Sci Parasitol 21(3):86-93, June 2020
ISSN 1582-1366

Seasonal dynamics of mosquitoes in a large urban area of Transylvania, Romania

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Abstract. In the context of urbanization and adaptability of mosquito to the human environment and resistance to pesticides, the aim of the present study was to bring new data on the mosquito population dynamics in the urban area of Cluj-Napoca. From April to October 2018, five trapping sites were sampled for 26 days using CDC light traps. Two trapping sites were located inside the campus of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (the Anatomy building trapping site and the Biodiversity building trapping site), one at the Ethnographic Museum and other two locations in two neighborhoods that corresponded to human households. The presence of mosquitoes was confirmed in 12 of the sampling days, with the peak in August. The variations of the number of mosquitoes, average temperatures and relative humidity throughout the study showed a very strong correlation between the minimum temperatures and number of specimens of collected mosquitoes ($r_s = 0.81591$, $p = 0$). A total number of 90 mosquitoes, 52 females and 21 males, belonging to three genera, were collected. The most common genus found in Cluj-Napoca was *Culex* (n=66), with *Cx. p. pipiens* as the only species collected, followed by *Aedes* (n=5), with *Ae. vexans* prevailing in this genus, and *Anopheles* (n=2), with one specimen of *An. maculipennis* complex. Most mosquitoes were collected from the Anatomy building trapping site followed by the Biodiversity building trapping site, both located in the University's campus, while the location at the Ethnographic Museum had the lowest capture of mosquitoes in the present survey. All of the species identified in the present study are known vectors of a variety of zoonotic pathogens. Therefore, regular surveillance studies, such as this one, should be accomplished to be able to predict trends of mosquito dynamics and to successfully act accordingly.

Keywords: Romania; Cluj-Napoca; Mosquito dynamics; Distribution; Vectors; *Culex pipiens*.

Dinamica populaţiei de țânțari dintr-o zonă urbană din Transilvania, România

Rezumat. În contextul urbanizării și adaptabilității țânțarilor la mediul uman, dar și al rezistenței la pesticide, obiectivul studiului prezent a fost de a aduce noi date despre dinamica populației de țânțari din zona urbană a orașului Cluj-Napoca. Capcane de capturare a insectelor de tip CDC au fost amplasate din aprilie până în octombrie 2018, în cinci zone urbane, timp de 26 de zile. Două locuri de capturare au fost amplasate în campusul Universității de Științe Agricole și Medicină Veterinară din Cluj-Napoca (un loc de capturare lângă clădirea Disciplinei de Anatomie Comparată și unul lângă clădirea Institutului de Biodiversitate), unul la Muzeul Etnografic și alte două în două cartiere care au corespuns gospodăriilor umane. Prezența țânțarilor a fost confirmată în 12 zile de capturare, cu cel mai mare număr de țânțari capturați în luna august. Variațiile numărului de țânțari, a temperaturilor medii și a umidității relative înregistrate pe parcursul studiului au arătat o corelație foarte puternică între temperaturile minime și numărul țânțarilor colectați (rs = 0,81591, p = 0). În total au fost capturați 90 de țânțari, 52 de femele și 21 de masculi, aparținând a trei genuri. Cel mai frecvent gen prezent în Cluj-Napoca a fost genul *Culex* (n = 66), cu *Cx. p. pipiens* ca singură specie colectată, urmată de genul *Aedes* (n = 5), cu *Ae. vexani* predominând în acest gen, și genul *Anopheles* (n = 2), cu un exemplar din complexul *An. maculipennis*. Majoritatea țânțarilor au fost colectați din cele două zone situate în campusul universității, în timp ce locația de la Muzeul Etnografic a avut cea mai mică captură de țânțari. Toate speciile identificate în studiul de față sunt vectori cunoscuți ai unei varietăți de agenți patogeni zoonotici. Prin urmare, ar trebui realizate studii regulate de supraveghere, ca și cel de față, pentru a putea prezice tendințele dinamicii țânțarilor și pentru a acționa cu succes în consecință.

Cuvinte cheie: România; Cluj-Napoca; Țânțari; Distribuție; Vectori; *Culex pipiens.*

Received 22.07.2020. Accepted 29.07.2020.

