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Temperature Characterization of Different Urban Microhabitats of *Aedes albopictus* (Diptera Culicidae) in Central–Northern Italy

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ABSTRACT *Aedes albopictus* (Skuse) is an invasive mosquito species that has spread to many countries in temperate regions bordering the Mediterranean basin, where it is becoming a major public health concern. A good knowledge of the thermal features of the most productive breeding sites for *Ae. albopictus* is crucial for a better estimation of the mosquitoes' life cycle and developmental rates. In this article, we address the problem of predicting air temperature in three microhabitats common in urban and suburban areas and the air and water temperature inside an ordinary catch basin, which is considered the most productive breeding site for *Ae. albopictus* in Italy. Temperature differences were statistically proven between the three microhabitats and between the catch basin external and internal temperature. The impacts on the developmental rates for each life stage of *Ae. albopictus* were tested through a parametric function of the temperature, and the aquatic stages resulted as being the most affected using the specific temperature inside a typical catch basin instead of a generic air temperature. The impact of snow cover on the catch basin internal temperature, and consequently on the mortality of diapausing eggs, was also evaluated. These data can be useful to improve epidemiological models for a better prediction of *Ae. albopictus* seasonal and population dynamics in central–northern Italian urban areas.

KEY WORDS temperature, population dynamics, breeding site, diapause, developmental rate

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Aedes albopictus (Skuse), a species native to southeastern Asia, was introduced into Europe over the past two decades, probably via the international trade in used tires, and spread rapidly in Southern Europe. This mosquito is a vector of many viruses (Mitchell 1991) and was responsible for a chikungunya outbreak in Italy during 2007 (Angelini et al. 2007, Rezza et al. 2007, Bonilauri et al. 2008) and the transmission of dengue in Croatia and France during 2010 (La Ruche et al. 2010, Gjenero-Margan et al. 2011).

Ae. albopictus can inhabit urban environments, often using artificial containers as breeding sites. Because variation in the climatic conditions experienced within these urban settings may potentially influence the development and life cycle of this mosquito (Fig. 1), it is difficult to model and predict population dynamics with the limited data currently available.

The urban climate is very complex, and there can be important thermal differences on a neighborhood scale. Airflow can be greatly perturbed by urban features, land use, or even by interactions with small objects and the land surface; hence, air temperatures may vary by several degrees within very short distances. A study conducted in Florence between 2004 and 2009 showed a mean difference of almost 2°C between the warmest and coolest urban areas of the city in all seasons (Petralli et al. 2011). Another study in Tel Aviv showed that vegetation can produce a cooling effect during summer, inducing a temperature decrease of 3–4°C (Shashua-Bar et al. 2010). Typical scales of urban microclimates, from less than one meter to hundreds of meters, are related to the size and density of buildings, trees, roads, streets, courtyards, and gardens (Oke 1988).

Studies have attested that survival rates and the length of each developmental stage of *Ae. albopictus* depend strictly on temperature (Alto and Juliano 2001a,b; Delatte et al. 2009). Daily mean air temperatures are used to identify the most suitable thermal condition for the life cycle activation or overwintering eggs deposition (Romi et al. 2001), and seasonal or annual mean air temperatures are used to determine threshold conditions for the survival of adult mosquitoes and overwintering eggs (Hanson and Creig1994, Kobayashi et al. 2002, Roiz et al. 2010). Thermal differences among microhabitats and within catch basins hence play a fundamental role in the estimation of developmental rates calculated either through theoretical thermodynamic enzymatic models (Schoolfield et al.

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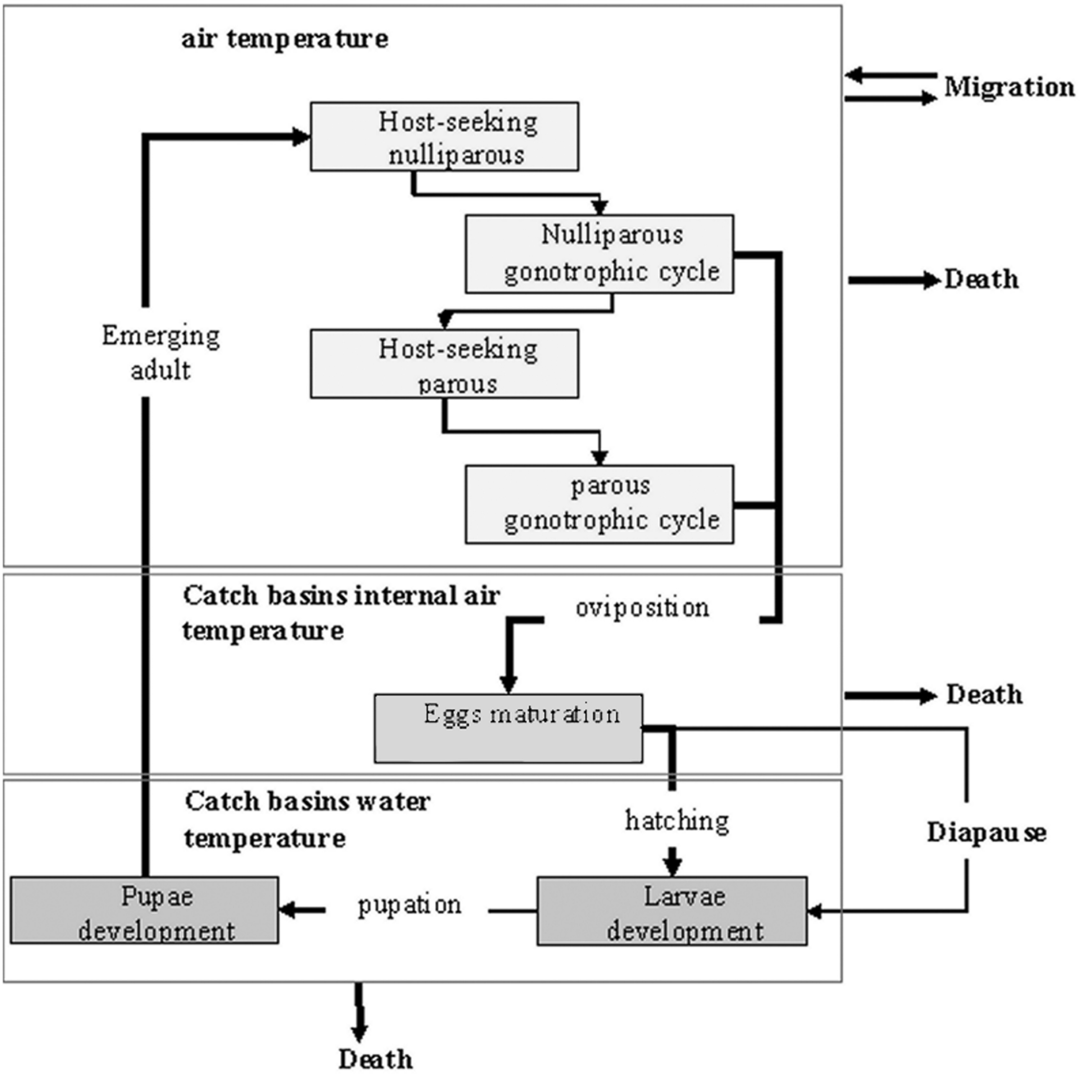

