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Aedes albopictus (Diptera: Culicidae) Monitoring in the Lazio Region (Central Italy)

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Abstract

The Asian tiger mosquito *Aedes albopictus* (Skuse 1894) is assuming an ever-increasing importance as invasive species in Europe and consequently as human health and nuisance concern. In Central Italy, the species has been recently involved in a chikungunya outbreak. A 3 yr *Ae. albopictus* monitoring was carried out in 21 municipalities of the Lazio region (Central Italy), belonging to three provinces. Samplings were performed on a weekly basis using ovitraps, in order to investigate climatic and spatial variables driving egg abundance and *Ae. albopictus* period of activity. A temperature of 10.4°C was indicated as lower threshold for the onset of egg-laying activity, together with a photoperiod of 13:11 (L:D) h. The whole oviposition activity lasted 8 mo (May–December), with 95% of eggs laid between early June and mid-November and a peak at the end of August. Egg abundance was positively influenced by accumulated temperature (AT) of the 4 wk preceding sampling and negatively by precipitation during the week before. Egg-laying activity dropped with decreasing AT, increasing rainfall, and with a photoperiod below 10:14 (L:D) h. Our results pinpointed the importance of fine-scaled spatial features on egg abundance. Some of these fine-scaled characteristics have been highlighted, such as the presence of vegetation and human footprint index. Our model estimated an almost doubled maximum number of laid eggs for the maximum value of human footprint. Compelling evidence of the relevance of fine-scaled characteristics was reported, describing cases where human-made breeding sites driven the abundance of *Ae. albopictus*.

Key words: mosquito, ovitrap, phenology, temperature, rainfall

The Asian tiger mosquito *Aedes albopictus* (Skuse 1894), indigenous to East Asia, is listed as one of the top 100 invasive species and can be considered the most invasive mosquito in the world (Benedict et al. 2007, Invasive Species Specialist Group 2009). Though the species is forest-dwelling and zoophilic in its native range, its strong ecological plasticity favored this mosquito to progressively adapt to rural, suburban, and highly dense urban areas as well as to alternative food sources, such as domestic animals and human blood, colonizing the tropical and temperate regions of all continents in the last 40 yr (Paupy et al. 2009, Bonizzoni et al. 2013). Its tendency to feed on different animal species, together

with its notorious daytime human-biting behavior, increases the medical relevance of *Ae. albopictus* as vector of several mosquito-borne pathogens. It is indeed known to be involved in the transmission of at least 25 human and zoonotic viruses, including dengue, chikungunya, Zika, and La Crosse (Gerhardt et al. 2001, Almeida et al. 2005, Vega-Rúa et al. 2014, McKenzie et al. 2019). Due to its high competence for these viruses, *Ae. albopictus* ranks second only to *Aedes aegypti* Linnaeus, 1762 in importance as diseases vector (Shroyer 1986, Knudsen 1995). Climate change, intensive growth of global transportation systems, arthropod adaptation to increasing urbanization, human population growth, degradation

of natural environments, and failure to contain mosquito population density are the main drivers, at a global scale, leading to the reemergence of mosquito-borne arboviruses (Rogers and Packer 1993, Cook 2008, Elbers et al. 2015, Gould et al. 2017). In Europe, five autochthonous chikungunya outbreaks were reported since 2007, in Italy and France, with the last one affecting some areas of Central and Southern Italy in 2017 (ECDC 2017). *Aedes albopictus*, first detected in Europe in 1979 (Adhami and Reiter 1998) and then in Italy in 1990 (Sabatini et al. 1990), was the proven vector involved in the Italian outbreaks (Venturi et al. 2017) and is nowadays considered an established invasive specie in Italy and in other European countries (ECDC 2019). The European Centre for Disease Prevention and Control (ECDC) has on several occasions pointed out the need of a comprehensive understanding of the vector-related risk concerning *Ae. albopictus* (ECDC 2009, 2012). Supporting this aim, the Italian Ministry of Health urged Public Health Services improving arboviruses surveillance and enacted the 'National Plan for the surveillance of the *Aedes* spp. borne arboviruses, with special reference to the chikungunya, dengue and Zika viruses' (Ministero della Salute 2018). Thereafter the enactment of the National Plan, the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana 'M. Aleandri' (IZSLT), in collaboration with the local public health services, started an *Ae. albopictus* monitoring by means of Oviposition Traps (OTs) in Central Italy. The use of OTs is considered an appropriate tool for mosquito surveillance (Velo et al. 2016), dispersal and spatial pattern detection (Honório et al. 2003), and control measures impact assessment (Mogi et al. 1990).

With a mean global air temperature projected to increase from 1.4 to 5.8°C by 2100 relative to 1990 (Houghton et al. 2001), climate variables are among the major issues to investigate, given their effect on mosquitoes ecology, development and behavior, as well as on transmission dynamics of mosquito-borne diseases (Brady et al. 2013). Moreover, as future trends indicate that urbanization process is expected to continue for decades and that the majority of humankind will likely be living in urban areas (United Nations 2019), expansion of urban environment and increasing population density will play a cornerstone role in spread, establishment and persistence of mosquitoes and mosquito-borne diseases.

Aim of the present study was to combine meteorological and environmental variables and density of human population to *Ae. albopictus* data, based on a 3-yr monitoring at regional scale, in order to identify potential spatiotemporal drivers determining mosquito presence, abundance, and seasonal activity.

