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Asian Tiger Mosquito *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) Overwintering in Montenegro, North Macedonia and Serbia

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Abstract: We confirmed occasions of outdoor reproductive activity of *Aedes albopictus* during the winter period in three Balkan countries. The main question was how long females of *Ae. albopictus* can stay active deposing overwintering eggs? In Podgorica (Montenegro), eggs were collected until 20th December; afterwards, no eggs were found until 28th March when the new season started. In Skopje (North Macedonia), eggs were observed until 22nd November; the activity in the new season started on 26th April. In Novi Sad (Serbia), eggs were found until 6th December; surprisingly, during wintertime, oviposition activity was observed at the end of December and during February. In the new season, the activity started on the 7th of June. During the winter period, we did not find any larvae in the ovitraps, and we were not able to hatch them in the laboratory from collected eggs. We conclude that long-lived females (belonging to the last seasonal generation) continue their activity during wintertime when favourable microclimate is available. Considering results and climate change predictions, surveillance should be planned during the whole year, at least in South European countries.

Key words: Asian tiger mosquito, surveillance, winter period, Balkan

Introduction

The Asian tiger mosquito *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) is a thermophilic invasive mosquito species originating from tropical regions of South-eastern Asia (Hawley 1988). It is a competitive vector able to transmit more than 20 viruses and filarial worms, which are pathogenic to animals and humans. The spread of these disease agents, besides SARS-CoV-2 and malaria, has become one of the major global health problems. It is predicted that climate change will affect the mosquitoes' distribution, which will allow them to bring pathogens to naive populations (Reinhold et al. 2018). In the last 20 years, the incidence of autochthonous mosquito-borne diseases like Chikungunya and Dengue have increased in Europe (Italy, France, Croatia); they are most likely mediated by well-adapted and established populations of *Ae. albopictus* (Rezza et al. 2007, Gjenero-Margan et al. 2011, Delisle et al. 2015, Succo et al. 2016, Calba et al. 2017). Populations of *Ae. albopictus* from subtropical and temperate regions overwinter by producing diapausing eggs, while tropical strains reproduce continuously during all seasons (Hawley et al. 1987). The ability of the species to survive winter temperatures is crucial for its establishment in temperate regions (Denlinger & Armbruster 2014). Photoperiodically-induced egg diapause is a key trait contributing to establishment and spread in temperate latitudes (Medley et al. 2019). Three are the critical climate parameters for the successful establishment and development of the species in Europe: January temperature above 0°C, annual mean temperature above 11°C and annual precipitation >500 mm (Cunze et al. 2016).

Aedes albopictus was firstly reported in Montenegro in 2001 (Petrić et al. 2001), then in Serbia in 2009 (Petrić 2009) and in North Macedonia in 2016 (Sokolovska et al. 2017). The species can overwinter in local environments and climates; further surveillance showed that it has become part of the entomofauna in these three Balkan countries (Petric et al. 2009, Pajovic et al. 2013, Kavran et al. 2019, Cvetkovikj et al. 2020). Having that in mind, our study was focused on the questions: (i) How long can females of *Ae. albopictus* stay active deposing overwintering eggs? (ii) Is the climate of the three Balkan countries suitable enough to provide species activity during the winter? Considering the previously published results from Southern Europe (Romi et al. 2006, Collantes et al. 2014), we need to answer these questions in line with the need for continuous surveillance of the species over the whole year.