Introduction

In the context of climate change, free trade and traveling, an increasing number of invasive mosquitoes have become established in various European countries such as Italy, France, Belgium, Netherlands or Germany (Medlock et al., 2012; Medlock et al., 2015). These mosquitoes together with the local species can spread a variety of diseases to animals and humans.

In Romania, there are over 50 species of mosquitoes, belonging to 7 genera (Păstrav et al., 2020). Some of these species are capable of transmitting tropical viruses such as Chikungunya, Dengue or West Nile virus and also filarioids, including *D. immitis* and *D. repens* (Calzolari, 2016).

There have been several of mosquito-borne diseases cases reported in humans in Romania in the past decade, including West Nile outbreaks (Lupșe et al., 2015).

The most recent study on the population of mosquitoes in Cluj-Napoca has taken place in 2015, in which 14 species of mosquitoes have been identified in 7 locations around the metropolitan area of the city (Török, 2017). Even though, the mosquitoes collected are known vectors for various diseases, no infections have been detected in the study. However, since then there have been no other studies regarding the mosquito population in Cluj-Napoca.

In this context of climate change, emerging vector-borne diseases, and lack of surveillance programs regarding the local population of mosquitoes, the present study aimed to bring new data on the mosquito population dynamics.

Materials and methods

Between April and October 2018, CDC light traps (Trappola per Monittoraggio Zanzare, IMT Original 2002, Italy and John W. Hock, model 512, Gainesville, FL, USA, USA) were placed in five sites in Cluj-Napoca city (figure 1).

