0001$).

Significant differences were found in the BI according to the level of maintenance of the open area ($F_{3, 2,915} = 67.83$; $P < 0.0001$), with the lowest value found, as expected, in the premises without green areas (PMI0 = 0.02 ± 0.21a) increasing with the decrease of the maintenance level (PMI1 = 0.50 ± 1.21b; PMI2 = 0.78 ± 1.62c; and PMI3 = 1.96 ± 3.42d).

The ANOVA showed that the PPI in premises without a yard (PMI0 = 0.22 ± 2.90a) was not different from the PPI found in courtyards with optimal maintenance (PMI1 = 16.86 ± 127.16ab) and medium

maintenance (PMI2 = 20.71 ± 117.62ab), whereas it was significantly lower with respect to the premises with badly maintained green areas (PMI3 = 37.67 ± 126.62b) ($F_{3, 2,915} = 3.66$; $P < 0.02$).

PSI. In 48.30% of the properties, green area was shaded for <25% of its extension (PSI1), in 33.13% the shaded area was in the range 25–50% (PSI2), and only in ≈4.93% of the inspected premises the shading covered >50% of the green area (PSI3). Significant differences were observed in the BI among the three shading categories ($F_{3, 2,915} = 23.72$; $P < 0.0001$) and in the HI between PSI1 and PSI3 ($F_{3, 260} = 28.84$; $P < 0.0001$). The PPI was higher in the highly shaded premises (PSI3, 52.71 ± 396.39 pupae per premises) with respect to other shading levels (PSI1, 16.15 ± 93.86 pupae per premises, PSI2, 17.05 ± 100.32 pupae per premises) ($F_{3, 2,915} = 6.40$; $P < 0.001$). As expected, the premises without a yard showed the lowest mosquito density irrespective of the index used to describe it.

Interaction between PMI and PSI. When premises maintenance and shading were considered as main factors for assessing the influence on the mean number of pupae per premises in houses with open areas,

Table 3. Pupae productivity of the different types of containers

Container	No. containers/premises	No. positive containers/premises	No. pupae/premises	No. pupae/ha	Container production (%)	Pupae production (%)
Catch basin	2.35 ± 2.96	0.33 ± 0.96	15.14 ± 123.14	237.77 ± 152.46	62.3	94.3
Plant saucer	5.79 ± 11.75	0.08 ± 0.57	0.10 ± 1.34	1.72 ± 1.67	15.1	0.7
Drum	0.09 ± 0.62	0.03 ± 0.27	0.42 ± 13.60	6.09 ± 11.13	5.7	2.4
Bucket	0.31 ± 1.16	0.03 ± 0.27	0.14 ± 2.59	2.08 ± 2.35	5.7	0.8
Tarpaulin	0.09 ± 1.06	0.00 ± 0.03	0.00 ± 0.06	0.01 ± 0.05	0.0	0.0
Tire	0.23 ± 9.75	0.00 ± 0.11	0.01 ± 0.38	0.13 ± 0.44	0.0	0.1
Bathtub	0.02 ± 0.23	0.00 ± 0.05	0.02 ± 1.00	0.36 ± 0.87	0.0	0.1
Containers <1 liter	0.28 ± 3.01	0.02 ± 0.20	0.03 ± 0.57	0.54 ± 0.64	3.8	0.2
Containers 1–10 liters	0.29 ± 1.07	0.03 ± 0.27	0.13 ± 2.08	2.28 ± 4.80	5.7	0.9
Containers >10 liters	0.09 ± 0.66	0.01 ± 0.14	0.08 ± 2.17	1.25 ± 1.89	1.9	0.5

Table 4. Results of the catch basin and container (categorized according to volume) inspection in the 2,919 premises (mean ± SD)

	Town	Total no.	Catch basins	Other containers		
				<1 liter	1–10 liters	>10 liters
No. infested containers/	Ravenna	706	0.26 ± 0.83b	0.10 ± 0.66a	0.05 ± 0.31a	0.02 ± 0.16a
premises	Cesena	695	0.45 ± 1.10c	0.07 ± 0.40a	0.07 ± 0.42a	0.05 ± 0.34ab
	Rimini	799	0.11 ± 0.58a	0.06 ± 0.36a	0.03 ± 0.22a	0.02 ± 0.34a
	Forlì	719	0.54 ± 1.19c	0.18 ± 0.94b	0.12 ± 0.64b	0.07 ± 0.40b
	Total	2,919	0.33 ± 0.96	0.10 ± 0.63	0.07 ± 0.42	0.04 ± 0.32
No. pupae/premises	Ravenna	706	12.62 ± 105.63ab	0.10 ± 0.98	0.08 ± 1.25a	0.12 ± 1.99
	Cesena	695	18.71 ± 175.39ab	0.11 ± 0.87	0.34 ± 3.60ab	0.27 ± 2.72
	Rimini	799	6.50 ± 88.85a	0.13 ± 1.42	0.15 ± 1.95a	0.91 ± 24.77
	Forlì	719	23.76 ± 109.50b	0.22 ± 2.16	0.56 ± 5.29b	0.74 ± 9.03
	Total	2,919	15.14 ± 123.14	0.14 ± 1.46	0.28 ± 3.38	0.52 ± 13.81