Fig. 1. Generic model diagram of mosquito population dynamics in urban area. The white section on the top shows the stages of *Ae. albopictus* life cycle influenced by air temperature (gonotrophic cycle). The central light gray section shows the stages influenced by the catch basin internal temperature (egg maturation). The dark gray section on the bottom shows the aquatic stages of mosquitoes influenced by the catch basin water temperature (pupae and larvae development).

1981) or the parametric function of temperature (Poletti et al. 2011).

A good estimation of developmental rates under a variety of temperature conditions is a key factor for their use in more complex population dynamics and epidemiological models (Focks et al. 1993, Dumont et al. 2008, Erickson et al. 2010, Poletti et al. 2011, Tran et al. 2013). Indeed, the CIMSiM model (Focks et al. 1993) for *Aedes aegypti* (L.), a closely related species to *Ae. albopictus*, implements an empirical formula for the estimation of maximum and minimum water temperature for 12 different types of containers based on air temperature and sun exposure.

In this study, micrometeorological monitoring was performed on three sites of a typical town in central–northern Italy called Cesena.Each site represents a particular urban microhabitat (permeable urban, impermeable urban, and permeable suburban microhabitats) that is also known to have different colonization and concentration levels of *Ae. albopictus* (Carrieri et al. 2011).

The study aimed to identify the main thermal characteristics in proximity to the land surface or inside what is considered to be the most productive breeding site, i.e., the ordinary urban catch basin (Carrieri et al. 2011) used to collect meteoric water or water from human activities; indeed, Carrieri et al. (2011) stated that in the towns of Emilia-Romagna region 90% of the larval population develop inside catch basins.

We focused on three types of temperature: air temperature at two meters, air and water temperature inside an ordinary catch basin. The air temperature drives oviposition, the gonotrophic cycle, and mainly determines the survival of adult females, a crucial factor in the ability of *Aedes* mosquitoes to transmit pathogens such as the dengue virus (Bhatt et al. 2013). Water temperature in the container influences the development of immature aquatic stages and also adult female size that can affect their capacity to vector arboviruses (Carrington et al. 2013; Westbrook et al. 2010). Air temperature inside the catch basin impacts on eggs maturation process, as it was noticed that *Ae. albopictus* eggs were often laid on the catch basin side wall (Carrieri et al. 2011, Hawley 1988). Also, the absolute minimum winter temperature that directly affects the overwintering eggs mortality (Hanson and Creig 1994, Thomas et al. 2012) and the snow cover insulating effect over the catch basin (Zhang 2005) were evaluated.

Air temperature and catch basin internal temperature were compared among the three microhabitats in order to evaluate their variability in the urban context.

Materials and Methods

Study Area and Environmental Data. The study was conducted in Cesena (latitude 44° 8′16.80″, longitude 12° 14′38.04″ and around 42 meters a.s.l.), a town in the Emilia-Romagna region, central–northern Italy, northeast of the Appennine mountains and 15 km from the Adriatic sea. Cesena has a warm-temperate sub-Mediterranean climate with warm summer, mild and quite wet winter, and wet autumn and spring (Kottek et al. 2006).

The study of breeding sites conducted by Carrieri et al. (2011) in Cesena showed that the most common and productive breeding sites for *Ae. albopictus* were the urban catch basins, which produced 96.3% of all the collected pupae. The catch basins in Cesena as well as in most central–northern Italian urban areas drain the meteoric water and are installed both on private property (54.3 catch basins per hectare in Cesena) and along the roadsides (7.9 catch basins per hectare).

In the town and its surroundings, three types of microhabitat were identified:

MH1—permeable urban microhabitat mainly consists of houses with private garden (59.6%). In 95.1% of these premises, the green areas or open courtyards are <50% shaded.

MH2—impermeable urban microhabitat consists of commercial and industrial areas (21.6%) where 94.6% of the car parks or courtyards are sunny and the shaded area is <25%.

MH3—permeable suburban microhabitat consists of detached houses with vegetable garden located in a periurban area (15.3%). Similarly to MH1, in the 94.2% of these premises <50% of the green areas are shaded.

The three microhabitats represent about the 97% of the entire urban and suburban area of Cesena (Carrieri, personal communication).

The micrometeorological study was conducted for each microhabitat in the proximity of a representative catch basin.