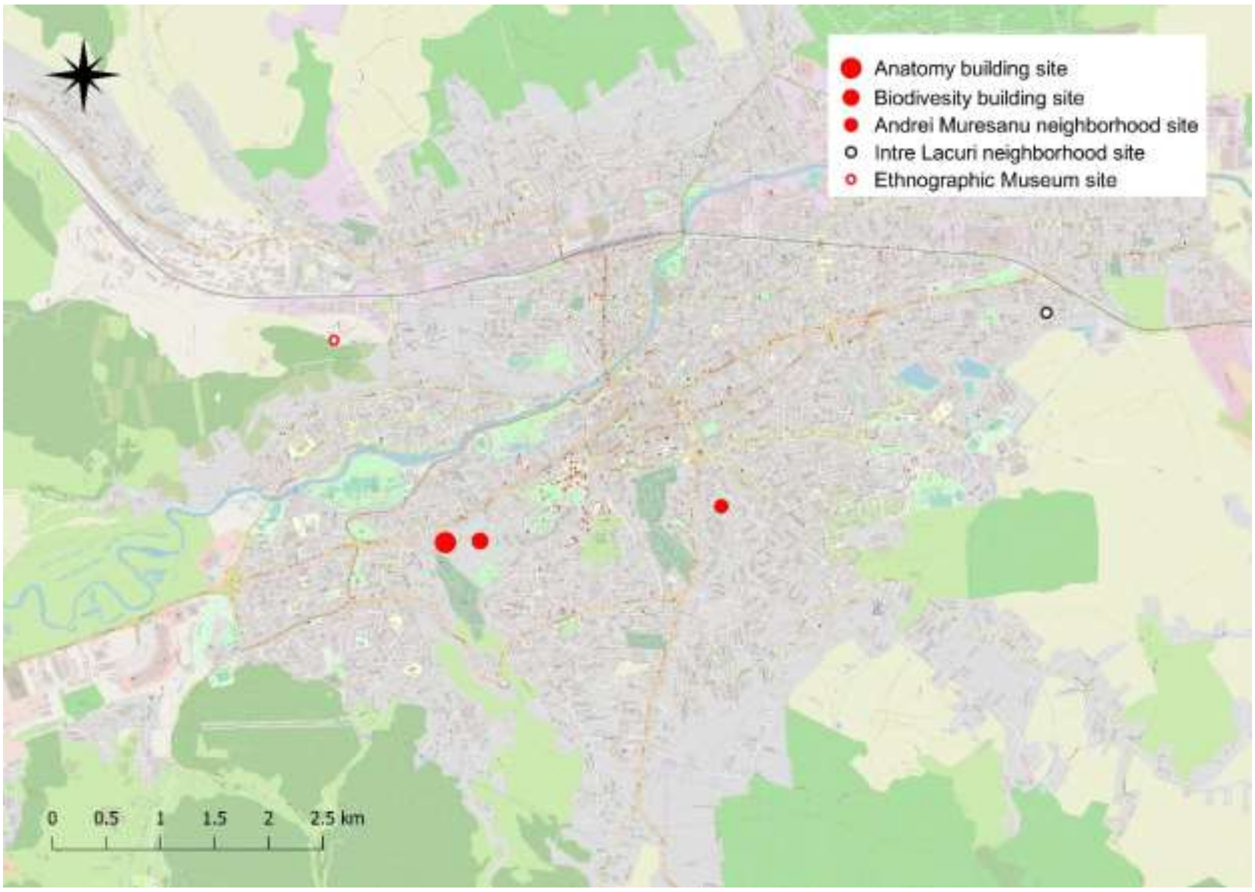

Figure 1. The sampled locations in the city of Cluj-Napoca, Romania in correlation with the total number of trapped mosquitoes

The traps were placed on one selected day of the week, each week for the length of the study. They were left overnight (19:00h-08:00h) and were collected the subsequent morning. Each trap was placed at approximately 1.5 m height from the ground. The mosquitoes were separated from other insects and placed in tubes with 70% ethanol. They were identified at the Department of Parasitology and Parasitic Diseases by morphological keys developed by Becker et al. (2010). Male mosquitoes were previously prepared for identification, by placing them in a 10% potassium hydroxide solution and bringing it to boil for 10 to 30 seconds until the exoskeleton had become very visible and all other tissues had melted. The last abdominal segments were separated from the body and placed on a slide in two drops of glycerin for examination under the stereomicroscope (Siverly and Shroyer, 1974; Rattanarithkul, 1982). Females required no additional preparation prior to the identification.

Trapping sites

In each trapping site, one CDC light trap was set overnight for the entire sampling period. The trapping sites aimed to cover a large area of the city's perimeter as well as to include different microhabitats suitable for mosquitoes. The trapping sites were: two in the campus of the University of Agricultural Studies and Veterinary Medicine of Cluj-Napoca: Anatomy building site (46.760280 N; 23.568199 E), Biodiversity building site (46.760193 N; 23.573477 E), one at the Ethnographic Museum (46.777537 N; 23.555137 E), one in Între Lacuri neighborhood (46.779648 N; 23.641241 E), and one in Andrei Mureșanu neighborhood (46.763281 N; 23.601473 E). The first trapping site was located in the University's campus, near a stream deviated from Someș River. The second trapping site was located as well in the campus, close to one small artificial pond, and under pine trees. The location at Ethnographic

Museum is inside the ethnographic museum's park and has the Hoia-Baciu Forest in the vicinity. The other two locations, Între lacuri and Andrei Mureșanu, correspond to human households. The Între Lacuri neighborhood is relatively close to the lakes in the neighborhood, but the trap location consisted of one house's backyard terrace, where the owner was raising peacocks. The Andrei Mureșanu household had an extensive garden, composed of various fruit trees such as walnut tree, cherry tree and apple trees.

Statistical analysis

The statistical analysis was performed using Social Statistics website (https://www.socscistatistics.com). The correlations between the number of specimens collected and temperature (min, max and average), relative humidity and household/environment was calculated using Spearman's Rho correlation test. The strength of correlation was established based on absolute value of r(s) coefficient, as follows: 0.00–0.19 "very weak" correlation; 0.20–0.39 "weak" correlation; 0.40–0.59 "moderate" correlation; 0.60–0.79 "strong" correlation; 0.80–1.0 "very strong" correlation.

Mapping

The map of the evaluated trapping sites was generated using QGis 3.6 software (www.qgis.org).