Different letters indicate significant differences at the 5% level.

the two-way ANOVA revealed a significant effect for the PSI ($F_{2, 2,512} = 3.09$; $P = 0.046$) but not for the PMI ($F_{2, 2,512} = 1.17$; $P = 0.31$) or for the interaction between the two ($F_{4, 2,512} = 0.52$; $P = 0.72$). Thus, the shading conditions played a significant role in favoring pupae productivity irrespective of the level of maintenance of the premises' open areas.

In particular, the mean number of pupae per premises in the badly maintained green areas was 24.63 when the shaded area was limited, 41.80 in intermediate shading conditions, and 80.07 when the shaded area was much extended (Table 6). This last category of highly productive premises, showing the optimal conditions for the development of *Ae. albopictus*, represented only 0.6% of total inspected premises.

Egg Density Estimation. In Fig. 4, the weekly mean number of eggs per ovitrap collected in the study towns by the Regional Monitoring Program together with the RV coefficient are reported. As normally happens in temperate regions, the seasonal pattern was a bell-shaped curve starting at week 22, peaking during weeks 27–37, and ending at week 45. The RV coefficient of the mean egg density in the summer period (weeks 26–35) was between 0.08 and 0.18, indicating good efficiency of the monitoring system (Southwood and Henderson 2000).

Immature Population Estimation: Indices Analyses. As shown in Table 7, all the premises population indices in the four towns seemed to be highly correlated with one to another but not with the PHI (number of pupae per hectare found in private and public areas). A correlation analysis was run between each popula-

tion index and the mean number of eggs per ovitrap laid, respectively, in the same week of the premises inspection, in the week before the inspection, and in the week after the inspection (Table 8). The analysis showed a strong correlation ($R^2 = 0.74$; $F_{1, 10} = 28.96$; $P < 0.001$) only between the PHI and the mean number of eggs collected by the ovitraps during the week after the inspection.

Relationship Between Female Population and Egg Density. We found a significant correlation between the number of adult females per hectare and the mean number of eggs collected in the ovitraps 7–14 d after the inspection ($R^2 = 0.62$; $F_{1, 10} = 16.37$; $P < 0.003$).

The mean females per egg rate (R_{FE}) was 1.22 ± 0.65 (95% CL = 0.81–1.63). The variability of R_{FE} was partially influenced by weather factors, as the Pearson product-moment correlation analysis revealed positive correlations between R_{FE} and relative humidity (min. relative humidity: $R = 0.71$, $P < 0.01$; mean relative humidity: $R = 0.74$, $P = 0.007$) and between R_{FE} and atmospheric pressure ($R = 0.66$, $P < 0.02$). The wind seemed to reduce R_{FE} ($R = -0.54$, $P = 0.07$), thus increasing the efficiency of the ovitraps. Furthermore, the number of eggs laid in the ovitraps did not influence R_{FE} ($R = 0.26$, $P = 0.42$) (no superoviposition effect).

The stepwise regression analysis ($R^2 = 0.86$; $F_{2, 9} = 28.64$; $P < 0.001$) showed that the number of females estimated through the PDS was correlated with the number of eggs laid in the ovitraps and with relative humidity. According to the equation in Table 9, an

Table 5. Density of public catch basins, catch basins with water, and infested catch basins and their productivity

Town	No. public catch basins/ha	No. public catch basins with water/ha (mean ± SD)	No. public catch basins with mosquito larvae and pupae/ha (mean ± SD)	No. pupae/ha (mean ± SD)
Ravenna	18.96	Not available	0.08 ± 0.09a	0.95 ± 1.64a
Cesena	7.00	3.02 ± 0.80a	0.04 ± 0.03a	0.21 ± 0.19a[a]
Rimini	14.04	9.80 ± 0.62b	1.48 ± 0.22b	848.90 ± 606.02b
Forlì	14.20	7.61 ± 3.20b	0.17 ± 0.17a	0.85 ± 1.70a

Different letters indicate significant differences at the 5% level within each column.

