Weather Factors Influencing the Population Dynamics of *Culex pipiens* (Diptera: Culicidae) in the Po Plain Valley, Italy (1997–2011)

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ABSTRACT The impact of weather variables on *Culex pipiens* L. (Diptera: Culicidae) population dynamics in the Po Valley, Northern Italy, a densely populated region containing the largest industrial and agricultural areas in Italy, was investigated. Monitoring of mosquitoes was carried out by using CO_2-baited traps without light, collecting data weekly from 1700 to 0900 hours during the period May–September, from 1997 to 2011. Daily minimum, average, and maximum relative humidity; daily minimum, maximum, and average temperature; rainfall; and hydroclimatic balance (rainfall-potential evapotranspiration) were obtained from three weather stations within the surveillance zone. The average population dynamic trend over the 15-yr period showed a bell-shaped curve with a major peak in June and a secondary peak at the end of August in the rural areas, whereas bimodality was not evidenced in the urban areas. The correlation analyses showed that the mosquito seasonal population and the population in the period of maximum West Nile virus circulation (August–September) was mostly affected by the relative humidity registered from March to July, particularly in May, and, to a lower extent, also by hydroclimatic balance registered in April–July, and by the rainfall occurred in June–July. In addition, the rate of increase of the population during the spring months influenced the development of the mosquito population of the following months.

KEY WORDS *Culex pipiens*, weather variable, relative humidity, population dynamic, correlation analysis

Culex pipiens L. is the most common mosquito species in the Po valley and shows large adaptability to changeable environmental conditions, and it colonizes road drains and sewer drains both in urban and suburban areas and seminatural environments, such as irrigation canals, hunting swamps, rice fields, and sewage lagoons. Many authors agree in saying that, in anthropized environments characterized by surface water pollution, as the plain of Bologna, the primitive eurygamous anautogenic species *Cx. pipiens pipiens* has evolved in the stenogamous autogenic form *Cx. pipiens molestus* that is mainly ornithophilic, but shows a relatively higher inclination for mammalian hosts, including humans, which may increase the human risk of West Nile virus (WNV) transmission.

Since 2008, in northern Italy, *Cx. pipiens* have arisen high public and veterinary health concern because it is the primary vector of WNV and it can vector also Usutu virus (Calzolari et al. 2010a, 2012). In addition, in Italy, *Cx. pipiens* is an important vector of *Dirofilaria immitis* (Leidy) and *Dirofilaria repens* (Railliet and Henry) (Cancrini et al. 2006).

Outbreaks of WNV occurred frequently in several European regions where the virus is maintained and amplified through transmission cycles involving several mosquito species (mainly *Cx. pipiens* and *Culex modestus* Ficalbi), birds (reservoir host), and humans and equines, which are considered dead end (they do not produce significant viremia; Hubalek and Halouzka 1999, Komar et al. 2003, Reisen et al. 2006, Jourdain et al. 2007, Votypka et al. 2008, Pradel et al. 2009).

In Italy, in 2008, after 10 yr during which no circulation of WNV flavivirus had been observed, eight human cases in three provinces in the Po Valley (Emilia–Romagna and Veneto regions) occurred. In 2009, 16 cases were registered in eight provinces of the same two regions (Barzon et al. 2009, Rizzo et al. 2009, Angelini et al. 2010). In 2010, 20011, 2012, and 2013, an increase of cases (respectively 3, 9, 35, and 55 cases) in Veneto and Emilia–Romagna regions was registered (Barzon et al. 2011 and http://ecdc.europa.eu/en/healthtopics/west_nile_fever/west-nile-fever-maps/pages/index.aspx). The recurrent WNV circulation in our regions (Barzon et al. 2013) and the influence of the vector population size on pathogen transmission cycle brought us to focus our study on the weather

