# Mosquitoes in double role: pollinators and virus vectors

In the 4 year of the project, we made data collection by relying on the combination of Mosquito monitoring with public participation. With massive media campaign inform the public about our study of tiger mosquito. Altogether in 2020-2024 we collect an identified 155077 mosquito individuals and 16246 of which were tiger mosquitoes.

We found that the number of validated Citize Science reports in a quadrat depends on the underlying sampling effort (i.e. number of total reports), but this relationship varies among species and study years. We created presence/absence map of invasive mosquitoes of Hungary. We demonstrated that conclusions about the local presence/absence of focal species based on citizen reports corroborate well the results of direct field sampling with conventional trapping protocols. (László Zsolt Garamszegi. Kornélia Kurucz, and Zoltán Soltész: Validating citizen science mediated surveillance of invasive mosquitoes in Hungary - Journal of Applied Ecology *60 : 7 pp. 1481-1494*)

We explores the association between urbanization level and the occurrence of invasive Aedes species, specifically Aedes albopictus, Aedes japonicus, and Aedes koreicus, in Hungary, using data from a community (or citizen) science program undertaken between 2019 and 2022. The association between each of these species and urbanized landscapes within an extensive geographic area was found to differ. Using the same standardized approach, Ae. albopictus showed a statistically significant and positive relationship with urbanization, whereas Ae. japonicus and Ae. koreicus did not. (Garamszegi, L.Z., Soltész, Z., Kurucz, K. *& Szentiványi T.:* Using community science data to assess the association between urbanization and the presence of invasive *Aedes* species in Hungary. *Parasites Vectors* **16**, 158 (2023).)

We evaluated the effect of an exhaustive list of environmental predictors on the distribution of three invasive species in Hungary (Ae. albopictus, Ae. japonicus, and Ae. koreicus) by using the same standards for data collection based on citizen science observations. Current distribution maps of these species were generated from a 5-year survey, then were compared with various predictor maps reflecting climate, habitat type, food supply, traffic, and interspecific competition by using a boosted regression trees approach that resulted in a subset of variables with the strongest impact. The best predictor sets were used to predict the probability of occurrence of the focal species for the whole country, and these predictions based on citizen science were evaluated against the results of an independent recent field surveillance. We uncovered species-specific patterns and found that different predictor sets were selected for the three different species, and only predictions for Ae. albopictus could be validated with direct trapping data. Therefore, citizen science informed distribution maps can be used to identify ecological predictors that determine the spread of invasive mosquitoes, and to estimate risk based on the predicted distribution in the case of Ae. albopictus. (László Zsolt Garamszegi, Zoltán Soltész, Tamara Szentiványi, Kornélia Kurucz, Gergely Nagy, Ákos Bede-Fazekas (2024): Identifying ecological factors mediating the spread of three invasive mosquito species: citizen science informed prediction. *Journal Pest Sciences* (2024).

We investigated if the occurence of the invasive species is associated with the composition of native mosquito fauna by controlling for several confounding factors, such as climatic, spatial and seasonal effects. *Ae. albopictus* was positively associated with the species richness and diversity of native mosquitoes, and most pair-wise associations between invasive and native species were positive. However, we identified a negative association between *Ae. albopictus* and *Anopheles plumbeus*, and between *Ae. japonicus* and *Ae. koreicus*, as well as between *Ae. albopictus* and *Ae. koreicus* which may indicate a role in competitive interaction between these pairs of species. We also performed an analysis of large-scale distribution maps, which revealed a significant overlap between invasive and several native mosquito species. Our study shows that invasive mosquitoes often thrive in areas where native species are already present, suggesting overlapping ecological niches. The identified co-existence patterns may help us understand why different invasive species disperse differently in the same geographic area. Importantly, *Ae. albopictus* seems to have an ecological effect beyond its prominent epidemiological role. (Zoltán Soltész, Zoltán Kenyeres , Gábor Markó, Gergely Nagy and László Zsolt Garamszegi:The co-existence patterns between native and invasive mosquito species in Hungary based on a field survey - D1 ecology/conservation biology journal )