Meteorological Data. Hourly temperature and relative humidity data were collected from 11 May 2007 until 30 September 2012 by installing a small urban network of sensors, sited in accordance with the WMO (World Meteorological Organization) guidance and recommendations (Oke 2006). Micrometeorological sensors were placed in each microhabitat (MH1, MH2, and MH3), with three types of installation (outside, inside the catch basin, and underwater) for either characterization or comparison purposes. The external installation consists of thermometer (TE—catch basin external temperature) and an hygrometer (RHE—catch basin external relative humidity) sited at 2 m above the ground almost 4 m away from the catch basin, and >2 m from buildings in order to obtain representative measurements in built-up areas (Oke 2006); the internal installation consists of thermometer (TI—catch basin internal temperature) and an hygrometer (RHI—catch basin internal relative humidity) attached to the side wall of the catch basin; the water installation consists of a thermometer (TW—catch basin water temperature) placed on a float under water. All the sensors were placed in MH1, while TW wasn't installed in microhabitat MH2 and MH3. The catch basin in MH1 and MH3 have analogous physical characteristics with a grate on top, while the one in MH2 is covered by a closed slab and water input is through a drainage channel. Three types of meteorological sensors (HOBO PRO Data Logger, Onset Computer Corporation, Pocassette, MA) were used as reported in other studies (Petralli et al. 2011). The acquisition interval was set at 1 h; all the sensors and all the local environmental characteristics around them remained unchanged throughout the monitoring period, thus avoiding time series inhomogeneities. Among measurements, the internal relative humidity (RHI) showed several unreliable values most likely due to the exposure to high humidity. The consequent lack of recovery from condensing conditions led to unavoidable inhomogeneities in the time series, so humidity data are not discussed in this article. In addition, a weather station sited in the Cesena urban area belonging to the regional hydro-meteorological network (Regional Agency for Environment Protection in the Emilia-Romagna Region, ARPA-EMR) was used either as a reference or comparison station, as the data recorded covered the whole study period. The station is equipped with a thermometer and hygrometer (hereinafter called T_ref and RH_ref) sited at 2 m above the ground. The sensor brands differed from those of the network in this study, but their technology and installation guaranteed trustworthy and reliable comparison. All the meteorological variables are listed in Table 1.

Seasonal data were calculated according to the WMO standard: winter is between December 1st and February 28th or 29th, spring season is between March 1st and May 31st, summer season is between June 1st and August 31st, and autumn is between September the 1st and the November 30th.

Statistical Analysis. Hourly data were averaged on a daily basis in order to calculate mean, maximum, and minimum values. A multiregressive linear model (MLM) was constructed for each temperature time series (mean, maximum, and minimum daily temperature were the MLM response variables) using ARPA-EMR daily mean, maximum, and minimum temperature and mean relative humidity as the predictor variables to achieve two goals: the first was to fill missing data in order to compare homogeneous time series and the second was to create a predictive statistical model either for air temperature in different urban microhabitats or for the specific temperature of a representative catch basin based on a single reference weather station (i.e., ARPA-EMR weather station). Also, the variable "month" was introduced as a numeric factor in order to weight the seasonal component in the regression analysis.

The MLM is expressed by the following formula:

$$T_{\min,\max,med} = \beta_1 \times T\_ref_{mean} + \beta_2 \times T\_ref_{\max} + \beta_3 \times T\_ref_{\min} + \beta_4 \times RH\_ref_{mean} + \varepsilon_{month\_i} + \varepsilon,$$

whereβ_1, β_2, β_3, and $\beta_4$are the correlation coefficients, $\varepsilon_{month_i}$is the monthly factor variable, and ε is the error term. Akaike information criterion (Akaike 1973) was then used for the statistical model parameters selection from a larger set with a stepwise approach in order to optimize model performances and give a better discrimination of the microclimatic variability among sites. Model parameters were selected above the 90% significance level. Performances of MLM calibration were evaluated through Pearson correlation coefficient and the residual standard error. Fitted daily temperatures were then averaged on a monthly and seasonal basis in order to calculate the average and standard deviation of monthly mean, maximum, and minimum values.

A multivariate analysis of variance (MANOVA) was conducted on daily temperature fitted through the MLM for either mean, maximum, or minimum temperature in order to test the thermal differences among the three microhabitats (two triplets of dependent variables *TE1*,*TE2*,*TE3*, and *TI1*,*TI2*,*TI3* were compared), among different kinds of sensor installations in MH1 (one triplet of dependent variables *TW1*,*TI1*,*TE1*), and also from the reference weather station ARPA-EMR. A Welch's t-test was then conducted in order to determine whether the means of daily temperatures were significantly different from each other either on the whole data set or on seasonal basis. Also seasonal data were showed and discussed through the use of box plot in order to compare results between different microhabitats and installations.

For the MLM and model selection, the statistical functions (LM and STEPAIC) were taken from MASS R package (Venables and Ripley 2002), while for MANOVA analysis functions were taken from CAR R package (Fox and Weisberg 2011).

Temperature Impacts on the Developmental Rates. Each developmental stage (lengths of egg maturation, larval and pupal development, and gonotrophic cycle) was computed through the parametric functions implemented in Poletti et al. (2011), which are interpolated functions of the duration of developmental stages of *Ae. albopictus*, as reported in Delatte et al. (2009).

Developmental stages as a function of the daily mean temperature were calculated in a dual mode over the whole collection period and then averaged on a seasonal basis. In the first mode, a generic daily mean air temperature (ARPA reference station T_ref) was used; in the second, a specific temperature within the catch basin located in the permeable urban microhabitat (MH1) was used for each life stage according to the model diagram of mosquito population dynamics in Fig. 1. More specifically, the catch basin external daily mean air temperature was used for the estimation of the gonotrophic cycle, the catch basin internal daily mean air temperature for eggs maturation stage, and the daily mean water temperature for both larval and pupal developmental rates. The mean seasonal length of each developmental stage calculated with the two approaches was then compared in order to show the differences when using a generic temperature instead of a specific one, and a Welch's t-test was conducted in order to determine whether the means of the daily developmental lengths were significantly different during spring, summer, and autumn.

Snow Cover Effect on the Catch Basin Internal Temperature. There was a very cold spell in the whole of central–eastern Europe between January and February 2012. Cesena was also affected and three

Table 1. List of temperature sensors and related variable names involved in this study

Microhabitat	Variable	Location	Latitude	Longitude	Altitude (m)
ARPA-EMR	*T_ref*, *RH_ref*	Outdoor 2 m. a.g.l.[a]	44° 8'16.80"	12° 14'38.04"	42.00
MH1	*TE1*	Outdoor 2 m. a.g.l., Proximity of the catch basin	44° 7' 57.80"	12° 16'2.20"	42.00
MH1	*TI1*	Inside the catch basin	44° 7'57.80"	12° 16'2.20"	42.00
MH1	*TW1*	Water inside the catch basin	44° 7' 57.80"	12° 16'2.20"	42.00
MH2	*TE2*	Outdoor 2 m. a.g.l., Proximity of the catch basin	44° 7'57.80"	12° 16'11.80"	42.00
MH2	*TI2*	Inside the catch basin	44° 7'57.80"	12° 16'11.80"	42.00
MH3	*TE3*	Outdoor 2 m. a.g.l., Proximity of the catch basin	44° 6'33.58"	12° 16'20.44"	42.00
MH3	*TI3*	Inside the catch basin	44° 6'33.58"	12° 16'20.44"	42.00

T_ref is the temperature of ARPA reference station; *RH_ref* is the relative humidity of ARPA reference station; *TE*, *TI*, and *TW* stand for the catch basin external, internal, and water temperature, respectively, while numbers *1*, *2*, and *3* stand for microhabitat 1, microhabitat 2, and microhabitat 3, respectively.