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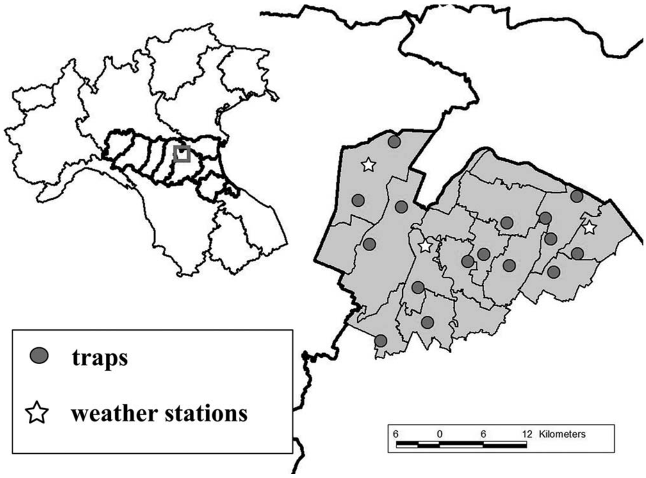

Fig. 1. Map of the study area with the position of the CO_2-baited traps.

factors affecting the mosquito seasonal development, the first necessary step to develop predictive models on the population dimension of *Cx. pipiens.*

The biology of mosquitoes suggests that weather can significantly affect adult and larval development and their survivorship, and many studies have focused on the effect of weather parameters on different aspects of mosquito bio-ecology (Shone et al. 2006, Reisen et al. 2008). According to Evans et al. (1987), to highlight the effects of weather conditions, the analysis should be extended over a range of time rather than considering data referred to single time points. Previous studies on the influence of weather parameters on the spread of mosquito-borne diseases found that temperature is the main parameter that governs larvae growth rate, female host-seeking activity, and fecundity (Moore 1985, Eisenberg et al. 1995, Reisen et al. 2008). The winter temperature influences the survival of overwintering females; rain intensity and frequency determine the quantity and quality of larval habitats; and the soil moisture and the relative humidity can affect the water persistency, the adult survival and dispersal, the female host-seeking activity and, as a consequence, the arboviral transmission by mosquitoes (Shaman et al. 2002, Shaman and Day 2005, Chaves and Kitron 2011).

It has been showed through the analysis of the data of WNV and Usutu virus infection rates in *Cx. pipiens*, recorded in the Emilia–Romagna region during 2009, that the growth rate of the viral circulation within the mosquito population increased from week 30 to week 39 (August–September; Calzolari et al. 2010b). At the same time, human cases occurred in Emilia–Romagna and Veneto from week 32 to week 35 (http://ecdc.europa.eu/en/healthtopics/west_nile_fever/west-nile-fever-maps/pages/index.aspx).

The current study focused on the impact of weather parameters on *Cx. pipiens* (the main vector of WNV in the Po Valley) population size, and the aim is to identify the physical and biotic environmental indicators that can enable to predict the seasonal population size. In particular, we investigated the influence of the meteorological parameters during the period May–July on the population density of the period August–September. This is the first necessary step for the development of epidemiological risk assessment models and preventative control programs.

Materials and Methods

Study Area. The study was performed in the plain area in the Bologna province (Emilia–Romagna region) where the weather is characterized by warm and wet summers, cold winters, rainy spring and autumn, and by low air circulation. The area is comprised between the latitude 44.646555°– 44.648048° and longitude 11.182993°–11.191504°. The study area is characterized by the presence of residential and industrial areas, surrounded by extensive agricultural ones, in which animal husbandry, ponds for fish farming, and hunting swamps are also present. The main breeding sites were drainage channels with sewage (including domestic, municipal, or industrial liquid waste products). Since 1997, sewage treatment programs have become fully operative in the urban areas of the Bologna province, thus significantly reducing surface water pollution and, in turn, leading to generalized decreased *Cx. pipiens* population density (www.gruppohera.it).

Sixteen CO_2 traps in fixed monitoring stations were activated in 12 municipalities (total surface of 665 km^2, resident population of 130,745 citizens), 10 localized in urban areas and 6 in rural areas (Fig. 1). The extension of the main breeding sites is reported in Table 1, whereas in Table 2, the typology of the breeding sites around the monitoring stations is summarized.

