Influence of tank colour on oviposition and development of *Aedes* sp. mosquito

Mia Bacalja, Andrea Brzica, Laura Zovko
Mihaela Marceljak Ilić
XV. gimnazija, Zagreb
Croatia
10th of January 2023

Abstract

The goals of this project are to investigate the influence of different colours of mosquito traps on the oviposition and development of mosquito and to determine whether the seasons will affect the number of mosquitoes found in mosquito traps. Three research questions have been formed:

- How does the trap colour (black, brown, dark blue, light blue, green, purple, red, orange, yellow, and white) influence oviposition and abundance of *Aedes* mosquitoes?
- Are there seasonal changes in oviposition and abundance of *Aedes* mosquitoes?
- Did the abundance and genera of observed mosquitoes change during the period between 2016 and 2021?

Using GLOBE Mosquito protocols and GLOBE Hydrology protocols, we conducted the project in two phases. In the first phase, mosquito traps were placed on the outside of the biology cabinet during April and May, while in the second phase, mosquito traps were placed in the same place, only during the summer months of August and September. In both phases of the study, there were clear differences in mosquito preferences toward a trap colour. In both seasons, there was an increase in the number of mosquitoes of the genus *Aedes* at all developmental stages in the darker coloured mosquito traps, while other genera did not appear at all in any mosquito traps. Also, research has found that the number of mosquitoes of the genus *Aedes* is higher during the summer than during the spring. In future investigations it would be necessary to conduct research in all four seasons, with an even greater number of traps in different locations and it would be desirable to determine the physical and chemical characteristics of the water after the end of the experiment.

Key words: Seasonal changes, GLOBE Mosquito protocols

Research Question and Hypothesis:

This research project aims to answer the following research questions:

- How does trap colour (black, brown, dark blue, light blue, green, purple, red, orange, yellow, and white) influence oviposition and abundance of *Aedes* mosquitoes?
- Are there seasonal changes in oviposition and abundance of *Aedes* mosquitoes?
- Did the abundance and genera of observed mosquitoes change during the period between 2016 and 2021?

It is hypothesised that the oviposition and abundance of mosquitoes will be greater in traps of darker colour. The assumption is made because of the observed preference of mosquitoes toward darker colours (Gilbert and Gouck, 1957). Through the review of literature, it is determined that mosquitoes are more abundant during the summer months (Balenghien et al., 2006), so we

assume that in our research we will observe changes in the oviposition and abundance of mosquitoes between the spring and summer period. Both of which affect the general mosquito population in cities like Zagreb. Due to mosquitos' adaptation to more urbanised environments like cities, it is important to know what affects their habitat in order to be better at regulating the spreading mosquito-borne diseases. Regarding the change of abundance and genera of observed mosquitoes from this year compared to the results of the 2016. research project, we assume that the general abundance of mosquitoes will be greater in 2021. but the genera of the observed mosquitoes will not change, said is assumed because of similar observations found by other Croatian researchers (Vrućina et al. 2020).

Introduction and Review of Literature

In 2016 GLOBE group of XV. gimnazija in Zagreb conducted research on the influence of physico-chemical parameters of water in mosquito traps of different colour and material on the appearance of mosquitoes. The results of that research made us intrigued and encouraged us to conduct research on the influence of plastic trap colour on the oviposition and development of the *Aedes* mosquito in 2021.

Our research project was conducted during the school years 2020/2021. and 2021/2022. The procedure of the collection and analysis of mosquito traps was done two times. The first time was during the spring of 2021 (April and May), and the second time was during the summer of 2021 August and September). The location of both repetitions of the experiments was XV. gimnazija in Zagreb.

The aim of this research project was to determine whether habitat (trap) colour and season influenced the abundance of mosquitoes and their development stages. Our goal was also to compare the results of this year’s research project with the results of the research the students of our school conducted in 2016 so we can determine whether there was a change in the abundance of mosquitoes and their genera in the area around XV. gimnazija. Such change could indicate a possibility of greater change of mosquito-borne diseases spreading in the Zagreb region which would impact the community severely. Croatian researchers in other parts of Croatia (Vrućina et al. 2020) have found that the number of mosquitoes has been steadily increasing with years, as well as the number of people infected with West Nile Virus which is one of the mosquito-borne diseases most prevalent in Croatia (Lužanac. 2022).