Results

Throughout this survey, from a total number of 26 days of sampling, the presence of mosquitoes was confirmed in 12 of them, with peak in August. A total number of 90 mosquitoes, 52 females and 21 males, belonging to three genera, were collected (table 1). The most common genus found in Cluj-Napoca was *Culex* (n=66), with *Cx. p. pipiens* as the only species collected, followed by *Aedes* (n=5), with *Ae. vexans* prevailing in this genus, and *Anopheles* (n=2), with one specimen of *An. maculipennis* complex. Unfortunately, a total number of 25 mosquitoes could not be distinguished beyond their genus and 17 individuals remained completely unidentified due to extensive body damage.

Table 1. Mosquito species identified in the present study

Month	Number of mosquitoes	Genus	Species	Collection point
April	0			
May	0			
June	11	*Culex*	*Culex* spp. (2)	2; 3; 4; 5
			Cx. pipiens s.l. (7)	
		Anopheles	*An. maculipennis* s.l. (1)	4
		Unknown	Unidentified (1)	1
July	19	*Culex*	*Culex* spp. (5)	1; 3; 5
			Cx. pipiens s.l. (9)	
		Aedes	*Ae. vexans* (3)	2; 5
		Unknown	Unidentified (2)	2; 5
August	45	*Culex*	*Culex* spp. (15)	1; 2; 3; 4; 5
			Cx. pipiens s.l. (17)	
		Aedes	*Aedes* spp. (1)	2
			Ae. vexans (1)	
		Anopheles	*Anopheles* spp. (1)	3
		Unknown	Unidentified (10)	1; 2; 4; 5
September	15	*Culex*	*Culex* spp. (1)	1; 2
			Cx. pipiens s.l. (10)	
		Unknown	Unidentified (4)	1; 2
October	0			

1 – Anatomy building site; 2 – Biodiversity building site; 3 – Ethnographic Museum site; 4 – Între Lacuri neighborhood site; 5 – Andrei Mureșanu neighborhood site.

Most mosquitoes were collected from the Anatomy building trapping site (figure 2), followed by the Biodiversity building trapping site, both located in the University's campus, while the location at the Ethnographic Museum had the lowest capture of mosquitoes in the present survey.

The variations of the number of mosquitoes, average temperatures and relative humidity throughout the study are shown in figure 3.

The statistical results show a very strong correlation between the minimum temperatures and number of specimens of mosquitoes collected (r = 0.81591, p = 0), a strong correlation between the average temperatures and number of mosquitoes (r = 0.70006, p = 7E-05) and a moderate correlation between the maximum temperatures and number of mosquitoes (r = 0.52281, p = 0.00614). The association between the two variables is therefore proven statistically significant.