Materials and Methods

For surveillance of *Ae. albopictus*, we used ovitraps described by the European Centre for Disease Prevention and Control (ECDC 2012) and Bellini et al. (2020), incorporating a new approach launched by COST Action CA17108 – *Aedes* Invasive Mosquitoes (AIM). One of the AIM objectives is the promotion of harmonisation (in terms of equipment, time and frequency of mosquito sampling) to ensure comparable outputs in terms of surveillance. AIM-COST protocol (AIM-COST 2020) was slightly modified to achieve comparability between the results obtained from different geographical regions of Montenegro, North Macedonia and Serbia. The first modification was monitoring extension towards the wintertime, until the beginning of mosquito activity next year. Black plastic containers filled with tap water and a standard tongue depressor as an oviposition substrate were used to collect the eggs. Tongue depressors were scratched with a sharp object on the exposed side, positioned and fixed on the inner wall of the ovitrap with paperclips. Traps were placed in shady and safe positions on the ground and kept at the same place during the surveillance period (26th October 2020 – 20th June 2021; 44th-24th week). Where two ovitraps were positioned at the same locality, distances between them were 15 to 100 m. The second modification was a reduction of ovitrap positions: instead of five ovitraps in three locations, we used two ovitraps in three locations and five ovitraps in five locations in North Macedonia. Ovitraps were checked biweekly for the presence of eggs and larvae. After each inspection, the traps were emptied, cleaned inside, the oviposition substrates replaced and the trap was refilled with water. Collected tongue depressors were marked with a unique code and collection date, wrapped with a slightly wet kitchen paper towel and stored in a plastic "zip-lock" bag. The bags were transported to the laboratory. The tongue depressors were checked for the presence of eggs under a stereomicroscope and proceeded to larval hatching. Importantly, besides latitude and longitude, altitude of traps positions was different too: 15 (42.321805; 19.282739) to 33 m a.s.l. (42.447923; 19.211396) in Podgorica; 249 (42.002903; 21.459275) to 252 m a.s.l. (42.003592; 21.45865) in Skopje and 76 (45.24044; 19.8046) to 78 m a.s.l. (45.2586; 19.81895) in Novi Sad (Table 1). We analysed the data in R (R Core Team 2020).

Table 1. Localities with latitude, longitude, altitude and abbreviations.

Code	Latitude	Longitude	Altitude
MNE-T1-2	42.447900	19.211846	33
MNE-T3-5	42.447923	19.211396	33
MNE-M1-2	42.321805	19.282739	15
MNE-M3-5	42.322158	19.282916	16
MNE-P1-2	42.425145	19.209422	33
MNE-P-3-5	42.425093	19.209227	33
NMKD-1	42.003592	21.45865	252
NMKD-2	42.003785	21.45898	252
NMKD-3	42.003792	21.460202	251
NMKD-4	42.003917	21.459897	250
NMKD-5	42.002903	21.459275	249
E-SRB-NS-1.2.	45.2586	19.81895	78
E-SRB-NS-3.7.9.	45.25866	19.81826	77
E-SRB-NS-11.12.	45.24002	19.80403	77
E-SRB-NS-13.14.15.	45.24044	19.8046	76
E-SRB-NS-21.22.	45.25128	19.84605	78
E-SRB-NS-25.28.29.	45.25032	19.84518	78