[a] Number of adult mosquitoes emerged from samples of pupae collected and brought to laboratory (pyriproxyfen was used in the control campaign).

Table 6. Number of containers with water, positive containers, and pupae per premises in relation to level of maintenance and shading of green area or open courtyard

Premise condition level		No.	% premises	No. containers with water/premises (mean ± SD)	No. positive containers/premises (mean ± SD)	No. pupae/premises (mean ± SD)
Maintenance[a]	Shading[a]					
0	0	398	13.63	0.19 ± 1.54	0.02 ± 0.21	0.22 ± 2.90
1	1	1,185	40.60	3.00 ± 3.83	0.48 ± 1.14	15.23 ± 91.27
1	2	674	23.09	3.50 ± 5.04	0.56 ± 1.36	14.80 ± 103.97
1	3	76	2.60	3.05 ± 3.92	0.36 ± 0.86	60.42 ± 504.55
2	1	162	5.55	3.73 ± 4.02	0.64 ± 1.46	19.56 ± 116.13
2	2	248	8.50	3.93 ± 4.14	0.79 ± 1.53	18.65 ± 76.49
2	3	53	1.82	4.81 ± 8.09	1.19 ± 2.34	33.91 ± 230.56
3	1	63	2.16	6.89 ± 10.95	1.98 ± 4.03	24.63 ± 77.23
3	2	45	1.54	6.93 ± 7.44	1.80 ± 2.68	41.80 ± 148.53
3	3	15	0.51	8.73 ± 7.27	2.33 ± 2.74	80.07 ± 203.02
All groups		2,919		3.06 ± 4.66	0.54 ± 1.42	16.08 ± 124.05

[a] The premises maintenance levels were as follows: 0, without yard; 1, optimal; 2, medium; 3, no maintenance or abandoned. The premises shading levels were: 0, without yard; 1, shaded <25%; 2, shaded 25–50%; and 3, shaded >50%.

increase in the relative humidity seemed to result in a decrease in the number of eggs laid in the ovitraps, reducing the efficacy of the ovitraps as a monitoring method.

Discussion

The catch basins in central and northern Italy are fundamental for the drainage of rainwater. They are always present in private building areas and usually occupy both sides of the roads at a distance of 10–20 m one from each other. During the summer, rainfall can easily reach 50 mm/h/m², and each catch basin collects and sends to the sewer system >1,000 liters/h. During the drier parts of the year, stagnant water in the catch basins could be used by mosquitoes for breeding. Indirect proof that catch basins constituted the most diffused and productive breeding sites for *Ae. albopictus* is in our finding that the females per egg rate did not seem to be affected by the presence of competitive breeding sites of any dimension, in any of the studied urban areas. Catch basins (both in public and private areas) produced 96.9% of the pupae, followed by drums (2.6%), buckets (0.9%), and medium containers used in vegetable gardens for irrigation (0.8%).

The courtyards and green areas were generally well maintained, and only 4.1% seemed poorly maintained or abandoned. In the latter premises category, we found the highest number of pupae per premises (37.67 ± 126.62), particularly in those with high shade coverage as well (on average 2.33 ± 2.74 positive containers per premises with 80.07 ± 203.02 pupae per premises). In all the other premises with optimal or medium maintenance levels, an average of 0.48 ± 1.23 active containers per premises was found, with a mean density of 15.13 ± 123.87 pupae per premises. A good level of maintenance of private and public green areas reduced the number of water containers in competition with the ovitraps as larval habitats to very low numbers (0.2 active containers per premises, catch basins excluded), concentrated in restricted areas.
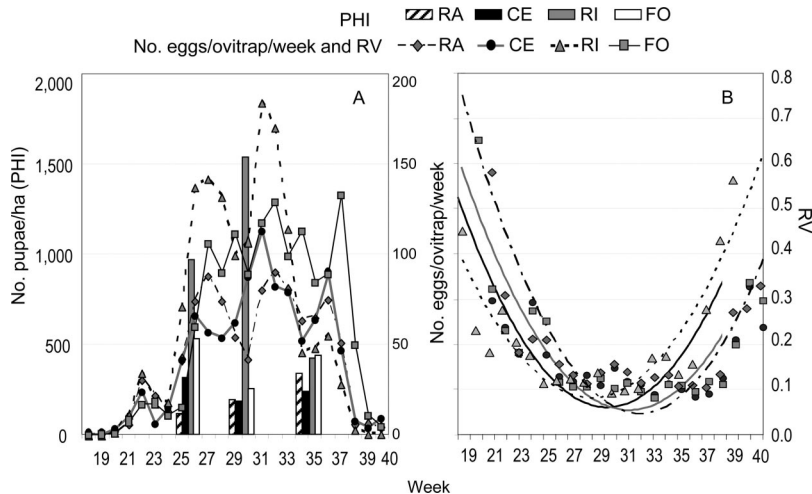

Fig. 4. (A) Weekly trend of the mean number of pupae per hectare (PHI) and of the mean number of eggs per ovitrap. (B) Weekly trend of the relative variation coefficients of the mean number of eggs per ovitrap.