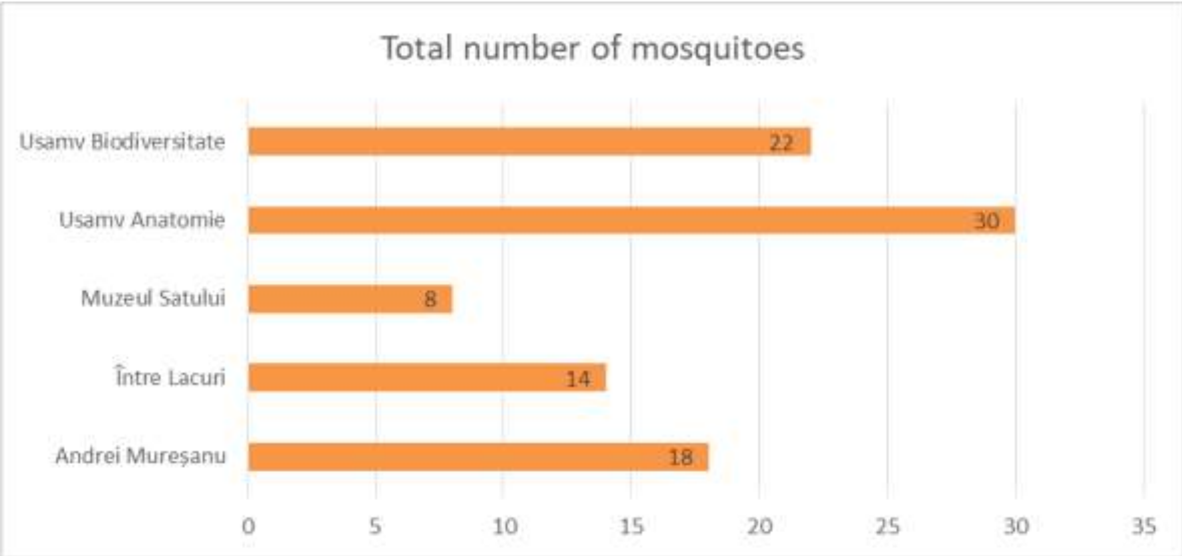

Figure 2 The total number of mosquitoes collected at each trapping site

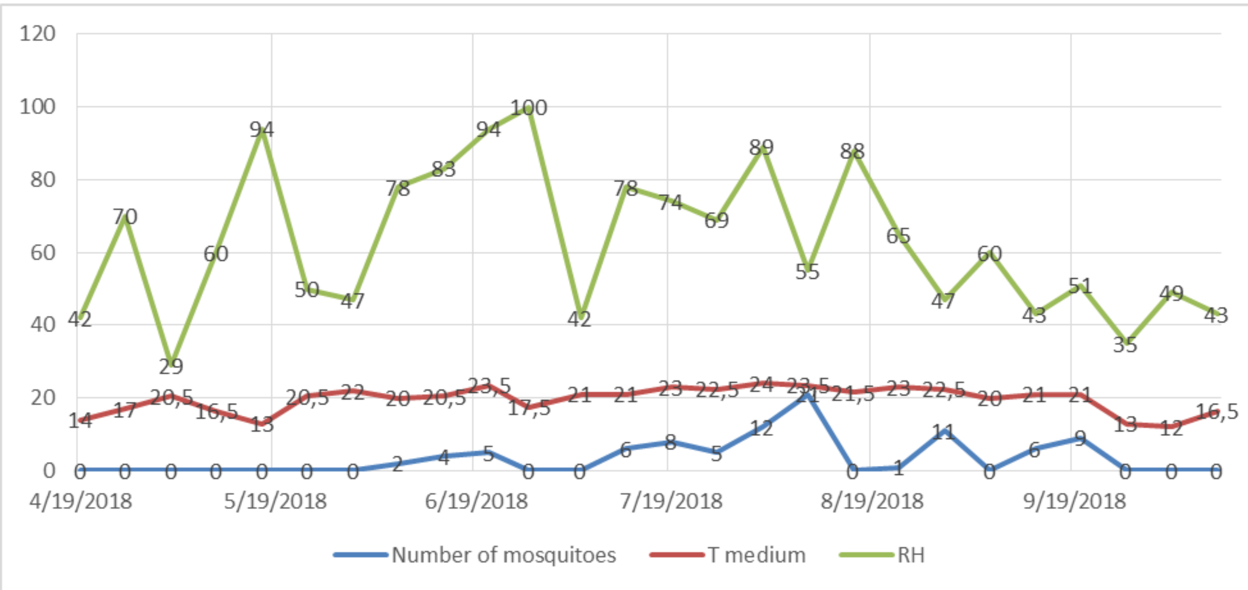

Figure 3. The number of mosquitoes, medium temperatures and relative humidity variations throughout the survey

Discussions

During the 7 months of our study, a low number of mosquitoes were caught and they seemed to only be present from June to September. One possible explanation could be the periodical mosquitoes control management that is performed in the city. Insecticide sprays are spread throughout Cluj-Napoca on a regular basis executed by Coral Impex SRL based on the contract signed with the City Hall of Cluj-Napoca (Proiect Hotărâre, 2018). The sites chosen in the present study were located in very urban areas meaning that any management program used by the city would have destroyed most mosquito populations in those areas. It would have been preferable to increase the number of sites and to have chosen a few remote areas as well. Secondly, the traps available for the present study were CDC light traps, usually used for catching mosquitoes during malaria surveillance and also arboviral surveillance (Medlock et al., 2018). The disadvantages of these traps include catching other insects and, more importantly, their low efficacy in the presence of another source of light, thus functioning at their best in completely dark environments while attracting mostly nocturnal mosquitoes (Medlock et al., 2018). Adding a source of CO2 or putting a UV light instead of a regular diode would have increased the efficacy of the contraptions and maybe led to a higher quota. Moreover, including a different type of trap, such as gravid traps designed to catch female mosquitoes in search of oviposition sites, might have improved the numbers as well.