[a] m. a.g.l. stands for meters above ground level.

consecutive snowfalls between February 1st and 15th led to a uniform snow cover of about 50 cm and a sharp air temperature decrease.

Differences between observed minimum temperature inside the catch basin and the fitted temperature through the MLM induced by the snow cover were minimized with the following method: the formula described in the statistical analysis section was inverted, such that the daily minimum internal temperature (the dependent variable became the independent one) and the mean relative humidity were both set to a constant value equal to the mean value recorded between February 1st and 15th. The independent variables T_ref_{mean}, $T\_ref_{\max}$, and $T\_ref_{\min}$ were all set constant and equal to the unknown variable X that was then calculated using the regression parameters shown in Table 2.

Results and Discussion

Statistical Model. For each kind of temperature sensor and installation (Table1), an MLM was calibrated over the whole data collection period either for mean, maximum, or minimum daily temperature, building up a total of 21 MLMs.

Model parameters were selected above the 90% significance level and are reported in Table 2, together with coefficient of determination R^2 and the residual standard error *SE*. The *p*-value resulted as being less than 0.001 for every MLM and also the *F*-statistic value and degrees of freedom always resulted as high, so there is a significant relationship between the independent variables (predictor variables) and the response variables (predictand variables) in the linear regression model.

Coefficient of determination R^2 resulted always higher than 0.93 for each type of installation (external, internal, and water installation) and for each microhabitat; thus, a very high correlation could be stated. Concerning the standard error *SE*, the lowest values were obtained for all kind of installation of MH1 (always lower than 1.60), while the highest values resulted up to 2.72 in MH2 and 2.33 in MH3.

Every statistical test derived from MANOVA analysis, either Hotelling–Lawley or Wilks or Pillai test criteria, confirmed the null hypothesis that all the dependent variables differed with a significance level above 99%. Therefore mean, maximum, and minimum daily temperatures resulted to be significantly different either between the three microhabitats and the three types of installations or from the reference external temperature ARPA-EMR. The Welch's t-test statistical significance is discussed in the following paragraphs where only significant differences with a confidence interval above 90% are argued (*p*-value resulted as being less than 0.1).

Catch Basin External Air Temperature. No significant differences in the daily mean temperature on the whole dataset were found between the three microhabitats and ARPA-EMR reference station. Instead some significant differences occurred on seasonal basis: *TE2* resulted a little higher than *T_ref*, while *TE3* a little lower in summer and in autumn (Fig. 2). Among the three microhabitats, *TE3* resulted a little lower than *TE2* and *TE1* in summer and autumn, and a little lower than *TE2* for the whole period.

Table 2. Parameters of the MLM for the three microhabitats

Variable[a]	β_1 [b]	β_2 [b]	β_3 [b]	β_4 [b]	ε_{feb} [c]	ε_{mar} [c]	ε_{apr} [c]	ε_{may} [c]	ε_{jun} [c]	ε_{jul} [c]	ε_{aug} [c]	ε_{sep} [c]	ε_{oct} [c]	ε_{nov} [c]	ε_{dec} [c]	ε [d]	R^2 [e]	*SE* [f]
TW1G	0.40		0.10	0.02		1.26	3.19	5.21	6.40	7.80	8.40	6.81	4.78	2.91	1.16	2.67	0.98	1.07
TW1M	0.40	0.03	0.06	0.01	0.43	1.54	3.51	5.65	6.74	8.42	9.02	7.33	5.06	2.96	1.17	3.21	0.98	1.09
TW1m	0.36		0.16		0.03	1.01	2.89	4.81	6.04	7.15	7.74	6.27	4.39	2.86	1.16	1.88	0.97	1.21
TI1G	0.64		0.08	0.01	0.26	1.46	2.89	4.62	5.52	6.98	6.83	4.70	2.80	1.35	0.33	1.26	0.98	1.13
TI1M	0.76	0.13	-0.15	-0.01	0.68	1.96	3.39	5.22	6.14	7.60	7.11	4.52	2.38	0.82		3.11	0.97	1.60
TI1m	0.51	−0.06	0.25	0.02		0.97	2.48	4.22	5.12	6.56	6.61	4.73	2.97	1.59	0.53		0.98	1.22
TE1G	0.80		0.13		−0.23	0.37	0.97	1.46	1.49	2.13	1.77	1.26	0.58			0.44	0.99	0.71
TE1M	0.57	0.62	−0.20	−0.01												1.97	0.99	1.07
TE1m	0.31	−0.22	0.79	0.03		0.35	1.31	2.21	2.58	3.23	2.78	2.31	1.20	0.40		−1.65	0.96	1.40
TI2G	0.79			−0.02		1.45	4.66	8.57	10.75	12.30	10.67	7.50	4.17	1.74		3.67	0.96	2.23
TI2M	0.66	0.28	−0.20		1.51	4.10	8.28	11.18	12.66	14.92	13.51	10.46	6.09	2.33		1.98	0.95	2.72
TI2m	0.56	−0.09	0.22			1.06	4.07	6.86	8.90	11.52	10.39	8.25	4.70	2.42	0.52	1.81	0.96	1.82
TE2G	0.93	−0.04	0.07	0.00	−0.20		0.56	1.17	1.35	1.53	1.33	0.92	0.27			0.66	0.99	0.64
TE2M	0.53	0.62	−0.14	0.00			0.47	0.91	0.99	0.79	0.53					0.61	0.99	1.03
TE2m		−0.07	0.87	0.01			0.75	1.44	1.80	2.19	2.13	1.82	0.65				0.97	1.14
TI3G	0.49		0.07	0.01		1.90	3.65	6.20	7.26	8.93	8.88	8.01	4.65	2.88	0.95	2.30	0.98	1.23
TI3M	0.51	0.09			0.71	2.96	3.80	6.39	6.94	9.04	8.81	7.59	4.47	2.47	0.77	3.10	0.97	1.49
TI3m	0.43		0.13	0.01		1.13	3.22	5.59	6.84	8.27	8.19	7.63	4.16	2.82	0.92	1.15	0.96	1.43
TE3G	0.60	0.12	0.21	0.01		1.07	1.30	1.25	1.13	1.57	1.80	1.32					0.98	1.08
TE3M	0.68	0.77	−0.47	−0.02		2.35			−2.39				−1.45	−1.33	−0.87	2.37	0.93	2.33
TE3m	0.31	−0.12	0.71	0.05			0.69	1.04	1.59	1.58	2.32	1.79	0.56			−4.23	0.94	1.52