Table 7. Pearson product moment correlations (R) among *Ae. albopictus* population indices

	HI	CI	BI	PPI	PHI
HI	—	0.9196**	0.9621**	0.7795**	−0.3095
CI		—	0.8758**	0.6994*	−0.3970
BI			—	0.8855**	−0.2750
PPI				—	−0.2106
PHI					—

HI: house index, percentage of houses with at least one positive container; CI: container index, percentage of infested containers; BI, Breteau index, number of positive containers per 100 houses; PPI: pupae per premises index, number of pupae per premises; and PHI, pupae per hectare index, number of pupae per hectare.

*, $P < 0.05$; **, $P < 0.01$.

In our study region, the most widely used *Stegomyia* population indices (HI, CI, and BI) applied to *Ae. albopictus* seemed well correlated one to another, in agreement with the data obtained by Tun-Lin et al. (1996) for *Ae. aegypti.* However, we found no correlation between the *Stegomyia* indices and the PHI (number of pupae per hectare), another indicator of adult density that Focks and Chadee (1997) considered reliable for estimating of *Ae. aegypti* adult density in Trinidad. This finding can be explained by the fact that the *Stegomyia* indices only consider the larval habitats within each premises, whereas PHI depends on the number of pupae found in both public and private areas, and in Italy the public catch basins represent the most uniformly diffused larval habitat and significantly affect the mosquito population density.

We obtained a highly positive correlation between the PHI and weekly mean of eggs per ovitrap collected 7–14 d after the inspection. This is in agreement with the model developed by Vallorani et al. (2010), who estimated that females that emerged from the sampled pupae should be ready to lay eggs 8–11 d later.

Many studies on mosquito female oviposition behavior have been conducted on *Ae. aegypti,* and several reports stated that this species is characterized by superoviposition and skip-oviposition behaviors. In

Table 8. Pearson product moment correlations (R) between mosquito pop indices and mean number of eggs per ovitrap per week collected the week before, the same week, and the week after the inspection

Pop index	Mean no. of eggs/wk/ovitrap (E)		
	E in the previous week	E in the inspection week	E in the week after inspection
HI	0.0867	−0.1117	−0.3778
CI	0.3194	0.0482	−0.4175
BI	0.0623	−0.1465	−0.4313
PPI	−0.0289	−0.2553	−0.5118
PHI	0.1703	0.3396	0.8622**

HI: house index, percentage of houses with at least one positive container; CI: container index, percentage of infested containers; BI, Breteau index, number of positive containers per 100 houses; PPI: pupae per premises index, number of pupae per premises; and PHI, pupae per hectare index, number of pupae per hectare.

**, $P < 0.01$.

particular, the number of eggs laid is influenced by the presence of conspecific eggs, container diameter, container volume and water surface (Apostol et al. 1994, Chadee 2009). The distribution and availability of competitive artificial containers modify the *Ae. aegypti* female oviposition behavior in ovitraps, thus influencing the relationship between the number of females and the number of eggs in the ovitraps, as described by Focks (2003), Harrington et al. (2008), and Chadee (2009).

According to our findings, which are in agreement with Allan and Kline (1998) and Carrieri et al. (2009), in *Ae. albopictus* the females per egg rate did not seem to be influenced by the egg density. In addition, we found a positive correlation between the PHI and mean egg density in the ovitraps, probably due to the lack of superoviposition behavior; if the number of females and number of eggs did not vary depending on the latter, then the number of eggs does not attract ovipositing females.

The uniform distribution of the most productive larval habitats (catch basins) and the lack of superoviposition behavior allowed the establishment of a correlation between the PHI (based on the PDS of public and private properties) and the number of eggs collected 7–14 d after the PDS. In our study, the R_{FE} was 1.22 ± 0.65. The most important consequence of this relationship is that the egg density data provided by the ovitraps can be used to determine the mean number of females per unit area.

We must note that the PDS value calculated in our study underestimated the number of females per hectare, because it did not consider the overlap between mosquito generations. If we hypothesize that the total number of females developed during the 2 wk preceding the sampling is proportional to the number of eggs sampled at day 14 and considering a mean daily survival of 0.82 (Otero et al. 2006), R_{FE} increases to 1.57 ± 0.77.