Materials and Methods

Study Area

During the chikungunya outbreak of 2017, an entomological surveillance by means of BG Sentinel traps was set and a pool of *Ae. albopictus* was found to be positive to the virus (Venturi et al. 2017). Shortly thereafter, a mosquito monitoring by means of OTs started on six sites in the town of Anzio, where the first symptomatic patients were recorded. During the following 2 yr (2018 and 2019), 77 sites were added to the former ones, leading to a total number of 83 OTs in three provinces of the Lazio region (Metropolitan City of Rome Capital, Latina, and Frosinone), distributed in 21 municipalities (Fig. 1a). OTs were set at least 300 m apart from each other, to avoid overlap of geospatial data (see *Geospatial data* section) and placed within urban areas in different site typologies, such as public, house or school gardens, hospitals, cemeteries, markets, train stations or inside seats of the local health service, riding clubs, churches, and senior centers, recovering the GPS coordinates of each site. OTs sites were agreed with Local Health Authorities and private owners, taking into account the availability of the Local health services operators.

Sampling Design

OTs consist of a black plastic container of 400 ml, filled three-quarter with tap water and equipped with a masonite strip (15 × 3 cm), where *Ae. albopictus* females lay eggs. OTs were placed at ground level in sheltered and shade places, to ease mosquitoes resting and eggs laying and were left in the same positions through the whole study period. Local health services operators dealt with the routine OTs management on weekly basis (collection and replacing of masonite strips and refilling OTs), delivering the samples to the Laboratory of Entomology of IZSLT. Eggs were counted under a stereomicroscope. To confirm *Ae. albopictus* specific identification, randomly chosen
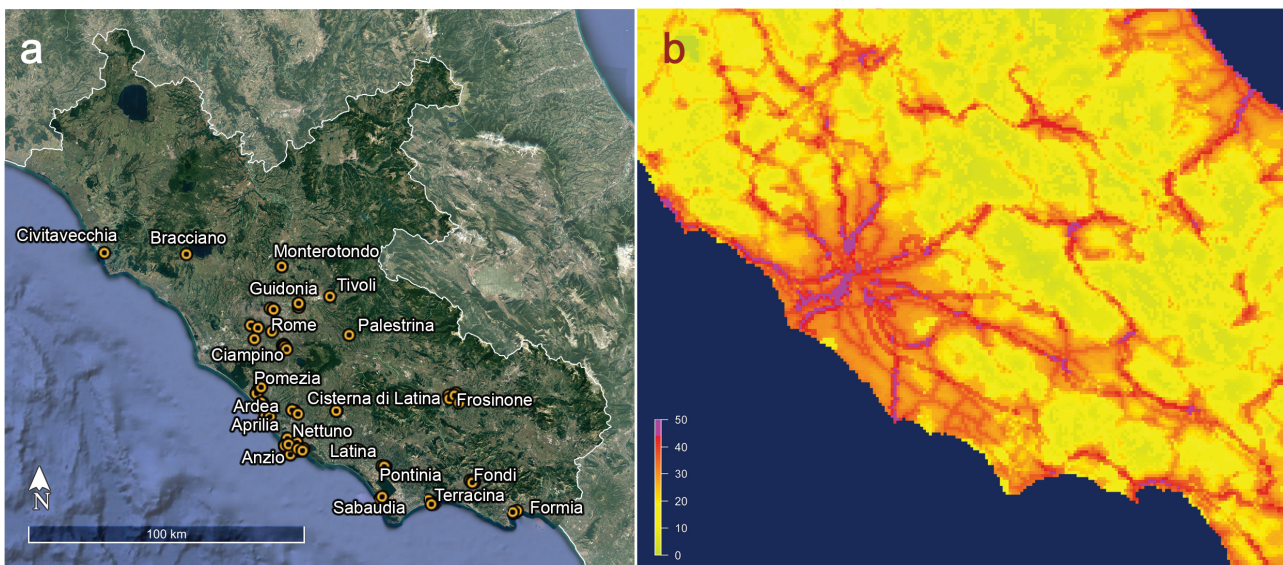

Fig. 1. (a) map of the Lazio region with the 21 municipalities where the 83 ovitraps were placed; (b) map of the human footprint index for the Lazio region.

masonite strips from each site were put in water with a source of food to allow eggs hatching and larval development to adults. Adult mosquitoes were morphologically identified using the identification keys of Severini et al. (2009) and Ree (2003). Sampling period and data for the present study ranged from September 2017 to December 2019.

Climate Data

Daily minimum and maximum air temperature and daily total precipitation for the years 2017, 2018, and 2019 were obtained from the website of the Lazio Region (http://dati.lazio.it/catalog/it/dataset). For this purpose, the closest weather station data were downloaded for each OT site (maximum, minimum, and mean distances were respectively: 12.60, 0.42, and 4.01 Km). Daily hours of light were obtained for each sampling date and site using the geosphere R package (Hijmans 2019), according to the formula by Forsythe et al. (1995). Since sampling was carried out on weekly basis, weekly mean temperatures (°C) were computed (i.e., mean of minimum, mean of maximum, and mean of average temperatures) and total weekly precipitation (mm) was calculated. Starting from sampling date and proceeding backward, mean temperatures were calculated for each of the four previous weeks: W_1, W_2, W_3, and W_4. Weekly mean minimum temperature was used to estimate the threshold temperature necessary for egg-laying activity to start and accumulated temperature (AT) was calculated by subtracting the identified threshold temperature from the daily mean temperature and summing the values per week (W_1–W_4) for each municipality (Kobayashi et al. 2002, ECDC 2009). Besides, following a commonly used procedure to account for climate variables influence on larval stage (Roiz et al. 2010, Manica et al. 2016), AT and rainfall cumulated data were calculated over the following time spans: $W_1 + W_2$; $W_1 + W_2 + W_3$; $W_1 + W_2 + W_3 + W_4$; $W_2 + W_3$; $W_3 + W_4$ and $W_2 + W_3 + W_4$.