We were involved in a social science analysis that analyzed hungarian citizen science (CS) projects. Nature conservation–related citizen science (NCCS) has grown rapidly worldwide in previous years. In Hungary, a few citizen science (CS) projects have been operating for years and some have only recently launched. Our aim herein is to assess the performance of eight Hungarian NCCS projects in three dimensions: a) science, b) nature conservation, and c) participants' development. An evaluation framework was developed for the assessment. Our results show that the Common Bird Monitoring Program performed the best overall. This is also the oldest NCCS project in the country. When comparing the performance per dimension, the majority of the projects tended to have good performances in the science dimension. Most of the projects ensure data quality using different strategies. However, the need for open data and processing the project results for generating scientific publications still needs to be tackled by some NCCS initiatives. Regarding the nature conservation dimension, data generated have been mostly used in monitoring species/ecosystems, whereas data is less commonly used for conservation policy-making. It was identified that the participants' development dimension has not received sufficient attention, and neither learning outcomes nor behavioral and attitude change has been evaluated by any projects. Our results of evaluating Hungarian NCCS initiatives in a complex way may offer insights for project managers and coordinators to identify which dimension are performing well and which areas need improvement. Also, our framework serves as a model that can be applied to current and future NCCS initiatives. (Soria Aguirre, JM, Váczi, O, Biró, M, Juhász, E, Soltész, Z, Barta, B, Márton, Z, Szép, T, Halpern, B, Szentirmai, I, Károlyi, B, Czeglédi, A, Bela, G and Tormáné Kovács, E. 2024. Citizen Science for Nature Conservation in Hungary A Three-Dimensional Approach. Citizen Science: Theory and Practice, X(X): X, pp. 1–15. DOI: https://doi.org/10.5334/cstp.762 )

We are also involved in an international collaboration that we report the results of AIMSurv2020, the first pan-European surveillance effort for Aedes invasive mosquitoes (Miguel et al published).

We are also involved in an international collaboration that target the genetic profiling of the invasive mosquito *Aedes koreicus* in Europe (Kurucz et al published).

We made an experiment revealed that high concentrations of sugar damage viral particles, indicating that WNV transmission via high-sugar nectar is unlikely. In the case of low sugar concentrations, the virus remains infectious only for a short duration (within 24 hours) of incubation. After further confirmatory repetitions, I plan to publish these findings in the journal *Parasites and Vectors* (Soltész Z., Marosi A. & Forgács P.: The possibility of West-Nile Virus transmisson through nectars.)

So far, 5 scientific publications have been published on the topic, and an additional 5 are expected to be released (currently in various stages of preparation). Moreover, 3 book chapters have been written about the three invasive species. The research findings have also resulted in 10 oral presentations and 4 posters.

## This Year

During the field season of 2024, we have collected 956 reports from the public either via the mobile application (566), and e-mail communication (379) and direct posting (9). The taxonomic validation of these observations revealed that 397 of these are true observations of the tiger mosquito. This year, in addition to community sample collection, we continued sampling with the BG trap Altogether, we trapped at 73 locations for 310 days, and we could successfully capture 18315 individuals, 1623 of which were tiger mosquitoes. Four permanent traps were installed (Budapest, Debrecen, Liszt Ferenc Reptér, Vácrátót) and monitored every 24 hours (except weekends). These traps caught 27855 mosquitoes, of which 4209 were tiger mosquito.