Weather factors can affect the availability of larval habitats. High temperature regimes increase water evaporation, thus reducing the number of containers with water available for oviposition, whereas rain and high relative humidity favor the presence of water in small containers (Favier et al. 2006). Despite that in Italian urban areas most of the catch basins (the main larval habitat for *Ae. albopictus*) always contained water, a relationship was found between weather parameters and the number of females per egg. Over the course of our study, R_{FE} was 1.40 ± 0.77 in June (mean $RH_{min} = 42.0\%$; mean $t_{min} = 18.0°C$) and 0.99 ± 0.72 in July (mean $RH_{min} = 30.2\%$; mean $t_{min} = 20.2°C$), increasing to 1.23 ± 0.27 in August (mean $RH_{min} = 31.7\%$; mean $t_{min} = 20.1°C$). If we take into consideration the overlap among mosquito generations, the estimated R_{FE} in the same periods were, respectively, 1.65 ± 0.84, 1.28 ± 0.87, and 1.74 ± 0.36. We hypothesize that humidity affected the gravid females' behavior, allowing for the exploitation of reproductive sites located in less shaded areas when the humidity level increases. In conditions of low relative humidity, females laid more eggs in the ovitraps because they

Table 9.   Values of significant effect in the stepwise regression

Effect[a]	Parameter	SE	t	P	CL −95%	CL +95%	β	SE
Intercept	−538.05	160.21	−3.36	<0.01	−900.48	−175.62		
E[b] (no. eggs/ovitrap/wk)	1.92	0.44	4.36	<0.01	0.92	2.92	0.64	0.15
Mean RH	8.91	3.28	2.71	<0.03	1.48	16.33	0.40	0.15
Equation	No. females = −538.05 + 1.92 E + 8.91 mean RH							

[a] Mean temperature, wind speed, and daily rainfall were pooled.
[b] Week after the premise inspection.

spent more time in the shaded areas where the ovitraps were placed. Thus, our findings suggest that to increase the reliability of the monitoring system and to reduce the seasonal variability of the R_{FE} value, it would be useful to place ovitraps in partially sunny locations as well.

In the public catch basins, control interventions against the larvae were conducted in all four towns, but with different results. In Ravenna, Cesena, and Forlì, the efficacy was high (as shown by the low frequency of infested catch basins), whereas Rimini showed the lowest efficacy (Table 5). Controlling the quality of catch basins treatments (i.e., the efficacy and the timing of execution), especially in shaded areas under trees along the roads (as found in Rimini), is crucial to reduce the mosquitoes population.

Concerning the private properties, our experience shows that the door-to-door campaigns repeated several times during spring and summer significantly reduced the number of pupae per premises. Making available control products free of charge at distribution points in the towns was less effective, but this strategy would be acceptable in years and periods when no epidemic occurs. In fact, if we compare the mosquito population of Rimini (five door-to-door control campaigns: one in the spring, four in the summer), Ravenna (one door-to-door control in spring and the offer of free larvicide to the citizens during summer), and Cesena (only one door-to-door control in spring) with that of Forlì (no control campaign in the private areas), we see that the percent reduction of the mosquito population with respect to that of Forlì was 72.7% in Rimini, 46.9% in Ravenna, and 21.3% in Cesena (data calculated from Table 3).

The correlation between the PDS and ovitrap data also might be related to the specific seasonal dynamic of *Ae. albopictus* population in temperate regions, showing a bell-shaped curve with a conspicuous increase of the population densities during the summer months followed by a dramatic decrease during the autumn-winter, with only a small portion of the population overwintering as eggs. In Italy, the *Ae. albopictus* life cycle in public catch basins is highly discontinuous and depends primarily on rainfall and the amount of water used to clean the roads (which differs from town to town). Therefore, the number of pupae sampled in these larval habitats is strongly influenced by the timing of sampling in relation to rains and road cleaning. If the sampling is carried out after a rainstorm, a few pupae and many positive containers are likely to be found. However, if the sampling is carried

out 5–7 d after heavy rainfall or a road cleaning, we would collect many more pupae. This aspect increased the variability of data obtained through the PDS in public areas. In the private areas, the variation of water volume in containers is less dependent on daily rain than in public areas, and the mosquito life cycle is highly dependent on human activities, such as irrigation, car washing, and other water-consuming activities.