Mosquito Control Program. A mosquito control program was started in 1988, and it is still being carried out over an area of ≈800 km^2 across 20 municipalities. The control program included monthly larvicidal treatments with temephos (until 2005) and diflubenzuron (since 2006) in catch basins and weekly treat-

Table 1. Extension of the main *Cx. pipiens* breeding sites in the study area

Breeding sites	Area/length
Hunting swamps	4.13 km^2
Lake fish farms	0.21 km^2
Special conservation areas	0.83 km^2
Floodplains and overflow river basins	0.37 km^2
Rice fields	0.86 km^2
Sewage lagoons of alimentary factories	1.54 km^2
Aquatic phytoremediation areas	0.03 km^2
Canals	153.37 km
Irrigation canals	1,037.48 km
Treated sewer canals	169.60 km

ments with *Bacillus thuringiensis* subsp. *israelensis* de Barjac (*B.t.i.*) in all the other larval habitats. From 1997–2011, the same organizations and service companies were in charge of the mosquito control activities (project organization, larvicide treatments, and quality control activities), and used identical protocols.

Mosquito Monitoring. The mosquito monitoring activity by using CO_2-baited traps without light (Bellini et al. 2003) was carried out, collecting data once a week from the beginning of May to the end of September from 1997 to 2011. The monitoring stations were established in shaded green areas both in urban and rural areas, and were maintained in the same place during the entire study period. The traps, positioned at 1.5 m from the ground level, were supplied with ≈0.5 kg of dry ice, that ensured a working period of 16 h (1700–0900 hours). The natural logarithm transformation was applied to the number of *Cx. pipiens* females weekly captured, then used to build up a population growth curve.

Weather Parameters. The following weather parameters were registered on a daily basis: daily minimum, average, and maximum relative humidity (RH) and the mean RH during the adult activity period (AAP RH) from 2100 to 0700 hours (Veronesi et al. 2012); daily minimum, average, and maximum temperature; temperature excursion (ESC = Tmax−Tmin); and rainfall. In addition, the hydroclimatic balance (HCB) was calculated on a daily temporal resolution, as follows:

$$HCB = Ra - ETP \qquad [1]$$

where *Ra* was the rainfall, *ETP* was the potential evapotranspiration (calculated through the Hargreaves equation; Hargreaves and Samani 1985). Negative data represent hydrologic deficit conditions, whereas positive values indicate a hydrologic surplus.

Data were obtained from three meteorological stations of the Hydro-Meteo-Climate Service of the Emilia–Romagna Regional Agency for Environmental Protection (ARPA-SIMC): San Pietro Capofiume (longitude 11.62264°, latitude 44.653776°), Padulle di Sala Bolognese (longitude 11.290563°, latitude 44.627752°), and Sant'Agata Bolognese (longitude 11.144931°, latitude 44.695002°; Fig. 1).

Statistical Analysis. Weekly *Cx. pipiens* catches were summed over a month from May to September, to mitigate the influence of daily weather factors' variation that could affect trap efficiency (Reinert 1989, De Gaetano et al. 2005). Block ANOVA was used to compare the mosquito population densities of urban and rural areas. We applied the same data transformation used in previous approaches (Lebl et al. 2013), and mosquito counts were standardized to females per trap per month, and these standardized counts were transformed by log(y+1) to normalize the data and control the variance. We adopted the Spearman's rank correlation, which is insensitive to transformation using monotonic functions. The Spearman correlation was calculated between the different weather parameters and: The seasonal *Cx. pipiens* density (i.e., the seasonal average of the number of females captured by CO_2 traps) in urban and rural areas. The monthly *Cx. pipiens* density in the whole (urban "plus" rural) area. The average *Cx. pipiens* density in the period of highest WNV transmission risk (August–September) in the whole area.

The same analysis was run to assess the correlation between *Cx. pipiens* density in August–September and the mosquito density registered in the previous months.

Table 2. The breeding site typologies around the monitoring stations

Monitoring station locality	Environment categorization	Catch basins	Drains and sewer drains	Irrigation canals	Sewage lagoons of alimentary factories	Rice fields	Hunting swamps
Argelato	Urban	×	×	×			
Baricella	Urban	×	×	×	×		×
Bentivoglio	Urban	×	×	×			
Altedo	Urban	×	×	×			
Malalbergo	Urban	×	×	×			×
Ponticelli	Rural			×	×	×	×
Minerbio	Urban	×	×	×			
S.Giorgio di P.	Urban	×	×	×			
Rubizzano	Rural		×	×	×		
Anzola dell'E.	Urban	×	×	×			
Calderara di R.	Urban	×	×	×			
Crevalcore	Rural		×	×	×		
Palata Pepoli	Rural		×	×			×
Amola	Rural		×	×	×		
Decima	Rural		×	×	×		×
Sala B.	Urban	×	×	×			