Geospatial Data

The 'map of the land use' provided by the Lazio region (from 2000 partially updated in 2016, http://dati.lazio.it/catalog/it/dataset/carta-uso-suolo-2016) is a higher spatial resolution version of the CORINE Land Cover dataset (CLC) and it was used to obtain landscape variables in a radius of 100 m around each OT, using ArcGIS 10.3 software by Esri. The land cover variables were extracted according to the third level of CLC detail. The radius was set considering the recognized limited active dispersal of *Ae. albopictus* (Hawley 1988, Rai 1991) and its dispersal ranges in urban areas (Honório et al. 2003, Bellini et al. 2010, Marini et al. 2010). In addition to land cover variables, the Shannon land cover index was calculated to describe landscape structure and diversity at small scale, namely for each OT site. Besides, during the OT placement, the operator checked for the presence of even small patches of low, dense vegetation such as hedges and shrubs, suitable resting and hiding sites for *Aedes* mosquitoes (Kamgang et al. 2012). The identification of the patches was made through a simple web gis created with Google Maps, using as background the very-high-resolution satellite imageries provided by this tool. Given the known human-biting behavior of *Ae. albopictus* and its aptitude to use artificial containers in suburban and urban environments as oviposition sites (Estrada-Franco and Craig 1995, Bonizzoni et al. 2013, Fontenille et al. 2007), data on global human pressure on the environment were downloaded from the NASA data center of Earth Observing System Data and Information System (EOSDIS). For this purpose, GeoTIFF data on human footprint were obtained (Venter et al. 2016, 2018)

and a raster value of human pressure on environment (from 0 to 50) was extracted for each OT site, using ArcGIS 10.3 software by Esri. Human footprint data take into account eight variables, including built-up environments, population density, electric power infrastructure, croplands, pasture lands, roads, railways, and navigable waterways. It is thus inherent in the index computation that the same value can derive from different combinations of these factors. Human footprint data are freely accessible at a spatial resolution of ~1 Km in Mollweide projection on EOSIDS (see Fig. 1b for graphical representation of human footprint of the Lazio region).

Statistical Analyses

Climate Data

To investigate the beginning and the end of egg-laying activity, samplings have been divided in two subsets: 1) 'onset', from the beginning of the year till the end of June; 2) 'end', from October till the end of the year. The whole dataset, also including the months July to September, was used for further analyses on the relationship between *Ae. albopictus* phenology and weather variables. A redundancy analysis (RDA) was performed to choose the weekly mean minimum temperature that best explains the variance in egg abundance during the onset of activity. Mean minimum temperatures from W_1 to W_4 before survey were set as independent variables and egg abundance [Ln (eggs n° + 1)] as dependent variable. Ordination was achieved with forward selection, using ordistep function (Blanchet et al. 2008), with the statistical software R version 3.5.1 (R Core Team 2018). Mean minimum temperature with highest F-statistic value and lowest Akaike Information Criterion (AIC) was retained as the most explanatory variable and used in linear regressions (Borcard et al. 2011). Linear regression models between egg abundance (log-transformed as above) and the chosen mean minimum temperature were performed to calculate the threshold temperature for egg-laying activity to start, for each municipality and for the pooled dataset. To compare threshold temperatures among municipalities the sma function was used in R (Warton et al. 2012). The function allows to test if several regression lines share a common intercept (threshold temperature in our study case) and to compute intercept of each regression if a difference among groups was highlighted (municipalities).

To quantify the threshold in hours of light needed for egg-laying activity to start, linear and nonlinear regression models were performed. Difference between models was tested thorough analysis of variance (ANOVA) and selection occurred on the basis of their AIC value; then the intercept of the best fitting model was computed. Relationship between climate variables, hours of light, and end of the egg-laying activity was investigated likewise, using the 'end' subset and choosing the explanatory climate variables as above described.

Phenology was investigated fitting a Gaussian model to the egg abundance data of the pooled dataset. The coefficient b of the function, indicating the position of the egg-laying activity peak along the season, was used to compute the time interval within which 95% of eggs have been laid, applying the three-sigma rule as follow: range limits = b ± 2*SD (i.e., SD). Where SD was computed as the square root of the coefficient c of Gaussian model (Squires 2001). Then a subset has been made, extracting data from samplings laying in-between the highlighted time interval. To investigate the influence of climate variables on egg abundance, this subset was used as response variable in a linear mixed model (LMM). RDA analysis with forward selection was performed to choose the climate variables that best explain the variance in egg abundance, using the retained ones as fixed effects in the LMM. In addition, OT site characteristics (e.g., market, cemetery, train station, etc.), municipality, year of sampling,

and OT identification code were taken into account as random factors in LMM. As OT code remained the same across time and several OTs shared the same site characteristic, these variables were specified in LMM, respectively, as crossed with years and nested within municipality, and the fixed effects coefficients were estimated using restricted maximum likelihood (Bates et al. 2015). Coefficient of the fixed effects and estimates of the variance explained by the random effects were discussed and *P*-values of the fixed effects were calculated using ANOVA function in R (Fox and Weisberg 2011).