Regarding the mosquito identification, the morphological aspects are not sufficient for distinguishing between *Cx. pipiens, Cx. quinquefasciatus* and *Cx. torrentium.* The same reasoning can be applied in the case of *An. maculipennis* complex's and *Cx. pipiens* complex's individuals, as the only way of differentiation is thorough molecular methods. Furthermore, due to the methods chosen for preservation (70% ethanol), the color and other morphological characteristics have faded, and therefore, in case of many female specimens the identification has stopped at the genus level.

The diversity of collected species during this survey is rather low, as compared to another study in Cluj-Napoca (Török, 2017) in which the number of species collected raised to 14. The difference resides in the trapping protocols applied, as they used gravid traps instead of CDC light traps, which appear to have been more efficient. Nevertheless, the most abundant species found was still *Cx. pipiens*, followed by *An. maculipennis* complex, both widely distributed throughout Europe (Becker et al., 2010).

All of the species identified in the present study are known vectors of a variety of pathogens. *Cx. pipiens*, the most prevalent, is a known vector for West Nile virus, which has both animal and human health importance (Calzolari, 2016). In humans, West Nile virus causes severe encephalitis, and has been reported in the South, and Southeast of the country since 1966 (Tsai et al., 1998; Hubálek, 2000). In 2016 the report from the National Institute of Public Health stated there have been 25 cases reported in humans from the South, South-East and East of the country, and one case from the Intra-Carpathian region of Transylvania (Mureș county) (Institute of Public Health, Romania, 2016). Furthermore, during a seroprevalence study on horses, sheep and poultry, conducted by the Romanian National Institute of Public Health, there has been a 33.4% seropositivity in horses, 8.6% in sheep and 13.5% in poultry in 2009 (ECDC/WHO, 2010). Nevertheless, *Cx. pipiens* is not the only species incriminated for transmitting West Nile virus, *An. maculipenis* complex and *Ae. vexans* are also known vectors (Wilkerson et al., 2015; Kampen and Walter, 2018). *Cx. pipiens* is also capable of transmitting various viral agents such as: Sindbis virus, Tahyna, Usutu, Rift Valley Fever, West Equine Encephalitis, St. Louis Encephalitis virus, Japanese Encephalitis virus and Batai; bacteria (*Francisella tularensis*) and even parasites (*Dirofilaria immitis*, *D. repens*) (Kampen et al., 2012; Zittra, 2015; Calzolari, 2016; Kampen and Walter, 2018).

Members of the *An. maculipennis* complex are known malaria vectors, and are namely represented in our country by *An. atroparvus*, *An. messeae, An. maculipennis* s.s. and *An.*

sacharovi (Nicolescu et al., 2004). However, in Romania malaria was eradicated in 1963 and it is unlikely to re-emerge in the present (Fălcuţă et al., 2011; Neghina et al., 2011). Other than *Plasmodium* spp, *An. maculipennis* complex is also capable of transmitting West Nile virus, Sindbis virus, Tahyna and *Dirofilaria* spp. (Kampen et al., 2012; Kampen and Walter, 2018).

Lastly, *Ae. vexans* is a known vector for various viral agents, out of which the most common are: Zika, Chikungunya, West Nile virus, Tahyna and East Equine Encephalitis (Wilkerson et al., 2015; Calzolari, 2016; Kampen and Walter, 2018). Furthermore, this species in capable of transmitting parasites, namely, dirofilariasis (*Dirofilaria immitis*), which cause severe cardiac diseases in carnivores and can also affect humans (Orihel and Eberhard, 1998; Kampen and Walter, 2018).

In the present study, the mosquito control protocols performed by the city resulted in the decrease of mosquito population in a part of the evaluated sites. The results of the present study suggest that the control program does not completely cover certain environments, like the University campus or private households, which would account for the greater proportion of specimens trapped there. Regular surveillance studies, such as this one, should be accomplished to be able to predict trends of mosquito dynamics in a certain area and act accordingly.

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