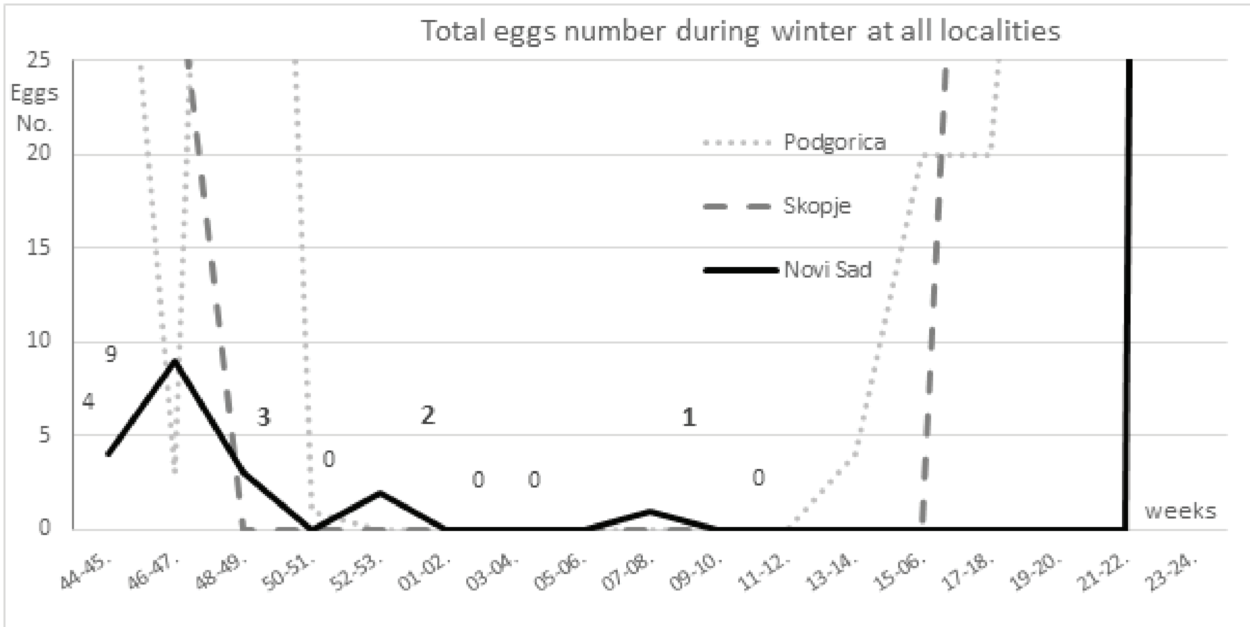

Fig. 1. Total number of eggs at all localities/countries (number of eggs at y-axis shown at 25 as maximum to highlight overwintering activities; data labels added only for the line representing Novi Sad data set; highlighted values for Novi Sad shows weeks when eggs were observed during the winter).

Results

In Podgorica (Montenegro), the activity of *Ae. albopictus* started to decrease from 26th October till 6th December. Between 7th and 20th December, only one egg was found in one locality. Afterwards, no eggs were found until 28th March at any of the localities. The new season started between 29th March and 11th April (13-14th week) (Fig. 1). We recorded the first larva during the period 9-12th week but in used tires in localities MNE-M1-2 and MNE-M3-5 and not in the ovitraps.

Under the continental climate and at the highest altitude, in Skopje (North Macedonia), the period with decreasing number of eggs ends 26th October – 8th November. During the period 9-22nd November, 31 eggs were recorded at one locality. Starting from 23rd November, no eggs were collected in any of the localities until 25th April. The activity starts in the period 26th April – 9th of May (17-18th week).

In the same type of climate but at significantly lower altitude, in Novi Sad (Serbia), stable and decreasing number of eggs in ovitraps were recorded from 26th October until 22nd November. During the period 23rd November – 6th December, activity was observed in a couple of localities with three eggs in total. After that, during wintertime, surprisingly, activities were observed at the end of December and during February. Actually, eggs were collected during the period 21st December – 3rd January (52-53rd week) at locality SRB13.14.15. and during the period 15-28th February (7-8th week) at locality SRB3.7.9. (Fig. 1). No activity was recorded for the rest of the period 7th December - 6th June. Unexpectedly, activity of *Ae. albopictus* started in Novi Sad three weeks after Skopje and five weeks after Podgorica; first eggs were collected during period 7-20th June (23-24th week).

Discussion

The three countries/areas of interest are considered as uniform by the Köppen-Geiger climate classification – Cfa (temperate climate, no dry season and hot summer) (Climate-Data.Org), but with some differences in terms of climate characteristics. Podgorica has an average January temperature of 4.2°C, the average annual temperature is 15.1°C and the rainfall average is 1956 mm per year. In Skopje, the average January temperature is 1.1°C, the average annual temperature is 13°C and the rainfall is 568 mm per year. In Novi Sad, average January temperature is 0.8°C, the temperature average is 12.7°C and the rainfall is 695 mm. Some values are almost at the edge of the limit that Cunze et al. (2016) suggested for the successful establishment of *Ae. albopictus* in Europe. Our surveillance supports their statements: in the three countries studied, *Ae. albopictus* has a well-established population with a capability for overwintering. Even being uniform (Cfa), the dif-