We have collaborated to a study by researchers at the University of Veterinary Medicine and Veterinary Medical Research Institute on flaviviruses in mosquitoes. To increase the probability of finding the virus in invasive biting mosquitoes: we sampled all settlements where there were human or equine West-Nile Virus cases in 2024. Altogether, we trapped at 34 location for 90 days, and we could successfully capture 4300 individuals, 521 of which were tiger mosquitoes. The detection of viruses has not yet been completed. West Nile virus has not been found yet in tiger mosquitoes, but we also tested the samples for Usutu virus, where there were positive poles. So far, no virus has been found in invasive mosquitoes in Hungary. We would like to publish these results in the journal Eurosurvaillance (First detection of Usutu virus in an invasive mosquito in Hungary)

The laboratory pollination experiments with *Aedes albopictus* ( self pollination, cross pollination and negative control/ the plants remained covered all the flowering time/ positive control/ plants were free to be visited by pollinators/) have been carried out on *Achillea millefolium* and *Cyanus segetum*. Unfortunately, little data was generated, which was not sufficient for statistical analyses. We will definitely continue the investigation.

We started this study in 2023 in connection the project virus transmission through nectar with 2 questions: How long does WNV retain its infectivity after being exposed to environmental effects, such as temperature and sunlight? and Does sugar concentration affect the sensitivity of WNV to environmental conditions? We made low-sugar (like cell medium) and high-sugar (like nectar) WNV suspensions, and they are disseminated into microcentrifuge tubes, and the aliquots are placed into an environment (considering BSL-3 regulations) that imitated the natural end-of-August climate, and 1 aliquot of each sugar content suspension was placed to -70 Celsius degree in the following times: 0 minute; 1; 2; 4; 8; 24; 72;168 hours. After the field season, we will test the virus with 2 methods: End-point titration: the virus suspensions will be inoculated to cell culture, and the infective titer (concentration of infective virus particles) will be determined by the cytopathogenic effects they induce. And Quantitative RT-PCR: determines the RNA degradation in the virus suspensions exposed to the natural environment. The decrease in the infective titer over time indicates that the chance of successful virus transmission through contaminated nectar sources is limited, which is further proven by RNA degradation over time. This study determines whether the hypothesis is worth further investigation.

It was revealed that the sugar caused damage to the virions during frozen storage, likely resulting in false outcomes during the virus titration. Additionally, it was found that the initial viral suspension contained a low virus titer, making the assessment of environmental conditions' effects statistically unreliable. Based on these findings, the experiment was redesigned.

Through serial passaging, we prepared a suspension with a higher virus titer, which was a time-consuming process due to several unsuccessful attempts. We also prepared low-sugar (cell medium-like) and high-sugar (nectar-like) WNV suspensions, which were aliquoted into microcentrifuge tubes and stored at room temperature in compliance with BSL-3 regulations. These suspensions were prepared to provide samples with incubation times of 0, 0.5, 1, 2, 4, 8, and 24 hours at the time of virus titration, thereby avoiding the false results caused by the effect of freezing. The experiment revealed that high concentrations of sugar damage viral particles, indicating that WNV transmission via high-sugar nectar is unlikely. In the case of low sugar concentrations, the virus remains infectious only for a short duration (within 24 hours) of incubation. After further confirmatory repetitions, I plan to publish these findings in the journal *Parasites and Vectors (*Soltész Z., Marosi A. & Forgács P.: The possibility of West-Nile Virus transmisson through nectars.)

We assessed the presence of kdr mutations L1014F and V1016G in *Culex pipiens* and *Aedes albopictus* mosquitoes, respectively, responsible for pyrethroid resistance. Mosquito

specimens were investigated retrospectively, i.e. collected from 2021-2023 within the framework of local monitoring programs run in urban areas representing five regions of Hungary. The mutations in mosquitoes were detected individually by allele-specific PCR and gel electrophoresis, following generally used protocols. Results: In *Cx. pipiens*, the kdr mutation was detected across all five collection sites, with resistance allele frequencies ranging from 18.1% to 36.3%. Resistance alleles were identified in homozygosity and in heterozygosity with the susceptible allele as well, resulting in 53% of the investigated mosquitoes showing resistance to pyrethroids in the Hungarian populations. In contrast, for *Ae. albopictus*, all the analysed individuals were found to carry only the susceptible alleles, indicating a homozygous susceptible genotype across all the investigated populations. (Rebeka Csiba, Zsaklin Varga, Dorina Pásztor, Bianka Süle, Zoltán Soltész, Brigitta Zana, Krisztián Bányai, Gábor Kemenesi, Kornélia Kurucz: Consequences of insecticide overuse in Hungary: assessment of pyrethroid resistance in Culex pipiens and Aedes albopictus mosquitoes - Parasites and Vectors, submitted)