Geospatial Data

To investigate the influence of geospatial variables on egg-laying activity, the maximum egg count of each OT and the average number of eggs, laid during the highlighted time interval (i.e., when 95% of eggs have been laid), were used as response variables. For the following analyses, the percentage of land cover variables was calculated on the total area of each buffer around OTs ($31{,}415$ m^2). Two RDA analyses with forward selection, one for each response variable (maximum and average egg count), were performed to choose the best explanatory variables among land cover variables, Shannon diversity index and footprint. Relationship between chosen variables and maximum and average egg number was then investigated with generalized linear models (GLMs), family Poisson. Finally, Mann–Whitney U tests were performed to highlight a significant difference in median number of maximum and average eggs among sites with or without low and dense vegetation.

Results

General Outcomes

A total of 2,896 samplings were carried out between September 2017 and December 2019, 64.8% of them positive (i.e., at least one egg was found on the masonite strip). All the identified adult mosquitoes (~ 9,000 specimens, about 5% of counted eggs), coming from each sampling site, were *Ae. albopictus*. Egg-laying activity started between early May and early June, ending between early October and the third decade of December, depending on the municipality. Two isolate, unexpected positive strips were recorded on the first days of January and on late February, respectively, in the municipalities of Fondi and Pontinia, Southern Lazio region. The mean number of activity days was 186, with five municipalities (namely Ciampino, Anzio, Fondi, Pontinia, and Sabaudia) exceeding 200 d. The maximum number of eggs laid on a single strip was 870, recorded during the last days of August 2019 on an OT placed inside a riding club near Rome. During this 3 yr study, 235 samplings (8.1%) exceeded 200 eggs, most of them (70.6%) between the last decade of August and October.

Statistical Analysis

Climate Data

Considering the onset dataset, ordination with RDA through forward selection indicated that the mean minimum temperature of W_2 best explains variance in egg abundance (df = 1; AIC = 340.79; $F = 319.37$; $P = 0.005$). Mean minimum temperature of W_1 was as well significant, but was discarded due to a ΔAIC > 2 respect to W_2 (df = 1; AIC = 337.46; $F = 5.33$; $P = 0.020$) (Burnham and Anderson 2002). The intercept of the linear regression model (df = 514; Adj. R^2 = 0.38; $P < 0.05$) indicated a lower threshold temperature of 10.4°C (SE: ± 0.2°C) for the onset of egg-laying activity (Fig. 2a). The comparison of intercepts among municipalities highlighted a significant difference in threshold temperature (df = 20;
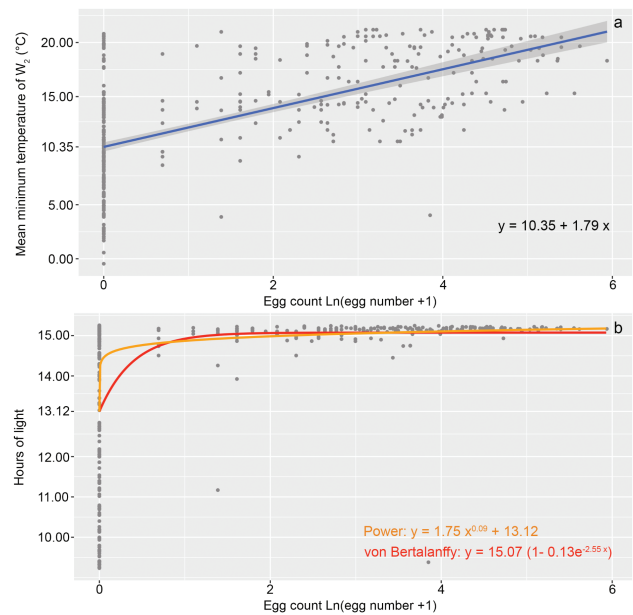
Fig. 2. Relationship between egg count and weather variables during the onset of egg-laying activity: (a) mean minimum temperature for the second week before sampling (W_2), with highlighted threshold temperature (10.35°C) and equation ($P < 0.05$); (b) hours of light with nonlinear models equations, which highlighted the same threshold value: 13.12 (L:D) h of light. The *P*-values of the nonlinear models coefficients were respectively: a ($P < 0.01$), b ($P = 0.52$), c ($P < 0.01$), for the Power equation; a ($P < 0.01$), b ($P < 0.01$), c ($P = 0.29$) for the von Bertalanffy.