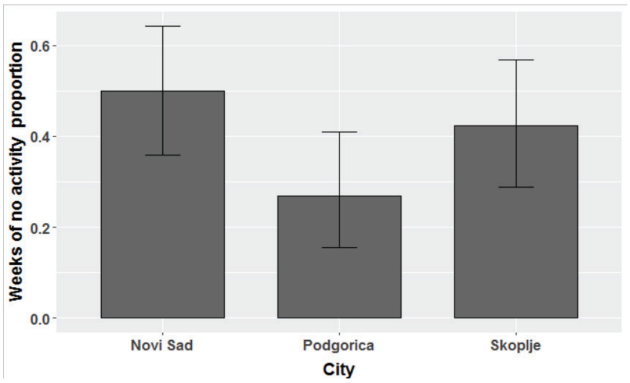

Fig. 2. Proportion considering weeks of diapausing between the three sampling sites in response to climate.

ferences in climate characteristics between countries appear statistically important for the analysis, considering the duration of diapausing at three sampling sites in response to climate: 14 weeks in Podgorica, 22 in Skopje and 26 in Novi Sad, respectively. Chi-squared analysis showed a significant difference between cities and the weekly activity during the year-long observed period ($\chi 2 = 5.9$, df = 2, P = 0.049), where the test for independence of all factors was $\chi 2 = 5.996$, df = 2, p-value = 0.04989 (Fig. 2).

The most interesting data from the surveillance appear in Novi Sad: a first record of outdoor reproductive activity of the Asian tiger mosquito during the winter period in December and February on the Balkans (Fig. 1). The record surprisingly appears farther north than expected, since we predicted that eggs should be found in a more favourable climate, i.e. in Podgorica or in Skopje, even with the influence of higher altitude. The answer can be a low number of ovitraps positioned in less favourable conditions. Nevertheless, this record corresponds with data of Romi et al. (2006) in the city of Rome and Collantes et al. (2014) in the region of Murcia, demonstrating a constant oviposition activity of females of *Ae. albopictus* during the wintertime. We checked for larvae in ovitraps during the monitored season and we stimulated hatching in the lab but, in all cases, including Novi Sad, we did not succeed to hatch eggs or develop larvae before the beginning of the new season. The phenomenon of diapause is induced mainly by photoperiod and temperature (Hawley 1988); on this basis, Romi et al. (2006) made two hypotheses: (1) long-lived female *Ae. albopictus* belonging to the last seasonal generation could have continued their activity for three months and (2) one or more cycles of reproduction have occurred at breeding sites with a favourable microclimate. Collantes et al. (2014) suggests that under the favourable winter conditions the first hypothesis is a fact. As we finding no larvae in nature/traps and were not able to hatch eggs in the laboratory, our results confirm the first hypothesis of Romi et al. (2006) and the conclusions of Collantes et al. (2014) that some females from the last generation in a season can extend overwintering eggs deposition during wintertime.

Conclusions

We confirmed rare occasions of outdoor reproductive activity of *Ae. albopictus* during the winter period (at end of December and in mid-February) in three countries with a similar Balkan climate within the limit values for its successful establishment in Europe. As we did not find any larvae in ovitraps and we were not able to hatch them from the collected eggs in the laboratory, we conclude that long-lived females, belonging to the last seasonal generation continuing their activity during wintertime in a favourable microclimate. Considering climate change predictions, it will be necessary to pay attention to invasive tropical mosquito species and their ability to continue development during less favourable winter conditions in Europe. The suggestion is to plan further surveillance, at least in South European countries, during the whole year. The surveillance during winter can be done with fewer traps per localities but will need to include, besides eggs presence, larval presence in the ovitraps and egg hatching stimulation in the laboratories.