## Publications :

### Published papers:

László Zsolt Garamszegi, Zoltán Soltész, Tamara Szentiványi, Kornélia Kurucz, Gergely Nagy, Ákos Bede-Fazekas (2024): Identifying ecological factors mediating the spread of three invasive mosquito species: citizen science informed prediction. *Journal Pest Sciences* (2024). https://doi.org/10.1007/s10340-024-01841-7

László Zsolt Garamszegi. Kornélia Kurucz, and Zoltán Soltész: Validating citizen science mediated surveillance of invasive mosquitoes in Hungary - Journal of Applied Ecology *60 : 7 pp. 1481-1494 https://doi.org/10.1111/1365-2664.14417*

Garamszegi, L.Z., Soltész, Z., Kurucz, K. *& Szentiványi T.:* Using community science data to assess the association between urbanization and the presence of invasive *Aedes* species in Hungary. *Parasites Vectors* **16**, 158 (2023). https://doi.org/10.1186/s13071-023-05780-7

Miguel Ángel Miranda, Carlos Barceló, Daniele Arnoldi, Xenia Augsten, Karin Bakran-Lebl, George Balatsos, Mikel Bengoa, Philippe Bindler, Kristina Boršová, Maria Bourquia, Daniel Bravo-Barriga, Viktória Čabanová, Beniamino Caputo, Maria Christou, Sarah Delacour, Roger Eritja, Ouafaa Fassi-Fihri, Martina Ferraguti, Eleonora Flacio, Eva Frontera, Hans-Peter Fuehrer, Ana L García-Pérez, Pantelis Georgiades, Sandra Gewehr, Fátima Goiri, Mikel Alexander González, Martin Gschwind, Rafael Gutiérrez-López, Cintia Horváth, Adolfo Ibáñez-Justicia, Viola Jani, Përparim Kadriaj, Katja Kalan, Mihaela Kavran, Ana Klobucar, Kornélia Kurucz, Javier Lucientes, Renke Lühken, Sergio Magallanes, Giovanni Marini, Angeliki F Martinou, Alice Michelutti, Andrei Daniel Mihalca, Tomás Montalvo, Fabrizio Montarsi, Spiros Mourelatos, Nesade Muja-Bajraktari, Pie Müller, Gregoris Notarides, Hugo Costa Osório, José A Oteo, Kerem Oter, Igor Pajović, John RB Palmer, Suncica Petrinic, Cristian Răileanu, Christian Ries, Elton Rogozi, Ignacio Ruiz-Arrondo, Isis Sanpera-Calbet, Nebojša Sekulić, Kivanc Sevim, Kurtesh Sherifi, Cornelia Silaghi, Manuel Silva, Nikolina Sokolovska, Zoltán Soltész, Tatiana Sulesco, Jana Šušnjar, Steffanie Teekema, Andrea Valsecchi, Marlen Ines Vasquez, Enkelejda Velo, Antonios Michaelakis, William Wint, Dušan Petrić, Francis Schaffner, Alessandra Della Torre, AIM-COST Consortium: AIMSurv: First pan-European harmonized surveillance of Aedes invasive mosquito species of relevance for human vector-borne diseases. GigaByte.:gigabyte57 2022, DOI: 10.46471/gigabyte.57

Kurucz K, Zeghbib S, Arnoldi D, Marini G, Manica M, Michelutti A, Montarsi F, Deblauwe I, Van Bortel V, Smitz N, Pfitzner WP, Czajka C, Jöst A, Kalan K, Šušnjar J, Ivović V, Kuczmog A Lanszki Z, Tóth GE, Somogyi B, Jakab F, Herczeg R, Urbán P, Bueno-Marí R, Soltész Z and Kemenesi G. 2021. Aedes koreicus, a vector on the rise: pan-European genetic patterns, mitochondrial and draft genome sequencing. PloS one, 17(8), e0269880. https://doi.org/10.1371/journal.pone.0269880