Wald = 109.20; $P < 0.05$) (see Supp. Table S1 [online only] for regressions results). Hence, the estimated thresholds have been used to compute specific AT for each municipality. A daylight threshold of 13.1 h (SE: ± 0.09) for the onset of egg-laying activity was estimated by the best fitting nonlinear model (Table 1, a). Trends of the first two ranked models on the basis of AIC model selection procedure were reported in Fig. 2b. A daylight threshold of 9.95 h (SE: ± 0.03) for the end of the egg-laying activity was identified by the best fitting model, with no significant difference highlighted by ANOVA between models (Table 1, b). RDA ordination through forward selection retained the following set of explanatory variables: mean maximum temperature of W_1; mean minimum temperature of W_2; ATW_1; ATW_{1+2} and cumulative precipitation of W_{1+2+3} (Table 2, a). Regression lines with estimated intercepts for the relationship between end of egg-laying activity, daylight, and climate variables were reported in Fig. 3a and b.

Phenology analysis showed a peak of oviposition around the 233rd day of the year (second-third decade of August), estimating that 95% of eggs have been laid from early June to half of November (Fig. 4). The results of LMM indicated that the random effect of year of sampling didn't influence egg abundance, being the variance explained by this variable indistinguishable from zero (5.26×10^{-5} ± 0.007). Conversely, OT site variables showed an increasing amount of explained variance from municipality (147.10 ± 12.13), through site characteristic (351.90 ± 18.76), up to OT identification code (1529.90 ± 39.11), namely the OT specific site. RDA ordination through forward selection indicated that the following climate variables best explain the variance in egg abundance, during the time interval of 95% activity: $ATW_{1+2+3+4}$, mean average temperature of W_3, total precipitation of W_1 and ATW_2 (Table 2, b). LMM results showed that $ATW_{1+2+3+4}$ had a positive effect on egg abundance and total precipitation of W_1 a negative one, as indicated by the

Table 1. Linear and nonlinear (Power and von Bertalanffy) models results, with estimated daylight threshold for onset (*a*) and end (*b*) of egg-laying activity

Model	df	AIC	|ΔAIC|	Estimated intercept ± SE	ANOVA results	
					F	*P*
a						
Power	513	2023.92	0.00	13.12 ± 0.09		
von Bertalanffy	513	2024.21	0.29	13.12 ± 0.26		
Linear	514	2043.43	19.51	13.23 ± 0.09	21.84	<0.05
b						
Power	1102	2145.93	0.00	9.95 ± 0.03		
Linear	1103	2146.01	0.08	9.97 ± 0.02		
Quadratic	1102	2146.30	0.37	9.96 ± 0.03		

ANOVA results were reported to highlight the significantly different model. AIC difference between models (\|ΔAIC\|); Akaike information criterion (AIC); *F* statistic (*F*); and *P* value (*P*).

Table 2. Result of the RDA ordination through forward selection performed on climate variables with egg abundance (log-transformed) as response variable, considering the end of egg-laying activity (a) and during the time interval of 95% of egg-laying activity (b)

Climate variable	df	AIC	*F*	*P*
a				
Mean maximum temperature W_1	1	1000.94	545.56	0.005
ATW_1	1	967.77	35.63	0.005
Mean minimum temperature W_2	1	956.33	13.48	0.005
Cumulative precipitation W_{1+2+3}	1	941.04	17.36	0.005
ATW_{1+2}	1	939.53	3.49	0.05
b				
$ATW_{1+2+3+4}$	1	18589	213.45	0.005
Mean average temperature W_3	1	18572	18.51	0.005
Total precipitation W_1	1	18560	14.73	0.005
ATW_2	1	18552	10.26	0.005

Akaike information criterion (AIC); *F* statistic (*F*); and *P* value (*P*). Week(s) preceding sampling (W).

variables coefficients (±SE): 0.49 (±0.10) for AT and −0.28 (±0.08) for rainfall. The significant effect of these climate variables on egg abundance was confirmed by the ANOVA test ($ATW_{1+2+3+4}$: df = 1, Wald = 24.14, $P < 0.05$; total weekly precipitation of W_1: df = 1, Wald = 12.93, $P < 0.05$) and their trends were reported in Fig. 4, along with egg abundance.

Geospatial Data

Ordination with RDA through forward selection indicated that variance in maximum egg count was explained by the following geospatial variables: permanently irrigated land, human footprint, and industrial and commercial units (Table 3, a). Variance in average number of eggs was explained by the same set of geospatial variables, with the addition of area earmarked for road and rail networks (Table 3, b). GLM results showed that footprint and irrigated land had a significant positive effect on maximum egg count, as indicated by their respective coefficients (±SE): 0.04 (±0.001) and 0.01 (±0.0004). Area destined for industrial and commercial units showed a weak but negative relationship with maximum egg count: −0.005 (±0.0002). GLM results confirmed the significance of estimated coefficients for the explanatory variables ($P < 0.05$). Considering the increasing urbanization and human pressure at global scale, we calculated the predicted maximum egg count whenever human footprint index assumed its maximum value. According to the GLM results, and considering 50 as the maximum human footprint value (Venter et al. 2016), the maximum egg count would reach 545, far

exceeding the average of maximum egg counts recorded in this study (314) (Fig. 5). GLM results indicated, as above, a positive significant relationship between average egg number, footprint (0.04 ± 0.002, $P < 0.05$) and irrigated lands (0.02 ± 0.001, $P < 0.05$). A negative effect of area earmarked for industrial and commercial units was highlighted (−0.005 ± 0.0004, $P < 0.05$), whereas the amount of area destined for road and rail networks had a positive effect on mean egg number (0.02 ± 0.001, $P < 0.05$). The Mann–Whitney *U* tests indicated that maximum egg count ($U = 359$, $P < 0.05$) and average number of eggs ($U = 393$, $P < 0.05$) were greater for sites with presence of at least a patch of low and dense vegetation than for sites that had not.