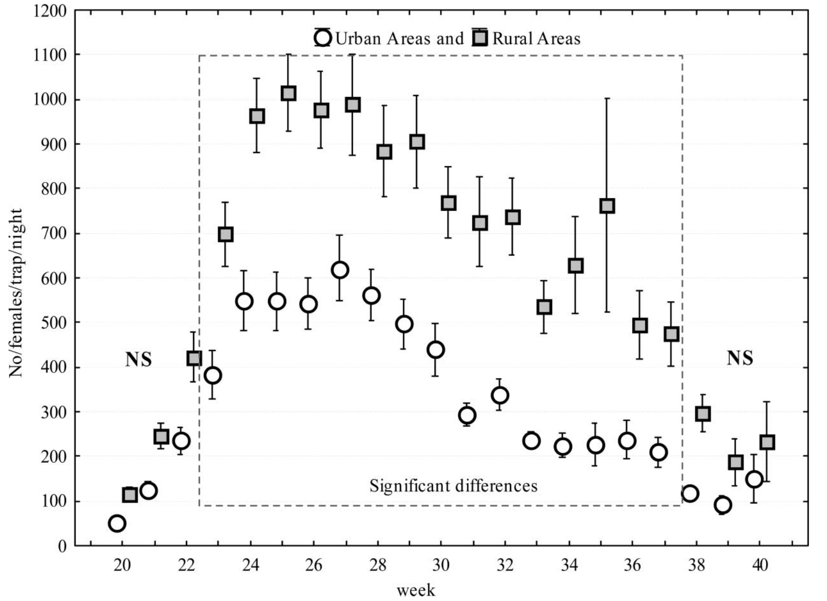

Fig. 2. Seasonal trend of *Cx. pipiens* female population density. The dotted line indicates the weeks during which statistically significant differences at the Duncan's test were found between the mean averages of the mosquito density population in urban and rural areas.

To evaluate the effectiveness of the correlations with the RH, given the small number (15 elements) we made a test based on Z-score using randomized assignments. For 1,000 times, we built random associations between *Cx. pipiens* density and mean and minimum RH by sampling data from the original data vector. Then we computed the Pearson correlation for all the 1,000 random vectors. Finally we computed the Z-score of the observed correlation with respect to the random distribution. We repeated this procedure 100 times (Spiegel et al. 2000).

Backward stepwise regression analysis was used to perform an explorative analysis of the influence of the weather parameters on mosquito catches in the whole area, in the period August–September. Furthermore, for this analysis, the Bonferroni correction was applied to counteract the problem of multiple comparisons and avoid errors of type I (Zar 1996).

If not differently indicated, mean data are followed by standard deviation.

Results

Mosquito Species. Ten mosquito species were collected, and *Cx. pipiens* accounted for the 97.07% of the total catches. Other species were *Aedes caspius* (Pallas) (2.65%), *Aedes albopictus* (Skuse) (0.22%), *Anopheles maculipennis* Meigen s.l. (0.04%), and *Aedes vexans* (Meigen) (0.01%). The 0.005% of the culicidic fauna was constituted by *Cx. modestus*, *Aedes geniculatus* (Olivier), *Culiseta annulata* Schrank, *Anopheles plumbeus* Stephens, and *Culex impudicus* Ficalbi.

The average number of *Cx. pipiens* females caught in the traps exhibited a bell-shaped curve that peaked in June (weeks 24–28), whereas a secondary peak occurred at the end of August. *Cx. pipiens* showed very similar trends in urban and rural areas for the first peak, whereas the secondary peak was more evident in rural areas (Fig. 2). Statistically significant differences ($F = 90.04$; df = 1,20; $P < 0.0001$) were observed in the population densities between the two different environments from week 23 to week 37, whereas no difference was found in the population densities of rural and urban areas in May and September.

Weather Parameters. *Rainfall.* In the study area, the annual rainfall ranged from 350.0 to 791.0 mm per year. During summer (from June to September), the rainfall ranged from 82.60 mm (in 2003, the driest year) to 342.20 mm (in 2002, the wettest year).