[a] Response variable names as reported in Table 1 with the addition of the letter "G," "M," or "m" that stand for mean, maximum, and minimum daily values, respectively.

[b] β_1, β_2, β_3, and $\beta_4$are the correlation coefficients selected above the 90% significance level; they multiply ARPA-EMR daily mean, maximum, and minimum temperature and mean relative humidity, respectively.

[c] ε_{feb}, ε_{mar}, ε_{apr}, ε_{may}, ε_{jun}, ε_{jul}, ε_{aug}, ε_{sep}, ε_{oct}, ε_{nov}, ε_{dec} are the monthly factor variables that refer to January.

[d] ε is the error term.

[e] R^2 is the coefficient of determination.

[f] *SE* is the residual standard error.

These results are probably due to the suburban location of MH3 that is not affected by the urban heat island effect. Slight and not significant differences between *TE1* and *TE2* (*TE2* was generally about 0.2–0.4°C higher than *TE1*) could be related to the features of the impermeable urban microhabitat (MH2) that mainly consists of concrete-based materials and almost no trees and green areas; the greater exposure to solar heat and the thermal capacity of concrete might cause a temperature increase during the whole daily cycle.

Although these differences are not very marked, the gonotrophic cycle could be influenced in a nonnegligible way and slight differences (not statistically significant) might be expected in the estimation of gonotrophic cycles through an enzymatic model (Fig. 3d).

Catch Basin Internal Air Temperature. Catch basin internal air temperature plays a key role in the *Ae. albopictus* life cycle. Daily mean values directly affect eggs maturation and may thus induce a delay or advance in the development cycle that contributes to

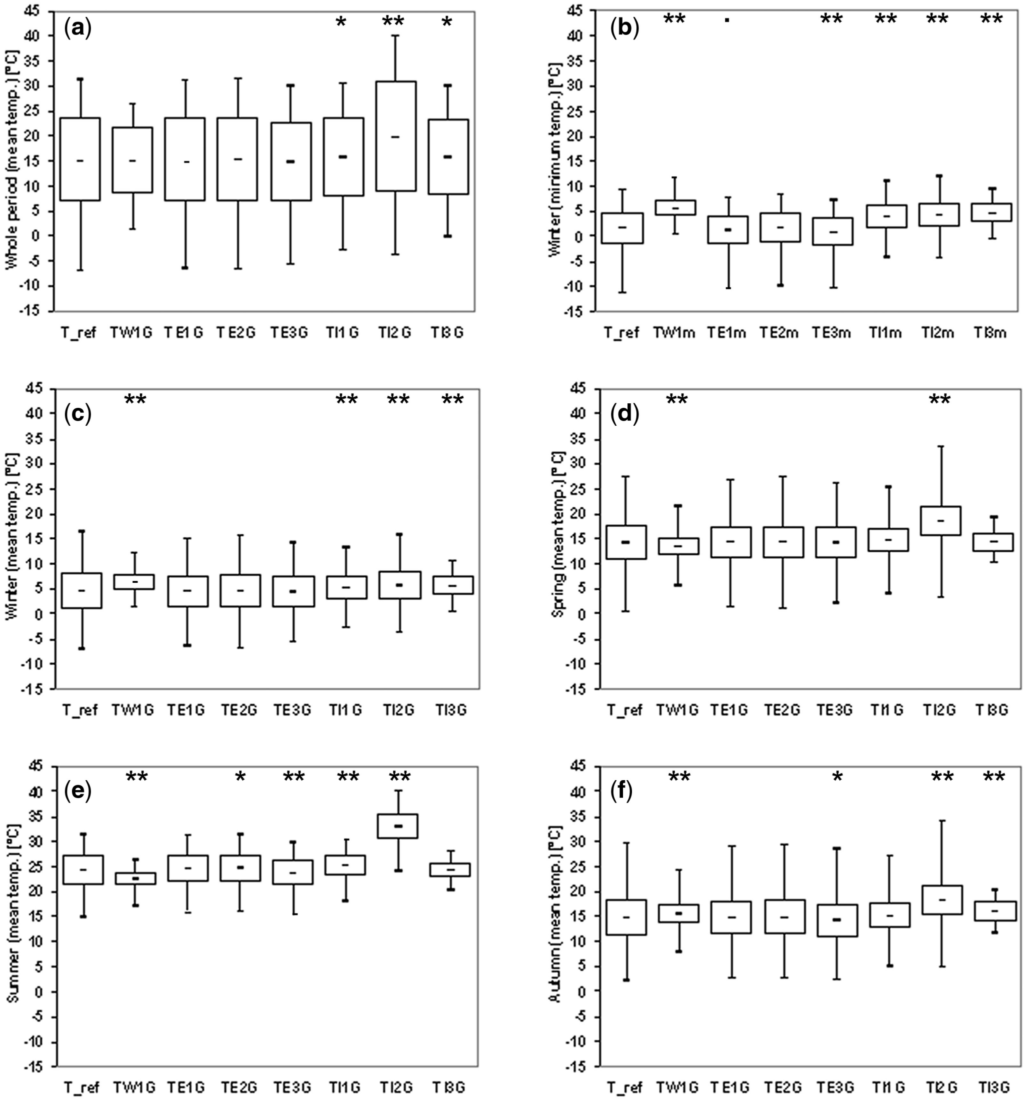

Fig. 2. Temperature comparison. Box plot consists of mean value, mean value plus and minus the standard deviation, and maximum and minimum absolute value averages over the whole period and on a seasonal basis. All graphs indicate the averaged mean value, except (b) where averaged minimum winter temperatures are shown. Sample size of daily data are 1,968 (a), 450 (b, c), 481 (d), 552 (e), and 485 (f). Dot or asterisks placed above the bars indicate a 90% (▪) 95% (*), and a 99% (**) confidence interval, reflecting a significant difference from the reference T_ref (Welch's t-test). Variable names on x-axis are defined in Table 1, with addition of the letter "G" in graph (a), (c), (d), (e), and (f) that stands for mean value, while addition of the letter "m" in graph (b) stands for minimum value.