Discussion

The outcomes here discussed represent the early steps of an *Ae. albopictus* monitoring began in 2017 in the Lazio region and still ongoing. As a matter of fact, notwithstanding an organized monitoring is crucial to achieve a comprehensive understanding of the vector-related risks for public health concerning this species, last data regarding *Ae. albopictus* in Central Italy go back up to almost 20 yr ago and refer only to Rome urban area (Romi et al. 1999). As expected, *Ae. albopictus* resulted to be widespread and abundant in the whole study area. As reported by many Authors, the main climatic drivers influencing *Ae. albopictus* life-cycle are photoperiod, air temperature, and rainfall. Notably, the identified
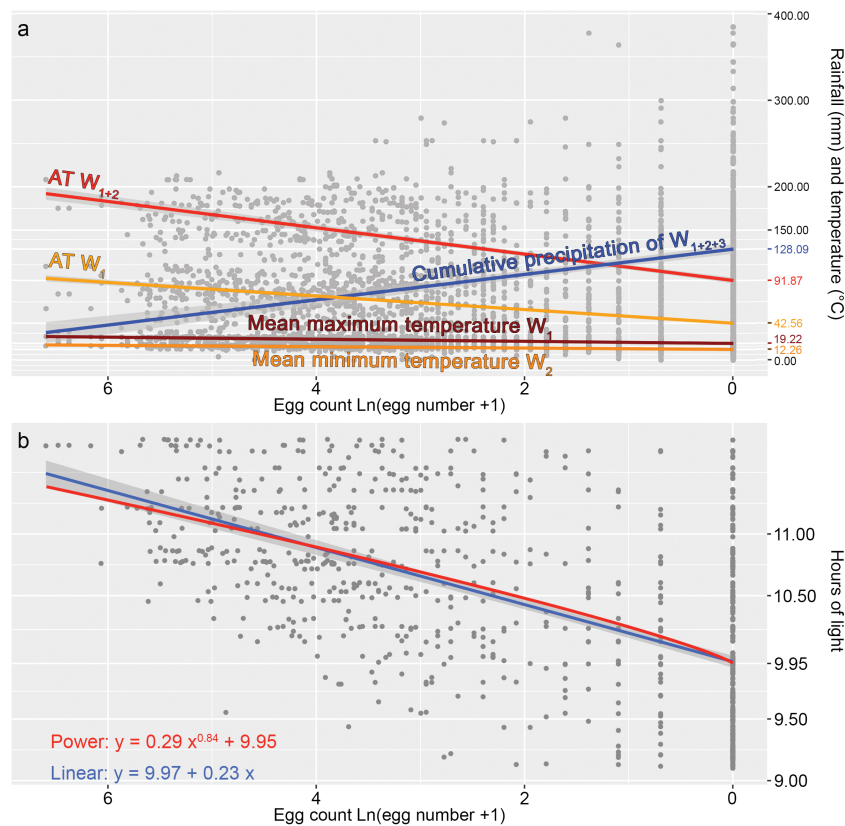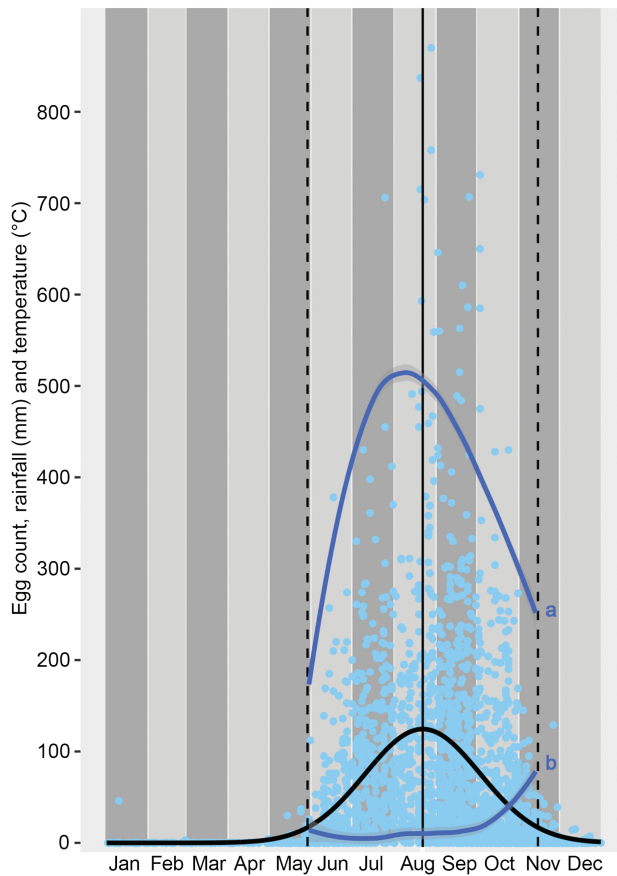
Fig. 3. Relationships between egg count and weather variables during the end of egg-laying activity: (a) cumulative precipitation ($P < 0.01$), AT (W_1: $P < 0.01$, W_{1+2}: $P < 0.01$), means of maximum ($P < 0.01$) and minimum ($P < 0.01$) weekly temperatures, with highlighted threshold values (see text for details); (b) hours of light with selected models equations. Thresholds values were 9.95 and 9.97 h of light, estimated respectively by Power and linear regression ($P < 0.01$) (Table 1, b). The P-values of Power coefficients were: a ($P < 0.01$), b ($P < 0.01$), c ($P < 0.01$).