We found a very low and nonsignificant negative correlation between the PHI (which considers the pupae production in public and private areas) and the other indices (which consider only the pupae produced in private areas). This finding might be due to the presence of water in the public catch basins being mainly caused by heavy rains, whereas in the private catch basins it is mainly related to irrigation, which occurs more frequently during the dry periods. This discrepancy is even more evident because the population indices are calculated based on a PDS, which is a discontinuous sampling method whose results largely depend on the timing of sampling with respect to egg hatching. Therefore, local factors can highly influence the water availability in both public and private catch basins, and in this sense both Rimini and Forlì are outliers. If Rimini were excluded from the analysis, the correlation between data collected through PDS and ovitrap monitoring would not be confirmed, whereas if Forlì were excluded the correlation level would be even higher. The current study has been planned in these very different four towns just to compensate for these contrasting tendencies.

The PDS is a reliable system for estimating the adult population of *Ae. albopictus.* However, considering the peculiar characteristics of breeding sites in northern and central Italy, its adoption as a monitoring method could be extremely costly, because the inspection of a representative sample of the main reproductive sites (Morrison et al. 2004), namely the catch basins, would be very time consuming.

Even if there is some risk of diffusion of the CHIK and dengue viruses in Italy and southern Europe—as the recent CHIKV epidemic (in Italy in 2007) and the dengue cases (in France and Croatia in 2010) showed—the conventional strategies of control seem too costly and the efficacy might prove insufficient without tight and continuous monitoring of the control measures. Based on the findings of this study, the Regional Plan for Tiger Mosquito Control and the prevention of CHIKV and dengue fever in 2010, in the absence of cases of diseases, planned to invest

available economic resources in: periodic larvicidal treatment of public catch basins; quality control of the disinfestation activities; distribution of larvicidal product to citizens; and public education and awareness-raising programs aiming to improve the management of mosquito population in private areas.

Monitoring by means of ovitraps constitutes an economically sustainable method to estimate the mosquito population density in a risk assessment plan. The results of our study show it is possible to shift to a monitoring program based on weekly (or biweekly) checks of the number of eggs laid within a network of ovitraps. Carrieri et al. (2011) showed that it is possible to set up large-scale ovitrap-based monitoring programs that would significantly reduce the financial investment, allowing continuous data collection throughout the reproductive season. The following paper in this series describes the determination of a risk threshold for CHIKV based on the mean egg density in ovitraps.

References Cited

Adhami, J., and N. Murati. 1987. Prani e mushkonjës *Aedes albopictus* në shqpëri. Rev. Mjekësore 1: 13–16.

Albieri, A., M. Carrieri, P. Angelini, F. Baldacchini, C. Venturelli, S. Mascali Zeo, and R. Bellini. 2010. Quantitative monitoring of *Aedes albopictus* in Emilia-Romagna, northern Italy: cluster investigation and geostatistical analysis. Bull. Insectol. 63: 209–216.

Allan, S. A., and D. L. Kline. 1998. Larval rearing water and preexisting eggs influence oviposition by *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae). J. Med. Entomol. 35: 943–947.

Apostol, B. L., W. C. Black, I. V., P. Reiter, and B. R. Miller. 1994. Use of randomly amplified polymorphic DNA amplified by polymerase chain reaction markers to estimate the number of *Aedes aegypti* families at oviposition sites in San Juan, Puerto Rico. Am. J. Trop. Med. Hyg. 51: 89–97.

Aranda, C., R. Eritja, and D. Roiz. 2006. First record and establishment of the mosquito *Aedes albopictus* in Spain. Med. Vet. Entomol. 20: 150–152.

Bellini, R., M. Carrieri, G. Burgio, and M. Bacchi. 1996. Efficacy of different ovitrap and binomial sampling in *Aedes albopictus* surveillance activity. J. Am. Mosq. Control Assoc. 12: 632–636.

Bonilauri, P., R. Bellini, M. Calzolari, R. Angelini, L. Venturi, F. Fallacara, P. Cordioli, P. Angelini, C. Venturelli, G. Merialdi, et al. 2008. Chikungunya virus in *Aedes albopictus*, Italy. Emerg. Infect. Dis. 14: 852–854.

Buntin, G. D. 1994. Developing a primary sampling program, pp. 99–115. *In* L. P. Pedigo and G. D. Buntin (eds.), Handbook of sampling methods for arthropods in agriculture. CRC, Boca Raton, FL.

Carrieri, M., M. Bacchi, R. Bellini, and S. Maini. 2003. On the competition occurring between *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae) in Italy. Environ. Entomol. 32: 1313–1321.

Carrieri, M., R. Bellini, S. Maccaferri, L. Gallo, S. Maini, and G. Celli. 2008. Tolerance thresholds for *Aedes albopictus* and *Aedes caspius* in Italian urban areas J. Am. Mosq. Control Assoc. 24: 377–386.