nonsimultaneous larval development in urban areas. The absolute minimum temperature is also a major determinant of overwintering mortality in eggs (Hanson and Creig 1994).

The catch basin internal temperatures in the three microhabitats were always significantly higher than the external reference temperature, except for *TI3* in summer and spring, and for *TI1* in autumn (Fig. 2). Also, microhabitats reciprocal differences were always significant, except for the differences with *TI1* and *TI3* for the whole period and between *TI2* and *TI3* in winter. Seasonal mean temperature *TI2* was always much higher than *TI1* and *TI3* (Fig. 2): differences were up to 8°C during summer, about 4°C in spring, 3°C in autumn, and just 0.5°C in winter. Furthermore *TI2* was also generally more variable than *TI1* and *TI3*, showing higher maximum values and lower minimum values in all four seasons.

All three microhabitats showed always significant differences between catch basin internal and external temperatures, except for MH1 and MH3 in spring. Over the whole collection period, differences between internal and external temperature were about 0.6°C in MH1, 4.5°C in MH2, and 0.9°C in MH3.

The differences in MH2 may have two causes. First the catch basins could differ in their physical characteristics that influence heat accumulation (as described in Materials and Methods section). Second is the environmental features of the impermeable microhabitat that are typical of industrial and commercial areas, where few trees and green areas together with high building density and concrete-based materials may increase local temperatures. The influence of these possible factors is supported by the observed differences recorded in MH2 between the internal and the external temperature, which reached a maximum of about 8.5°C during summer due to the high insolation and long photoperiod, while the minimum of about 1.1°C was reached in winter.

It can therefore be stated that this type of catch basin, particularly if located in an impermeable urban environment, could induce an earlier development of the first generation of mosquitoes during spring, support the autumn colonization due to a faster larval and pupal development, and favor the oviposition of diapausing eggs. On the other hand, during summer too high temperature and lack of green areas could limit the colonization and spreading of this mosquito.

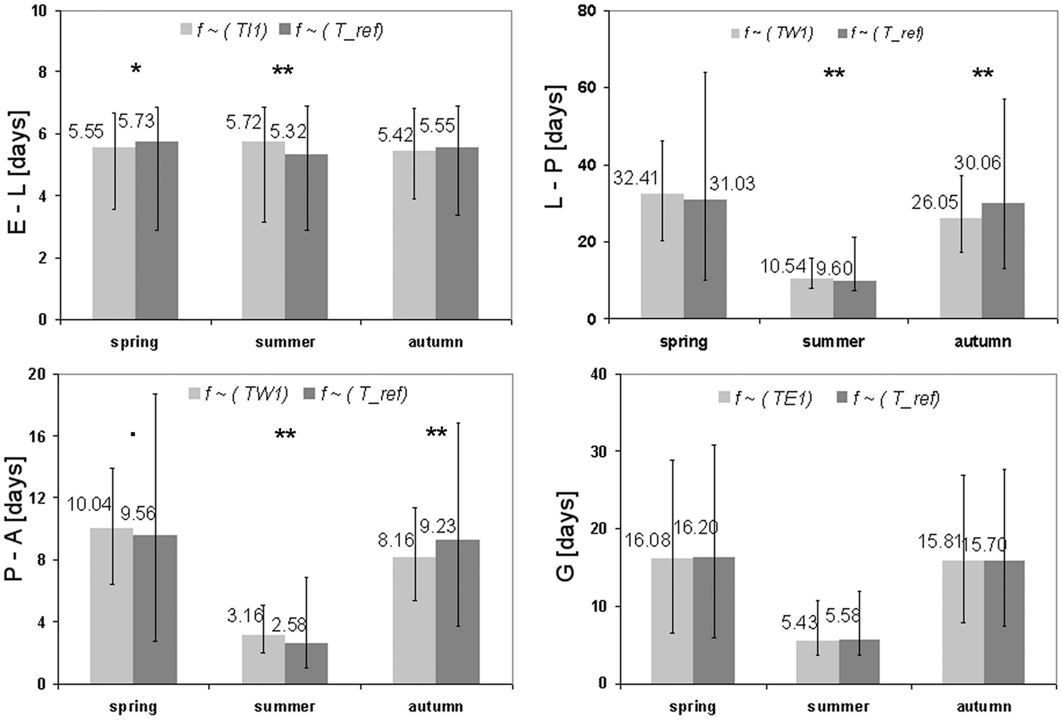

Fig. 3. Comparison of developmental time length. Seasonal mean values are represented by gray histograms, while maximum and minimum values are represented by black bars: (a) egg maturation time length, calculated either as a function of catch basin internal temperature—TI1 (light gray) or the reference temperature T_ref (dark gray); (b) larva to pupa developmental time length, calculated either as a function of catch basin water temperature—TW1 (light gray) or reference temperature T_ref (dark gray); (c) pupa to adult developmental time length, calculated either as a function of catch basin water temperature—TW1 (light gray) or reference temperature T_ref (dark gray); (d) gonotrophic cycle time length, calculated either as a function of catch basin external temperature—TE1 (light gray) or reference temperature T_ref (dark gray). Sample size of daily data are 481 (spring), 552 (summer), and 485 (autumn). Dot or asterisks placed above the bars indicate a 90% (▪), 95% (*), and a 99% (**) confidence interval reflecting a significant difference from the developmental time length calculated with the reference temperature T_ref (Welch's *t*-test).