## Planned publications:

Soria Aguirre, JM, Váczi, O, Biró, M, Juhász, E, Soltész, Z, Barta, B, Márton, Z, Szép, T, Halpern, B, Szentirmai, I, Károlyi, B, Czeglédi, A, Bela, G and Tormáné Kovács, E. 2024. Citizen Science for Nature Conservation in Hungary A Three-Dimensional Approach. Citizen Science: Theory and Practice, X(X): X, pp. 1–15. DOI: https://doi.org/10.5334/cstp.762 (Accepted)

Rebeka Csiba, Zsaklin Varga, Dorina Pásztor, Bianka Süle, Zoltán Soltész, Brigitta Zana, Krisztián Bányai, Gábor Kemenesi, Kornélia Kurucz: Consequences of insecticide overuse in Hungary: assessment of pyrethroid resistance in Culex pipiens and Aedes albopictus mosquitoes - Parasites and Vectors ( submitted)

Zoltán Soltész, Zoltán Kenyeres , Gábor Markó, Gergely Nagy and László Zsolt Garamszegi:The co-existence patterns between native and invasive mosquito species in Hungary based on a field survey - D1 ecology/conservation biology journal (the text of the manuscript is ready, negotiations with co-authors are underway)

Soltész Z., Marosi A. & Forgách P.: The possibility of West-Nile Virus transmisson through nectars. *Parasites and Vectors* (the text of the manuscript is mostly ready, the experiment needs to be repeated due to statistical analysis)

Erdélyi K, Forgách P, Horváth E.. & Soltész Z.,: First detection of usutuvirus in an invasive mosquito in Hungary. – Eurosurvaillance (PCR tests have been completed, confirmatory sequencing and sequence analysis are still required)

## Book chapter:

Soltész Zoltán, Kurucz Kornélia: *Koreai szúnyog - Aedes koreicus (Edwards, 1917)*, In: Haraszthy, László (szerk.) Özönállatfajok Magyarországon, Duna-Ipoly Nemzeti Park Igazgatóság; Külgazdasági és Külügyminisztérium (2022) pp. 223-225., 2022

Soltész Zoltán, Török Edina: *Ázsiai bozótszúnyog Aedes japonicus (Theobald, 1901)*, In: Haraszthy, László (szerk.) Özönállatfajok Magyarországon, Duna-Ipoly Nemzeti Park Igazgatóság; Külgazdasági és Külügyminisztérium (2022) pp. 226-229., 2022

Soltész Zoltán, Garamszegi László Zsolt*: Ázsiai tigrisszúnyog Aedes albopictus (skuse, 1894)*, In: Haraszthy, László (szerk.) Özönállatfajok Magyarországon, Duna-Ipoly Nemzeti Park Igazgatóság; Külgazdasági és Külügyminisztérium (2022) pp. 219-222., 2022

## Oral presentation in conferences:

Julie Augustin, Zoltán Jánki, Sándor Zsebők, Péter Seffer, Istvan Balog, Zoltán Soltész, Vilmos Bilicki, László Zsolt Garamszegi: *Using mosquito sounds and machine learning to identify invasive mosquito species of medical importance,* XXVII International Congress of Entomology, 2024

L. Z. Garamszegi, Z. Soltész, T. Szentiványi, K. Kurucz, G. Nagy, Á. Bede-Fazekas: *Citizen science informed prediction of the distribution of three invasive mosquito species in Hungary*, 1st Debrecen Online Conference on Infectious Diseases in a One Health context, 202

László Zsolt Garamszegi, Zoltán Soltész, Tamara Szentiványi, Kornélia Kurucz, Gergely Nagy and Ákos Bede-Fazekas: *Identifying ecological factors mediating the spread of three invasive mosquito species: citizen science informed prediction*, 23rd European Society for Vector Ecology (ESOVE) Conference, 2024

Soltész Z.: *Szúnyogprobléma hazánkban*, III. Pest Control ’24 konferencia, 2024

Soltész Z. Idegenhonos inváziós csípőszúnyogok észlelése a közösség erejéve. A Magyar Biológiai Társaság XXXIII. Vándorgyűlése,Pécs, Hungary, 2024. június 6–7 (Abstract book: p. 27.)