Relative Humidity. The minimum RH showed the largest seasonal variability among the measured weather parameters. The average minimum RH in the summer months was 35.97% (dry season) and increased to 63.40% in autumn (rainy season). During winter and spring, minimum RH showed intermediate values: 61.22 and 41.66%, respectively. The driest month was July, with an average value of minimum RH = 34.20%. The lowest values were registered in 2003 (24.34%) and 2007 (23.25%), whereas the highest one in 2002 (44.13%; Fig. 3). Mean and maximum RH reflected the trend of the minimum RH, but within smaller ranges of variability. A highly significant correlation was found between the mean daily RH and the RH measured during the nighttime ($R^2 = 0.91$; $F_{1,38} = 399.48$; $P < 0.0000$; SE = 1.35).

Hydroclimatic Balance. The highest hydroclimatic deficit occurred in July and the value registered in 2007 was the lowest ever registered in the 15-yr period of study.

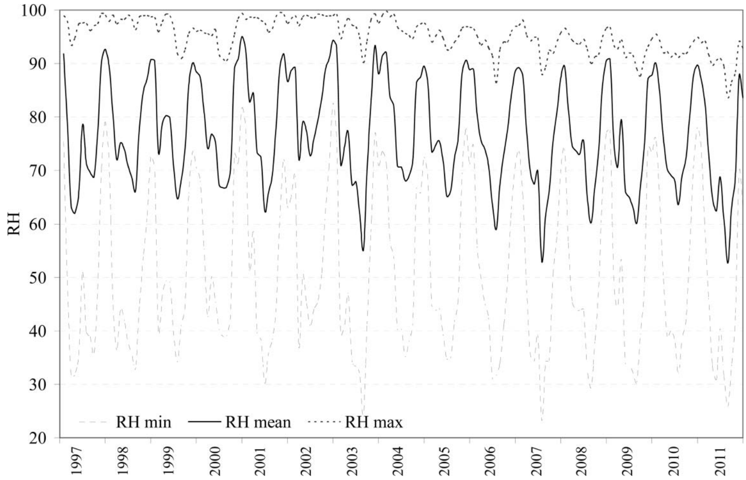

Fig. 3. Seasonal trends of minimum RH, mean RH, and maximum RH (averages of the years 1997–2011).

Temperature. The analysis of the seasonal trend of temperature data showed that December, January, and February were the coldest months, whereas the highest temperatures, above 35°C, occurred in July and August.

The coldest winters occurred in 2009 and 2010, with the minimum temperatures as low as −12.4°C and −11.8°C, whereas the mildest one occurred in 2007, when minimum temperatures never slowed down below −5°C. The highest summer (June–September) temperatures were registered in 2003, when the average was 25.87 ± 2.24°C; the coolest summer occurred in 1997 (average temperature, 21.94 ± 2.46°C).

Number of Days With Average Temperature Below 0°C. For any year of study, the months of November and December of the previous year were included in the calculation of the number of winter days with mean temperature below 0°C. The mildest winters (no >5 d with mean temperature below 0°C) were registered in 1998, 2001, 2007, and 2008. The coldest winters (>20 d with mean temperature below 0°C) were 2002, 2005, 2006, and 2010.

Wind. Average wind speed (measured at 10-m height) was 2.18 ± 0.72 m/s, with peaks of 6.26 m/s.

Factors Affecting the Seasonal Trend of the Mosquito Population Density. *Correlations Between* Cx. pipiens *Population Density and Weather Parameters.* Statistically significant correlations between the weather parameters and the seasonal and monthly average catches of *Cx. pipiens,* both in urban and rural monitoring stations, are reported in Table 3.