Moreover, a mean summer value of about 33.05°C in MH2 could hinder its development (Briegel et al. 2001 and Delatte et al. 2009).

Compared with the reference air temperature *T_ref* (Fig. 4a), the temperatures within the catch basin resulted in mean egg maturation times that were a little faster during spring of about 0.2 d (T = −2.200; df = 954.799; $P < 0.05$), slower during summer of about 0.4 d (T = −5.256; df = 1077.755; $P < 0.001$), and just a little faster during autumn of about 0.13 d (not statistically significant). Collectively, these differences might result in an overall increase in the estimated length of *Ae. albopictus* development season.

The absolute winter minimum air temperature inside the catch basin is a key factor for evaluating the overwintering eggs mortality as pointed out by Hanson and Creig (1994) and Thomas et al. (2012). Hanson and Creig (1994) verified in Japan that for a minimum air temperature of −1°C the overwintering eggs mortality was about 9%, for −5°C about 15%, and for −10°C about 21%. Thomas et al. (2012) found that the lowest temperature threshold tolerated by European eggs of *Ae. albopictus* undergoing diapause was −10°C for long-term exposures (12 and 24 h) and −12°C for 1-h exposure. During the cold spell in December 2009, the reference air temperature *T_ref* reached an absolute minimum temperature of about −11°C, a value of about 6°C lower than those reached inside the catch basin in the three microhabitats. The use of air temperature instead of catch basin internal temperature would therefore cause an overestimation of mortality, thus resulting in an underestimation of the availability of overwintering eggs at the beginning of the development season.

Comparing daily mean values of the winter minimum temperature, microhabitats reciprocal differences resulted always significant as well as the difference between the three microhabitats and the reference external temperature. The catch basin internal temperature in the three microhabitats was much higher than *T_ref* and MH3 was higher than MH1 and MH2. In contrast, the absolute minimum winter temperature in suburban microhabitat MH3 was almost 3.5°C higher than those in MH1 and MH2. This evidence was far from it might be expected and couldn't be related only to microhabitat differences. The anomalous behavior of the MLM in computing the absolute minimum winter temperature in MH3 could only be explained by the lack of data during the coldest period of the whole measurement campaign, when temperature of about −3°C were measured by both sensors in MH1 and MH2 around December 20th 2009. Because MLM in MH3 is trained by observed data always higher than −1°C, the MLM couldn't obtain temperatures as low as in MH1 and MH2.

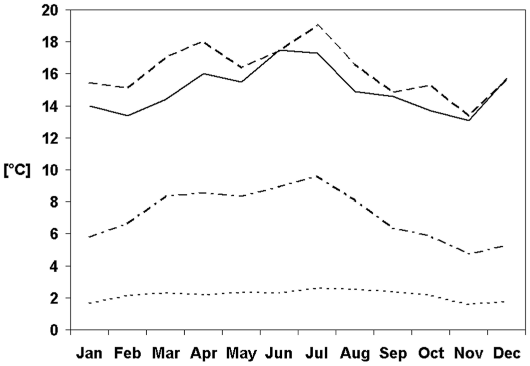

Fig. 4. Comparison of temperature excursion in microhabitat MH1. Daily excursions were calculated as the difference between the maximum and minimum daily temperature and averaged on a monthly basis: solid line is the excursion of the reference air temperature (*T_ref*), dashed line is the excursion of the catch basin external temperature (*TE1*), dash-dotted line is the excursion of the catch basin internal temperature (*TI1*), and dotted line is the excursion of the catch basin water temperature (*TW1*).

Catch Basin Water Temperature. The catch basin water temperature was measured only in the permeable urban microhabitat (MH1). The mean water temperature, which is the parameter conditioning all the immature stages, gave the most striking results.

TW1 resulted significantly different either from the external reference temperature (Fig. 2) or internal and external temperatures in the three microhabitats; the only exceptions were the differences with TI1 and TI3 in autumn and with *T_ref* and the external temperatures in all the three microhabitats for the whole period. Mean value of *TW1* was about 0.8°C and 1.7°C lower than *T_ref* in spring and summer, respectively, while it was about 1°C and 2°C higher than *T_ref* in winter and autumn, respectively (see Fig. 2). These strongly affected the development of immature stages (Fig. 3b and c). The larval developmental stage as a function of catch basin water temperature was on average about 1 d slower during spring (not statistically significant) and summer (T = 5.736; df = 1039.188; $P < 0.001$), but up to 4 d faster during autumn (T = −4.225; df = 847.379; $P < 0.001$). Similarly, the pupal developmental stage was about half a day slower during spring (T = 1.824; df = 900.881; $P < 0.1$) and summer (T = 8.574; df = 991.245; $P < 0.001$), and about 1 d faster during autumn (T = −3.798; df = 856.605; $P < 0.001$). Hence the faster larval and pupal developmental stages during autumn could produce additional generations of mosquitoes, an overall increase in the length of *Ae. albopictus* development season, and also an increased production of diapausing eggs.

Moreover mean seasonal values of water temperature were much less dispersed than the catch basin external or internal air temperature throughout the year, due to the much greater thermal capacity than that of the air. Hence, it can be stated that water temperature is much less affected by air temperature fluctuations; this could led to great prediction variability in the estimation of larval and pupal developmental stages, and much more uncertainty on a daily basis could be expected using the generic air temperature.

Finally, another relevant outcome is that the absolute minimum temperature was never below 0°C and absolute maximum never exceeded 27°C (data not shown).

Temperature Fluctuations. Temperature fluctuations were evaluated through the monthly mean temperature excursion that was calculated in MH1 for either the catch basin external (*TE1*), internal (*TI1*), or water temperature (*TW1*) as the difference between the maximum and minimum daily temperature averaged on a monthly basis (Fig. 4). The highest excursion occurred for *TE1* (i.e., *TE1M*−*TE1m*) as well as *T_ref* (i.e., *T_ref_M*−*T_ref_m*), varying between 13.4°C in November and 19°C in July; the excursion was lower for *TI1* (i.e., *TI1M*−*TI1m*), and it varied from 4.7°C in November and 9.6°C in July. Instead, water temperature excursion *TW1* (i.e., *TW1M*−*TW1m*) was very low and almost steady throughout the year, varying between 1.6°C in November and 2.6°C in July (Fig. 4).