threshold photoperiod for egg-laying activity to start was 13:11 (L:D) h (mid April), which can be considered a reliable threshold for the whole Lazio region, since northernmost site (Lat. 42° 05′ 53″ N) and southernmost one (Lat. 42° 15′ 15″ N) differed by less than one latitudinal degree (i.e., ~ 111 km). Besides, considering that developmental time can take more than three weeks from egg hatch to adult stage at low temperatures (Hawley 1988), we can assume that overwintering eggs hatched in early March, namely when the photoperiod was 11.6:12.4 (L:D) h, in accordance with Toma et al. (2003). Female trophic activity lasted until the photoperiod dropped below 10:14 (L:D) h (mid November), when it is likely that concomitant temperature factors trigger adult females to lay diapausing eggs (Waldock et al. 2013). Regarding *Ae. albopictus* developmental zero temperature, a threshold of 10.4°C has been identified, consistently with the commonly accepted lower threshold of 11°C (Hawley 1988, Kobayashi et al. 2002). It is worth noting that the threshold temperature highlighted for an *Ae. albopictus* Northern Italy population was 13°C (Roiz et al. 2010) and that our results reported a significant difference in threshold temperature among municipalities. Geographically separated *Ae. albopictus* populations are likely to respond to different temperature thresholds, as it happens for critical photoperiod variations (Medlock et al. 2006) or for the overwintering ability achieved by temperate *Ae. albopictus* strains (Gubler 1970, Hawley et al. 1987). AT resulted to be among the relevant factors, together with cumulative precipitation and daily temperature, influencing abundance of *Ae. albopictus* eggs, hence the extent of its trophic activity. The AT over the 4 wk before sampling positively influenced egg abundance. Notably, the estimated

peak of egg abundance showed a few weeks time delay respect to the AT peak (Fig. 4). Furthermore, our results showed that when the AT over the 2 wk before sampling dropped below 91.8°C (or that of the week directly preceding sampling drop below 42°C), oviposition activity ceased. The positive effect of temperature on female activity is consistent with the results of previous studies (Estrada-Franco and Craig 1995, Roiz et al. 2010).
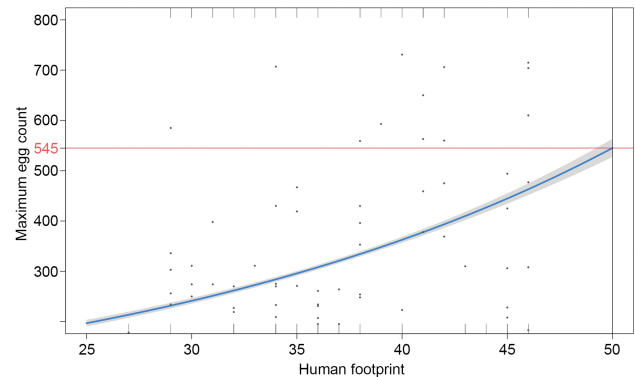
Precipitations showed a negative effect on oviposition activity. This relationship is quite controversial. As a matter of fact, for the biology itself of *Ae. albopictus*, the flooding of larval breeding sites is crucial to allow eggs submersion. Though, many Authors reported a negative effect of rainfall (Roiz et al. 2010, Dieng et al. 2011). It is worth noting that in Central Italy heaviest rainfalls occur in November, when also temperatures dropdown. Hence, the observed negative relationship between egg abundance and rainfall could be a consequence of the temporal coincidence between decreasing temperatures and autumn rainfalls. Besides, after rainfalls oviposition could be diverted towards manmade containers, usually abundant in urban and suburban areas, decreasing egg number in experimental containers. On the other hand, during summer months, egg abundance increased until reaching its peak even though no variation in cumulated precipitation occurred (Fig. 4). As underlined by Waldock et al. (2013), precipitation is one of the most difficult environmental variables to model, considering that in urban areas *Ae. albopictus* can give rise to rain-independent populations (Toma et al. 2003), due to the occurrence of many rain-independent water supplies linked to human activities. Our results show that in the study area, trophic activity of

Ae. albopictus, and the consequent arboviruses transmission risk, are mainly concentrated in the period June–November, when 95% of eggs were laid. Nevertheless, the whole activity can last up to 8 mo (depending on the municipality), whereas in northernmost regions, the whole trophic activity of the species is concentrated in our 95% time interval (Romi et al. 2008). This means that central regions of Italy could be particularly prone to sustain outbreaks