The analysis of the correlations between the seasonal abundance of *Cx. pipiens* and the weather parameters recorded in different preceding periods showed that the mean RH, the minimum RH, and the AAP RH averages registered in May were highly and positively correlated with the seasonal average of *Cx. pipiens* catches (respectively, $r_s = 0.89$, $r_s = 0.91$, and $r_s = 0.86$; all $P < 0.001$), and good positive correlations were also observed between the humidity level in May and the *Cx. pipiens* population density in June, July, and August. The minimum z-score obtained for minimum and mean RH were respectively 3.24 and 3.17, whereas the average z-score values were 3.46 ± 0.07 SD and 3.30 ± 0.06 SD. The observed z-score are in line with the *P* values and indicate that there is a very high probability that the correlations found are not random. Positive, but weak, correlations were also found between the seasonal *Cx. pipiens* population density and, respectively, the rainfall occurred in the period May–September ($r_s = 0.60$; $P = 0.018$), HCB registered during the period April–September ($r_s = 0.60$; $P = 0.017$), and potential evapotranspiration during the period April–May ($r_s = 0.77$; $P = 0.0008$). The minimum temperatures registered in May positively affected the population density, which was negatively correlated with the wind speed recorded in the same month (Fig. 4). No correlation was found between the *Cx. pipiens* average population density in May and the number of days with mean temperature below zero during the previous winter, whereas a positive—even if limited—influence of the mean seasonal population measured during the previous year on the population density in May emerged ($r_s = 0\ 0.71$; $P < 0.003$).

Correlations Among Monthly Population Density Data. The population density registered in May was weakly correlated with that of June, and no correlation was found with data registered in the following months (Fig. 5), but during the course of the season the influence of this parameter progressively increased. In fact the data collected in June, July, and August were correlated with those collected in the following month.

Factors Affecting *Cx. pipiens* Population Density in August–September. The mosquito population density in the period of maximum WN virus circulation within the mosquito population (August and September) seemed to be affected by RH, HCB, and temperature recorded in July. The stepwise regression analysis ($R^2 = 0.83$; df = 2, 12; $F = 29.63$; $P < 0.000023$) showed

Table 3. Statistically significant Spearman's correlations between weather parameters and seasonal and monthly average catches of *Cx. pipiens* in urban and rural monitoring stations

Weather parameters		No. of *Cx. pipiens* females captured							
		Seasonal avg			Monthly avg				
Month	Parameter	Total area	Urban area	Rural area	May	June	July	Aug.	Sept.
April	min RH					0.59*			
	mean RH			0.52*		0.64*			
	max RH			0.56*		0.58*			
	AAP_RH			0.54*		0.69**			
	ESC		−0.56*	−0.53*		−0.63*			
May	min RH	0.91***	0.85***	0.89***		0.80***	0.82***	0.70**	0.60*
	mean RH	0.89***	0.84***	0.84***		0.82***	0.76***	0.65**	0.59*
	max RH							0.53*	
	AAP_RH	0.86***	0.87***	0.78***		0.86***	0.70**	0.69**	0.64**
	min T	0.66**							
	mean T				0.66**				
	max T		−0.51*				−0.67**		
	Rain		0.60*				0.64**		
	Wind				−0.62*				
	EPT	−0.66**	−0.70**	−0.65**		−0.59*	−0.73**	−0.59*	
	HCB	0.54*	0.63*				0.72**		
	ESC	−0.78***	−0.74**	−0.77***		−0.74**	−0.71**	−0.58	
July	min RH	0.53*		0.60*				0.83***	0.72**
	mean RH	0.52*		0.56*				0.86***	0.78***
	max RH	0.59*							
	AAP_RH	0.55*	0.54*	0.60*			0.53*	0.89***	0.70**
	mean T							−0.54*	
	max T							−0.57*	
	Rain							0.66**	
	Wind	−0.53*	−0.70**				−0.73**	−0.53*	
	EPT			−0.52*					
	HCB			0.55*				0.71**	
Aug.	min RH								0.61*
	mean RH								0.56*
	max RH							0.64**	0.64*
	AAP_RH								0.55*
	Wind	−0.62*	−0.55*	−0.66*					−0.68*
Sept.	min RH	0.62*	0.59*	0.57*					0.64*
	mean RH	0.58*	0.59*	0.54*					0.64**
	max RH								0.56*
	AAP_RH	0.54*	0.54*	0.52*					0.64*
	Wind	−0.66*	−0.63*	−0.71**					−0.62*
	HCB		0.56*						

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

that the minimum RH in May (Beta = 0.41 ± 0.12 SD) and July (Beta = 0.65 ± 0.12 SD) were effective factors of variability. In addition, *Cx. pipiens* population density in the period of maximum viral circulation was slightly affected by the mosquito density itself, measured in July (Table 4).