Larval development could hence be favored inside an ordinary catch basin, as the water temperature remains almost constant throughout the year, as stated by Carrington (Carrington et al. 2013), for *Aedes aegypti* (L.), unlike in small epigean breeding sites where the water temperature fluctuates more likely than the air temperature due to a reduced thermal capacity and insulation (Jacobs et al. 2008). Besides, small temperature fluctuations in larval environments significantly influence the development time, larval survival, females size, and the adult female reproductive output (i.e., the energy allocated to egg production and oviposition), and epidemiological factors like arbovirus infection rate can also be affected (Carrington et al. 2013 and Westbrook et al. 2010).

Snow Cover Effect Over the Catch Basin. In the permeable urban microhabitat (MH1), the measured air temperature inside the catch basin, which is the relevant temperature during winter affecting the mortality of overwintering eggs (Hawley 1988, Carrieri et al. 2011), was strongly influenced by the snow cover that persisted above the catch basin between February 1st and 15th 2012. As shown in Fig. 5, daily minimum internal temperature never fell below 0°C and its fluctuations were significantly reduced, varying between 0.9 and 1.6°C for 2 wk until the snow melted. On the contrary the catch basin external air temperature dropped to very low values with a minimum value of −11.2°C being recorded by the external sensor. It can thus be stated that the snow cover had a sort of insulating effect on the catch basin preventing exposure of the overwintering eggs to very low temperature, thus allowing the survival of diapausing eggs during winter. The insulating effect could strongly affect the estimation of the absolute winter minimum temperature and thereafter the mortality of overwintering eggs, like stated by Hanson and Creig (1994) and Thomas et al. (2012).

Daily values of the minimum internal temperature fitted with the parameters in Table 2 wrongly showed values below 0°C with a trend that could be expected without the snow cover. The correction of the MLM was performed as previously described in Materials and Methods in order to obtain more trustworthy prediction outcomes in case of future snow-covered periods: the linear mathematical formula was inverted, the regression parameters were set as shown in Table 2, and the daily minimum internal temperature (*TI1m*) and external relative humidity of the reference station (RH_ref_{mean}) were set to the mean daily values measured during the snow cover: 1.25°C and 79.87% respectively. As a result, the fitted daily mean external temperature was −0.49°C; this represents the fictitious value that must be used in the MLM during a snow-cover period in order to obtain a more trustworthy value of the catch basin internal minimum temperature.

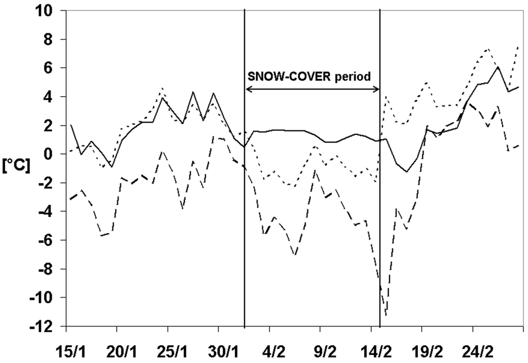

Fig. 5. Snow cover effect on the catch basin internal temperature in microhabitat MH1. The solid line is the observed daily mean temperature inside the catch basin, dashed line is the observed daily mean temperature outside the catch basin, and dotted line is the daily mean temperature inside the catch basin fitted through the multiregressive linear model without the correction concerning the snow cover effect.

The main purpose of this work was to give a precise definition of the thermal characteristics within one of the main breeding sites for *Ae. albopictus* in central–northern Italian urban areas (Carrieri et al. 2011), i.e., the catch basin. This work highlights the importance to identify thermically different urban microhabitats, as significant differences were found among the catch basin internal temperatures in the three different urban microhabitats taken into account (permeable and impermeable urban, and permeable suburban); significant differences occurred also between the catch basin external, internal, and water temperature. This study can thus be useful for better estimations of the length of *Ae. albopictus* developmental stages, mortality rates, and diapausing effect inside a catch basin.

Catch basin internal temperature and especially water temperature had the most evident impacts on the estimation of eggs maturation process, the length of immature stages, and the overwintering eggs mortality. Eggs maturation inside the catch basin resulted faster during spring, slower during summer, and slightly faster during autumn. Also larval and pupal developmental stage under the water of a catch basin was a little bit slower during spring and summer, but much faster during autumn. Hence, an ordinary catch basin could breed more generations of *Ae. albopictus* than a small epigean container, besides an increased production of diapausing eggs.

Furthermore, the absolute minimum winter temperature inside the catch basin was much higher than the external temperature especially during a snow cover period; hence, the adult females' habit of laying eggs on the side walls of catch basins enables them to overcome harsh winters more easily and to potentially recolonize large areas in early spring. Basically, if the ordinary catch basin represents the most common breeding site in an urban area, it can be stated that all the catch basin thermal characteristics might induce an increase in the estimated length of *Ae. albopictus* development season compared with taking into account a generic air temperature.

Furthermore the enhancement of all thermodynamic processes involved in the mosquito life cycle can allow a better set-up of population dynamics and epidemiological models, two important tools for control and preventive measures that public health authorities should take into account.

The study focused on Cesena, a small town in Emilia-Romagna Region, could be reasonably and approximately applied on every central–northern Italian urban area as well as others around the Mediterranean where the catch basin may represent one of the main breeding site for *Ae. albopictus*.

We would therefore strongly suggest using the specific temperature instead of a generic air temperature in order to properly estimate different developmental stages, mortality rates, or other aspects like overwintering eggs mortality and female size.

In order to reach this goal, we constructed a statistical model to predict external, internal, and water temperature for three kinds of microhabitats in Cesena using a reference weather station belonging to the Regional Agency for Environment Protection in the Emilia-Romagna Region. A correction was also introduced into the statistical algorithm in order to take into account the snow cover effect over the catch basin which was shown to be highly relevant for the estimation of diapausing eggs mortality. Calibration tests on the MLMs implemented in this work were striking, showing coefficients of determination always above 0.93 for all the models.

Finally, it is important to point out that the statistical analysis and MLMs are related to an ordinary catch basin with a capacity of about 40 liters; hence, obvious nonlinear variations are expected according to the catch basin capacity and physical characteristics, besides the environment surrounding the catch basin.

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