of *Aedes*-borne arboviruses, also since according to some Authors (Romi et al. 2006), females of this species could be able to extend their trophic activity to the coldest months of the year. It is thus possible, considering also the unexpected oviposition events in January and February here reported, that a favorable urban microclimate could extend the adult activity of *Ae. albopictus*. Spatial analysis indicated that fine-scaled characteristics influence the abundance of *Ae. albopictus*. Indeed, going down in detail from the municipality level until the specific OT site, an increasing explanatory power of the spatial variable (i.e., trap site) was highlighted. Despite indices describing landscape structure and heterogeneity might be useful to understand mosquito ecological requirements (Vanwambeke et al. 2007, 2011; Whiteman et al. 2019), Shannon diversity index did not explain variation in egg abundance as much as some land cover variables, presence of small patches of low and dense vegetation and human footprint. Some fine-scaled characteristics could be more relevant than spatial heterogeneity, meeting specific ecological requirements of the species. We highlighted that the presence of an even small patch of low and dense vegetation, providing shelters and potential resting sites, has a positive effect on *Ae. albopictus* abundance, consistently with previous findings (Kamgang et al. 2012, Manica et al. 2016). Furthermore, human footprint index was positively correlated with egg abundance. Human footprint, as calculated by Venter et al. (2016), takes into account built environments, population density, electric infrastructure, crop lands, pasture lands, roads, railways, and navigable waterways to define global human pressure. This variable can thus summarize different ecological

Fig. 4. Annual egg count trend over the 3 yr sampling. Solid black line represents the estimated *Ae. albopictus* phenology according to the Gaussian model, with the peak indicated by the vertical solid black line. Dashed thin vertical lines indicate the extent of the time interval within which 95% of eggs have been laid. Upper line (a) shows the trend for the AT over the 4 wk before sampling and lower line (b) indicates the cumulated precipitation for the week before sampling, during the 95% time interval. Shaded areas indicate the 95% confidence intervals of the lines.

Fig. 5. Relationship between maximum egg count and human footprint according to the GLM result, with indicated (by horizontal line) the expected count for the higher threshold value of human footprint and shaded areas indicating the 95% confidence interval.

Table 3. Result of the RDA ordination through forward selection performed on geospatial variables with maximum egg count (a) and average number of laid eggs during the time interval of 95% of egg-laying activity (b) as response variables

Geospatial variable (CLC third level code)	df	AIC	F	P
a				
Permanently irrigated land (212)	1	873.68	9.32	0.015
Footprint	1	869.67	6.01	0.020
Industrial or commercial units (121)	1	863.80	7.86	0.005
b				
Permanently irrigated land (212)	1	659.94	9.74	0.025
Road and rail networks and associated land (122)	1	655.46	6.49	0.015
Footprint	1	651.87	5.51	0.035
Industrial or commercial units (121)	1	648.13	5.58	0.015

Akaike information criterion (AIC); *F* statistic (*F*) and *P* value (*P*).

requirements of *Ae. albopictus* such as presence of breeding sites, proximity to human hosts and mild microclimate even during the coldest months of the year. It has long been known the connection between urbanization and milder weather conditions (Oke and Maxwell 1975). Furthermore, it has been shown that a rapid and unplanned urbanization process increases larval habitats, larval development rate, and survival of *Ae. albopictus* (Li et al. 2014, Samson et al. 2015). Hence, human footprint index should be taken into consideration in future analyses, since maximum number of laid eggs can almost double when human footprint reaches its higher threshold value. As for land cover categories resulting to have a significant influence on *Ae. albopictus* abundance, i.e., presence of permanently irrigated land (positive effect) and presence of industrial and commercial units (negative effect), very low scale and spatially restricted situations could explain these findings. The positive effect of permanently irrigated lands would seem to contradict what is well known for this species, i.e., the fact that in Europe it is a mainly urban and suburban species. As a matter of fact, analyzing individually the sites influencing this result, it was possible to ascertain that even if main part of the buffers were interested by this kind of land cover, there were at least small portions with some kind of buildings (an hospital and an amusement park for children in two of these sites), together with the presence of many man-made larval breeding sites. On the other hand, most part of the buffers with industrial and commercial units were wholesale markets and hospitals, characterized by the absence of even a small patch of low vegetation. Given these results, it is questionable if the use of geographic variables, even if considered at a very low scale such 100 m of radius, can be really useful in understanding and defining the drivers affecting *Ae. albopictus* abundance, which would be influenced by fine-scaled situations. As examples, IZSLT entomologists were asked by the local public health services to investigate why egg counts at times exceeded 300 eggs/OT in a big hospital in Rome and in a railway station in Ciampino municipality. In both cases, it was possible to ascertain that in the strict proximity of the OTs there were many manmade breeding sites, in a small dump internal to the hospital and in an abandoned courtyard in the railway station. In the first case, it was possible to obtain the elimination of the many small water collections and, as expected, this intervention was quite immediately followed by a decrease in the number of eggs in the OTs. Thus, site-specific and very fine-scaled characteristics can lead to high *Ae. albopictus* abundance. Nevertheless, though fine-scaled, such situations are evenly distributed in urban and suburban areas.

In conclusion, we consider our findings relevant to deepen *Ae. albopictus* ecology in urban areas and useful to model its distribution and consequent risk of related arboviruses spread. In a public health perspective, clear recommendations include the reinforcement of surveillance activities between June and November in Central Italy and those areas with the same yearly pattern of rainfall/temperature, paying particular attention to the AT, which can predict the increase of vector abundance. Besides, in a risk reduction perspective, Local Health Authorities and governance should carry out awareness campaigns to help community eliminating and controlling mosquito breeding sites in private properties.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

Table S1. Results for the lower threshold temperature of each municipality (intercept) for the onset of egg-laying activity, together with R^2 and p-value of the performed linear regressions.

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