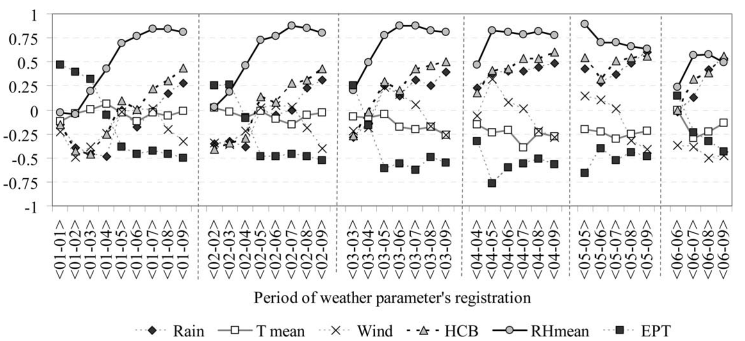

Fig. 4. Correlation between the average seasonal *Cx. pipiens* population density and the most relevant weather variables, measured during different periods. On the y-axis, the s values of the Spearman's correlation test are reported. On the x-axis, the period of registration of the weather variable; for example, <1–1> and <1–5> mean that the variables have been measured, respectively, during January and during the period January–May. The mean relative humidity registered during May gives the best positive correlation with the seasonal mosquito population density.

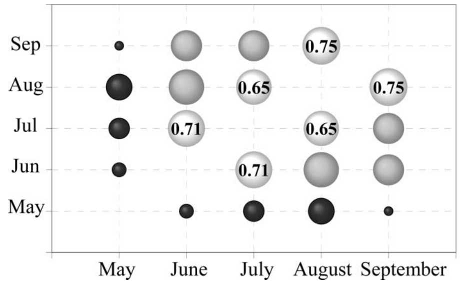

Fig. 5. Spearman's rank correlation between *Cx. pipiens* population density registered in any month and that one of each other month.

Discussion

In this study, we investigated the influence of weather factors and of the *Cx. pipiens* population density itself on the population density in the Bologna province (Po Valley, northern Italy) in the period 1997–2011. To this aim, we choose as end points the mean seasonal population density (from May to September) and the population density in the period of highest risk of human WN virus transmission (August–September).

It is known that, owing to the complexity of the interactions among biotic and abiotic factors, it is extremely difficult to recognize which is the key factor, if any exists, affecting the mosquito population growth in the field. For this reason, it may appear at first surprising the finding that the RH of May is the key factor affecting the mean population density both of the whole season, and of the period of maximum viral circulation; the latter is also influenced by the population density, the HCB, and the mean temperature registered in the previous—even though the amount of variability owing to these parameters is not very high.

However, why should the RH level in May be the key factor affecting the seasonal population density and the density registered in August–September? In May, environmental conditions become suitable for the development of mosquito population, as temperatures become favorable for mosquito reproductive behavior, egg laying, and larval development (mean temperature in May in the region of the study: 17.94 ± 2.73°C). The analysis of the *Cx. pipiens* population trend in the 15-yr period under investigation shows four phases (Fig. 6). In the first phase, at the end of the spring (from week 20 to week 24), an exponential growth rate occurs, and the rate of increase depends on the carrying capacity of the environment and on the weather conditions. The first phase strongly affects the following development of the population, as in the second phase, at the beginning of the summer (weeks 24–29), the growth curve stops to increase and becomes flat; in the third phase (weeks 29–34), a slight decrease of the population can be observed, whereas at the end of the summer (from week 35) a severe decrease occurs. So, the rate of increase of the first generations during Spring (May) seems to be of crucial importance for the development of the mosquito population in the following summer season. The minimum RH in May affects the population growth because it exerts a positive effect on female fertility and survival, whereas the host-seeking activity and the dispersal capacity are influenced by the RH during the night period, when females are actively looking for their bloodmeal (Kaul et al. 1984, Takken et al. 1997, Shaman et al. 2002, Chaves and Kitron 2011). In addition, the RH level affects the environment carrying capacity, as it influences the availability and extension of the breeding sites and the mosquito ability to colonize new habitats.