Carrieri, M., A. Masetti, A. Albieri, B. Maccagnani, and R. Bellini. 2009. Larvicidal activity and influence of *Bacillus thuringiensis var. israelensis* on *Aedes albopictus* oviposition in ovitraps during a two-week check interval protocol. J. Am. Mosq. Control Assoc. 25: 149–155.

Carrieri, M., A. Albieri, P. Angelini, F. Baldacchini, C. Venturelli, S. Mascali Zeo, and R. Bellini. 2011. Surveillance of Chikungunya vector *Aedes albopictus* (Skuse) in Emilia-Romagna (Italy): organizational and technical aspects of a large-scale monitoring system. J. Vector Ecol. 36: 108–116.

Chadee, D. D. 2009. Oviposition strategies adopted by gravid *Aedes aegypti* (L.) (Diptera Culicidae) as detected by ovitraps in Trinidad, West Indies (2002–2006). Acta Trop. 111: 279–283.

Chadee, D. D., R. Doon, and D. W. Severson. 2007a. Surveillance of Dengue fever cases using a novel *Aedes aegypti* population sampling method in Trinidad, West Indies: the cardinal points approach. Acta Trop. 104: 1–7.

Chadee, D. D., B. Shivnauth, S. C. Rawlins, and A. A. Chen. 2007b. Climate, mosquito indices and the epidemiology of Dengue fever in Trinidad (2002–2004). Ann. Trop. Med. Parasitol. 101: 69–77.

Dalla Pozza, G., and G. Majori. 1992. First record of *Aedes albopictus* establishment in Italy. J. Am. Mosq. Control Assoc. 8: 318–320.

Favier, C., N. Degallier, P. de Tarso Ribeiro Vilarinhos, M. do Socorro Laurentino de Carvalho, M. A. Cavalcanti Yoshizawa, and M. Britto Knox. 2006. Effects of climate and different management strategies on *Aedes aegypti* breeding sites: a longitudinal survey in Brasília (DF, Brazil). Trop. Med. Int. Health 11: 1104–1118.

Flacio, E., P. Lüthy, N. Patocchi, F. Guidotti, M. Tonolla, and R. Peduzzi. 2004. Primo ritrovamento di *Aedes albopictus* in Svizzera. Boll. Soc. Ticinese Sci. Nat. 92: 141–142.

Focks, D. A. 2003. A review of entomological sampling methods and indicators for Dengue vectors infectious disease analysis. World Health Organization, Gainesville, FL.

Focks, D. A., and N. Alexander. 2006. Multi-country study of *Aedes aegypti* pupal productivity survey methodology: findings and recommendations. Special Programme for Research and Training in Tropical Diseases, World Health Organization, Geneva, Switzerland.

Focks, D. A., and D. D. Chadee. 1997. Pupal survey: an epidemiologically significant surveillance method for *Aedes aegypti:* an example using data from Trinidad. Am. J. Trop. Med. Hyg. 56: 159–167.

Focks, D. A., D. G. Haile, E. Daniels, and G. A. Mount. 1993. Dynamic life table model for *Aedes aegypti* (Diptera: Culicidae): analysis of the literature and model development. J. Med. Entomol. 30: 1003–1017.

Focks, D. A., R. J. Brenner, J. Hayes, and E. Daniels. 2000. Transmission thresholds for Dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility

in source reduction efforts. Am. J. Trop. Med. Hyg. 62: 11–18.

Fontenille, D., A. B. Failloux, and R. Romi. 2007. Should we expect Chikungunya and Dengue in southern Europe?, pp. 169–184. *In* W. Takken and B.G.J. Knols (eds.), Emerging pests and vector-borne diseases in Europe. Wageningen Academic Publishers, Wageningen, The Netherlands.

Harrington, L. C., A. Ponlawat, J. D. Edman, T. W. Scott, and F. Vermeylen. 2008. Influence of container size, location, and time of day on oviposition patterns of the dengue vector, *Aedes aegypti*, in Thailand. Vector Borne Zoonotic Dis. 8: 415–423.

Hawley, W. A. 1988. The biology of *Aedes albopictus.* J. Am. Mosq. Control Assoc. 4 (Suppl): 1–40.

Klobucar, A., E. Merdic, N. Benic, Z. Baklaic, and S. Krcmar. 2006. First record of *Aedes albopictus* in Croatia. J. Am. Mosq. Control Assoc. 22: 147–148.

Kuno, E. 1991. Sampling and analysis of insect populations. Annu. Rev. Entomol. 36: 285–304.