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References

Bellini R., Michaelakis A., Petric D., Schaffner F., Alten B., Angelini P., Aranda C., Becker N., Carrieri M., Di Luca M., Falcuta E., Flacio E., Klobucar A., Lagneau C., Merdic E., Mikov O., Pajovic I., Papachristos D., Sousa C. A., Stroo A., Toma L., Vasquez M. I., Velo E., Venturelli C. & Zgomba M. 2020. Practical management plan for invasive mosquito species in Europe: I. Asian tiger mosquito (*Aedes albopictus*). Travel Medicine and Infectious Disease 35: 101691–101697. https://doi.org/10.1016/j.tmaid.2020.101691

Calba C., Guerbois-Galla M., Franke F., Jeannin C., Auzet-Caillaud M., Grard G., Pigaglio L., Decoppet A., Weicherding J., Savaill M. C., Munoz-Riviero M., Chaud P., Cadiou B., Ramalli L., Fournier P., Noel H., De Lamballerie X., Paty M. C. & Leparc-Goffart I. 2017. Preliminary report of an autochthonous chikungunya outbreak in France, July to September 2017. Eurosurveillance 22 (39):17–00647. doi: 10.2807/1560-7917. ES.2017.22.39.17-00647. PMID: 29019313; PMCID: PMC5709952.

Climate-Data.Org https://en.climate-data.org/europe/macedonia/skopje/skopje-1768/; HTTPS://EN.CLIMATE-DATA.ORG/EUROPE/MONTENEGRO/PODGORICA/PODGORICA-1324/; https://en.climate-data.org/europe/serbia/vojvodina/novi-sad-1020/; (accessed 6th October 2021).

Collantes F., Delgado J. A., Alarcón-Elbal P. M., Delacour S. & Lucientes J. 2014. First confirmed outdoor winter reproductive activity of Asian tiger mosquito (*Aedes albopictus*) in Europe. Anales de Biologia 36:71-6. doi: http://dx.doi.org/10.6018/analesbio.36.12

COST CA17108 Action – Aedes Invasive Mosquitoes (AIM). https://www.aedescost.eu/aimsurv; (accessed 14th November 2021).

Cunze S., Kochmann J., Koch L.K. & Klimpel S. 2016. *Aedes albopictus* and its environmental limits in Europe. PLoS ONE 11 (9): e0162116. https://doi.org/10.1371/journal.pone.0162116

Cvetkovikj A., Djadjovski I., Krstevski K., Popova Z., Rashikj Lj., Atanasova K., Cvetkovikj I. & Stefanovska J. 2020. New Records of the Asian Tiger Mosquito in North Macedonia. Macedonian Veterinary Review 43 (2): 125–129. https://doi.org/10.2478/macvetrev-2020-0025

Delisle E., Rousseau C., Broche B., Leparc-Goffart I., L'Ambert G., Cochet A., Prat C., Foulongne V., Ferre J. B., Catelinois O., Flusin O., Tchernonog E., Moussion I. E., Wiegandt A., Septfons A., Mendy A., Moyano M. B., Laporte L., Maurel J., Jourdain F., Reynes J., Paty M. C. & Golliot F. 2015. Chikungunya outbreak in Montpellier, France, September to October 2014. Eurosurveillance 20 (17): 21108. doi: 10.2807/1560-7917.es2015.20.17.21108. PMID: 25955774.

Denlinger D.L. & Armbruster P.A. 2014. Mosquito diapause. Annual Review of Entomology 59: 73–93. https://doi.org/10.1146/annurev-ento-011613-162023

European Centre for Disease Prevention and Control (ECDC) 2012. Guidelines for the surveillance of invasive mosquitoes in Europe. Stockholm: ECDC. 95 p. Available from: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/TER-Mosquito-surveillance-guidelines.pdf

Gjenero-Margan I., Aleraj B., Krajcar D., Lesnikar V., Klobucar A., Pem-Novosel I., Kurecic-Filipovic S., Komparak S., Martic R., Duricic S., Betica-Radic L., Okmadzic J., Vilibic-Cavlek T., Babic-Erceg A., Turkovic B, Avsic-Zupanc T., Radic I., Ljubic M., Sarac K., Benic N. & Mlinaric-Galinovic G. 2011. Autochthonous dengue fever in Croatia. Eurosurveillance 16 (9): 19805. PMID: 21392489.

Hawley W. A. 1988. Biology of *Aedes albopictus*. Journal of the American Mosquito Control Association Supplement 1: 1–39. PMID: 3068349.