Zoltán Soltész, Kornélia Kurucz, László Zsolt Garaszegi: A surveillance program of invasive mosquitoes based on citizen science in Hungary - 10th International Congress of Dipterology USA, Reno, 16-21. VII.2023 Abstract book p:191.

Tamara Szentiványi, Laura González Velasco, Zoltán Soltész, László Zsolt Garamszegi: Estimating Dirofilaria (Railliet & Henry) (Nematoda: Onchocercidae) occurrence and distributionin Hungary using different approaches. - 10th International Congress of Dipterology USA, Reno, 16-21. VII.2023 Abstract book p:197.

Károly Erdélyi, Zoltán Soltész, Anna Nagy, László Zsolt Garamszegi, and Petra Forgách (2023) Confirmation of seasonal flavivirus activity at suspect localities through targeted mosquito trapping and testing. 15th EPIZONE Annual Meeting, 26-28 April 2023. Novi Sad, Serbia. ISBN- 978-86-82871-47-7

Soltész, Z, Báldi, A, Garamszegi LZ. 2021. Az ázsiai tigrisszúnyog feltérképezése a lakosság bevonásával – Egy citizen science projekt bemutatása. Talk. XII. CONGRESS OF THE HUNGARIAN ECOLOGISTS, 2021. August 24–26. Vác, Hungary. (Abstract book: p. 103.)

Garamszegi LZ, Bede-Fazekas, Á, Somodi, I. Soltész, Z. 2021. Három inváziós csípőszúnyogfaj hazai elterjedését meghatározó környezeti tényezők: térképezés citizen science adatok alapján. Talk. XII. CONGRESS OF THE HUNGARIAN ECOLOGISTS, 2021. August 24–26. Vác, Hungary. (Abstract book: p. 50.)

## Poster in conferences:

Zoltán Soltész, Tamara Szentiványi, Kornélia Kurucz, László Zsolt Garaszegi: The association between urbanization and the presence of invasive mosquito species in Hungary based on community science - 10th International Congress of Dipterology USA, Reno, 16-21. VII.2023 Abstract book p:192.

Soltész Z, Kurucz K., Szentiványi T. and Garamszegi LZ. 2022. Hol vannak inváziós csípőszúnyogok Magyarországon?. Poster. 13rd HUNGARIAN CONFERENCE ON CONSERVATION BIOLOGY, 2022. August 28–31. Pécs, Hungary. (Abstract book: p. 100.)

Soltész Z, Plankó E and Szöllősi E 2022. Milyen csípőszúnyog vektorok terjesztik a madármalária thazánkban?. Poster. 13rd HUNGARIAN CONFERENCE ON CONSERVATION BIOLOGY, 2022. August 28–31. Pécs, Hungary. (Abstract book: p. 101.)

Kurucz K, Zeghbib S, Manica M, Arnoldi D, Marini G, Michelutti A, Montarsi F, Deblauwe I, Smitz N, Jöst A, Kalan K, Kuczmog A Lanszki Z, Tóth GE, Bueno-Marí R, Soltész Z, Jakab F, Kemenesi G. 2021. Az inváziós koreai szúnyog (Aedes koreicus, Culicidae) európai terjedésének vizsgálata genetikai megközelítéssel. Poster. XII. CONGRESS OF THE HUNGARIAN ECOLOGISTS, 2021. August 24–26. Vác, Hungary. (Abstract book: p. 176.)