Morrison, A. C., H. Astete, F. Chapilliquen, G. Ramirez-Prada, G. Diaz, A. Getis, K. Gray, and T. W. Scott. 2004. Evaluation of a sampling methodology for rapid assessment of *Aedes aegypti* infestation levels in Iquitos, Peru. J. Med. Entomol. 41: 502–510.

Otero, M., H. G. Solari, and N. Schweigmann. 2006. A stochastic population dynamics model for *Aedes aegypti*: formulation and application to a city with temperate climate. Bull. Math. Biol. 68: 1945–1974.

Petric, D., I. Pajovic, A. Ignjatovic, A. Cupina, and M. Zomba. 2001. *Aedes albopictus* (Skuse, 1894) new mosquito species (Diptera, Culicidae) in the entomofauna of Yugoslavia, p. 29. *In* Proceedings, Symposia of Serbian Entomologists, 26–29 September 2001, Belgrade. Entomological Society of Serbia, Belgrade, Goč-Serbia.

Rezza, G., L. Nicoletti, R. Angelini, R. Romi, A. C. Finarelli, M. Panning, P. Cordioli, C. Fortuna, S. Boros, F. Magurano, et al. 2007. Infection with Chikungunya virus in Italy: an outbreak in a temperate region. Lancet 370: 1840–1846.

Romi, R., M. Di Luca, and G. Majori. 1999. Current status of *Aedes albopictus* and *Aedes atropalpus* in Italy. J. Am. Mosq. Control Assoc. 15: 425–427.

Sabatini, A., V. Raineri, G. Trovato, and M. Coluzzi. 1990. Aedes albopictus in Italia e possibile diffusione della specie nell'area mediterranea. Parassitologia 32: 301–304.

Samanidou, A., A. Voyadjoglou, E. Patsoula, G. Spanakos, and N. C. Vakalis. 2005. Confirmation of *Aedes albopictus* (Skuse) (Diptera: Culicidae) in Greece. Eur. Mosq. Bull. 19: 10–12.

Schaffner, F., W. Van Borstel, and M. Coosemans. 2004. First record of *Aedes* (*Stegomyia*) *albopictus* in Belgium. J. Am. Mosq. Control Assoc. 20: 201–203.

Scholte, E. J., and F. Schaffner. 2007. Waiting for the tiger: establishment and spread of the Asian tiger mosquito in Europe, pp. 241–260. *In* W. Takken and B. Knols (eds.), Emerging pests and vector-borne diseases in Europe. Wageningen Academic Publishers, Wageningen, The Netherlands.

Southwood, T.R.E., and P. A. Henderson. 2000. Ecological Methods, 3rd ed. Blackwell Publishing.

Taylor, L. R. 1961. Aggregation, variance and the mean. Nature 189: 732–735.

Taylor, L. R. 1984. Assessing and interpreting the spatial distributions of insect populations. Annu. Rev. Entomol. 29: 321–357.

Tun-Lin, W., B. H. Kay, and A. Barnes. 1995a. The premise condition index: a tool for streamlining surveys on *Aedes aegypti.* Am. J. Trop. Med. Hyg. 53: 591–594.

Tun-Lin, W., H. Kay, and A. Barnes. 1995b. Understanding productivity, a key to *Aedes aegypti* surveillance. Am. J. Trop. Med. Hyg. 53: 594–601.

Tun-Lin, W., B. H. Kay, A. Barnes, and S. Forsyth. 1996. Critical examination of *Aedes aegypti* indices: correlations with abundance. Am. J. Trop. Med. Hyg. 54: 543–547.

Urbanelli, S., R. Bellini, M. Carrieri, P. Sallicandro, and G. Celli. 1999. Population structure of *Aedes albopictus* (Skuse): the mosquito which is colonizing Mediterranean countries. Heredity 84: 331–337.

Vallorani, R., G. Messeri, A. Crisci, F. Piani, C. Venturelli, S. Mascali Zeo, M. Carrieri, R. Bellini, A. Albieri, P. Angelini, et al. 2010. A biometeorological population dynamic model for *Aedes albopictus* (Skuse) adapted to the urban Italian (Emilia-Romagna region) context, p. 89. *In* Proceedings, Emerging Vector-borne Diseases in a Changing European Environment, 10–12 May, Le Corum, Montpellier, France.

Williams, C. R., S. A. Long, R. C. Russell, and S. A. Ritchie. 2006. Optimizing ovitrap use for *Aedes aegypti* in Cairns, Queensland, Australia: effects of some abiotic factors on field efficacy. J. Am. Mosq. Control Assoc. 22: 635–640.

[WHO] World Health Organization. 1972. A system of world-wide surveillance for vectors. Wkly. Epidemiol. Rec. 47: 73–84.