Hawley W. A., Reiter P., Copeland R.S., Pumpuni C.B. & Craig G.B. 1987. *Aedes albopictus* in North America: probable introduction in used tires from northern Asia. Science 236 (4805): 1114-1116. DOI: 10.1126/science.3576225

Kavran M., Ignjatovic Cupina A., Zgomba M., Zunic A., Bogdanovic S., Srdic V., Dondur D., Pudar D., Marinkovic D. & Petric D. 2019. Invasive mosquito surveillance and first record of *Aedes japonicus* in Serbia. Proceedings of The IXth EMCA conference. La Rochelle p.78. doi:10.13140/RG.2.2.19326.43846

Medley K. A., Westby K. M. & Jenkins D. G. 2019. Rapid local adaptation to northern winters in the invasive Asian tiger mosquito *Aedes albopictus*: A moving target. Journal of Applied Ecology 56 (11): 2518–2527. https://doi.org/10.1111/1365-2664.13480

Pajovic I., Petric D., Bellini R., Dragicevic S. & Pajovic Lj. 2013. *Stegomyia albopicta* Skuse, 1894 (Diptera: Culicidae) on Luštica peninsula 2011-2012 (Montenegro). Archives of Biological Science 65 (3): 829–838. https://doi.org/10.2298/ABS1303829P

Petric D., Pajovic I., Ignjatovic Cupina A. & Zgomba M. 2001. *Aedes albopictus* (Skuse, 1894) a new mosquito species (Diptera; Culicidae) in the entomofauna of Yugoslavia. In Proceedings of the Symposia of the entomologists of Serbia, Belgrade, Serbia, pp. 26–29 (in Serbian).

Petrić D. 2009. Monitoring of invasive vector mosquitoes and vector-borne diseases. Report to Administration for Environmental Protection. Novi Sad. 9 p.

Petric D., Zgomba M., Ignjatovic Cupina A., Pajovic I., Merdic E., Klobucar A., Zitko T. & Landeka N. 2009. Invasion of the *Aedes albopictus* to Eastern Mediterranean Area. International Symposium on the Asian Tiger Mosquito *Aedes albopictus* and its distribution in relation to Bionomy and Climatic factors. German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety. Speyer, invited lecture, p. 38–39.

R Core Team 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org.

Reinhold J. M., Lazzari C. R. & Lahondere C. 2018. Effects of the environmental temperature on *Aedes aegypti* and *Aedes albopictus* mosquitoes: A Review. Insects 9 (4):158 1–17. doi: 10.3390/insects9040158

Rezza G., Nicoletti L., Angelini R., Romi R., Finarelli A. C., Panning M., Cordioli P., Fortuna C., Boros S., Magurano F., Silvi G., Angelini P., Dottori M., Ciufolini M. G., Majori G. C. & Cassone A. 2007. Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet 370 (9602): 1840–1846. doi: https://doi.org/10.1016/S0140-6736(07)61779-6

Romi R., Severini F. & Toma L. 2006. Cold acclimation and overwintering of female *Aedes albopictus* in Roma. Journal of the American Mosquito Control Association 22 (1): 149–151. DOI: 10.2987/8756-971X(2006)22[149:CAAOOF]2.0.CO;2

Sokolovska N., Kostovska J., Musa S., Lazarevska L., Bajrami L. & Arsenievski Z. 2017. Our experience in collecting tiger mosquitoes using ovitraps in Strumica, Gevgelija and the border crossing to Greece. Journal of Health Science 5 (6): 345–349. https://doi.org/10.17265/2328-7136/2017.06.010

Succo T., Leparc-Goffart I., Ferre J. B., Roiz D., Broche B., Maquart M., Noel H., Catelinois O., Entezam F., Caire D., Jourdain F., Esteve-Moussion I., Cochet A., Paupy C., Rousseau C., Paty M. C. & Golliot F. 2016. Autochthonous dengue outbreak in Nîmes, south of France, July to September 2015. Eurosurveillance 21 (21): 30240. https://doi.org/10.2807/1560-7917.ES.2016.21.21.30240

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