The population density in August–September is also influenced by the HCB measured in July, whereas the HCB measured in the period April–July affected the global seasonal trend. July was the driest month, showing the highest evapotranspiration, and this condition could have affected the extension of the water surface availability, the water quality of some larval breeding site typology, or both. For example, in the canals receiving sewage waters, evaporation could increase organic matter concentration to a level negatively affecting the larval development.

Rainfall did not affect the seasonal trend of mosquito populations. The main breeding sites in the Bologna plain are constituted by artificial environments, where the water presence is mostly connected with human activities. In addition, the rain can positively or negatively affect mosquito development according to precipitation intensity and to breeding site typology, and its effect, being potentially either negative or positive, is not easily identifiable.

To summarize, the air RH is a weather parameter strictly related to other weather parameters, such as temperature, rainfall, soil moisture, wind, and temperature excursion, and for this reason it can be considered a sort of synthesis of the combination of the effects owing to different environmental parameters, each one of which, in turn, affects different aspects of the reproductive cycle and the habitat characteristics (food quality and abundance, predator, parasite incidence, etc.).

The winter temperature variations seemed to have no influence on the mosquito population density reg-

Table 4. Statistically significant Spearman's correlations between the average *Cx. pipiens* density recorded in the period of highest WNV transmission risk (August–September) and, respectively, the mosquito population density of the previous months and the weather parameters

Factor of correlation with Aug.-Sept. population	Period of measurement	r
Monthly mosquito density	June	0.59*
	July	0.61*
	Aug.	0.96***
	Sept.	0.85***
Min. RH	May	0.66**
	July	0.85***
	Sept.	0.52*
Mean RH	May	0.62**
	July	0.90***
	Sept.	0.55*
HCB	July	0.69**
Mean temperature	July	−0.59*
Max temperature	July	−0.61*

* $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$.

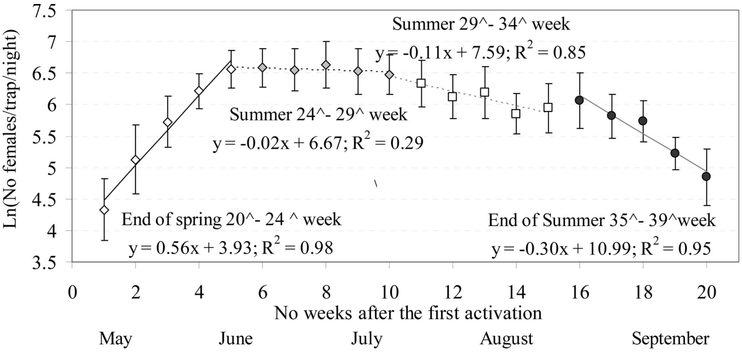

Fig. 6. Seasonal *Cx. pipiens* population trend. Data averaged over 15 yr (from 1997 to 2011).

istered in May and on the seasonal population density. This finding can be explained by the fact that *Cx. pipiens* overwinters in the adult stage, retained in protected sites (houses, stables, and cellars), at least partially protected from the effects caused by extremely low temperatures. The spring and summer temperatures did not directly affect the seasonal population density, and we could observe only a slight negative correlation between the temperature recorded in June and July with the number of mosquitoes caught in the following months. This finding is probably related to the multiple and contrasting effects of this weather factor on the mosquito population development: on one side, high temperatures enhance the mosquito population development by shortening the larval development time, but, on the other side, they can lead to a reduction of the extension of flooded areas (i.e., of the availability of larval habitats) by promoting water evaporation. As expected, the wind negatively influenced the number of mosquito captured, but it is unclear whether this parameter affected female flight behavior or the efficiency of CO_2 traps, or both.

The correlation analyses run on the data collected in rural and in urban areas showed very similar results, demonstrating that in the Bologna plain, a highly anthropized environment, the weather factors affected mosquito populations irrespectively of the level of urbanization.

To the best of our knowledge, this is the first time that a relevant correlation between RH of a precise period of the year (May) and the mosquito population density in the following months has been provided. We believe that the aggregation analysis of our data will be useful to develop a mathematical model able to predict the seasonal pattern of *Cx. pipiens* on the basis of the meteorological variables. Such a model would be a very precious tool for the further development of epidemiological models to forecast West Nile virus circulation and its intensity.

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