Research Methods and Materials

In order to successfully conduct the research project, firstly the traps mosquitoes needed to be constructed. The instructions for the construction of the traps were given as a resource during the GLOBE Mosquito campaign in which 27 students from 1.B class of our school participated. GLOBE activities and protocols were implemented in teaching while the students of 1.B class constructed mosquito traps that were later on used for this research project.

Materials used for the construction of traps were transparent plastic bottles (all from the same brand), gridded mesh, scotch tape, coloured paper, elastic band, aluminium foil and water (again the same brand in every mosquito trap). All mosquito traps were made using the same materials

and every one of them had the same type of water poured into them. During both repetitions of the research project, the mosquito traps were made the same way and of the same material.

The constructed mosquito traps were placed in boxes and onto the outer side of the biology cabinet's window. The geographical coordinates of the location of the mosquito traps are 45°49'10.0"N 16°00'26.9"E. During the first period of the research (spring of 2021) mosquito traps were placed at the location in April. And during the second period of the research (summer of 2021) the mosquito traps were placed at the location in August. During the first period of the research, 30 mosquito traps were used, 3 of each colour. During the second period of the research, 10 mosquito traps were used, 1 of each colour. Due to the lack of available material, only 10 new mosquito traps were constructed.

During the period while the mosquito traps were exposed to the outer nature, the same type of water ("Jana") was poured into them whenever evaporation of a certain amount of volume was observed. Our mosquito traps are shown in Figure 1.

Figure 1. Plastic mosquito traps on the outer side of the window of the school's biology cabinet

The first period when this research was conducted was between 13.4.2021. and 14.5.2021. . The second period when this research was conducted was between 4.8.2021. and 4.9.2021. The periods were chosen because during these two months, looking at the ten-year period between 1994. and 2004. mosquitoes were most present in Croatia (Bogojević et al., 2004).

When the research period ended, the mosquito traps were brought inside, where the number of mosquito eggs, larvae, and adult mosquitoes was counted. If the number of those was too great to be counted at once, the whole contents of the mosquito trap was placed into alcohol, to preserve the contents. One of the found larvae is shown on Fig. 2.

Figure 2. Mosquito larvae found in mosquito traps.

To determine whenever the population of larvae and eggs found in the mosquito traps were truly mosquitoes, the school's microscope was used to look at the individual mosquito stages closer. All data on the found mosquito eggs, larvae and adults was uploaded to the GLOBE Observer app during the Mosquito Habitat Mapper Challenge.

Applying GLOBE Hydrosphere protocols, physico-chemical analysis was conducted with the “Jana” water used in the mosquito traps. Following the GLOBE Protocols, the presence of dissolved oxygen (O_2) phosphates (PO_4-P), nitrates and nitrites (NO_2, NO_3) were tested. Also, the pH of the water was determined, followed by determination of the total water hardness (CH) and carbonate hardness (CH). The results of the described measurements are shown in Table 1.

Table 1. The initial results of physico-chemical analysis of Jana water

$PO4^{-}$ P [mg/L]	0,0
$NO2^{-}$ [mg/L]	0,0
$NO3^{-}$ [mg/L]	0,0
pH	7,5
CH [°]	8,0
TH [°]	8,0
O2 [mg/L]	10,0

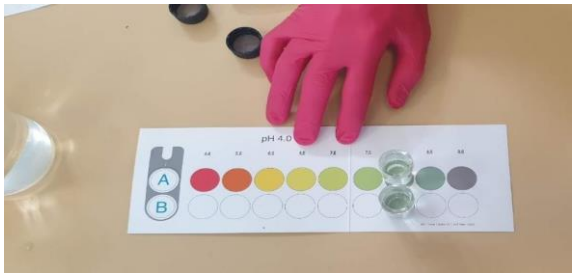

Figure 3. Physico-chemical analysis of water in the research project

During the analysis collected data, the computer program Microsoft Excel was used for data entry

and processing. The data analysis of the first research period (spring 2021) included the collection of data on the number of eggs, larvae and adults of the genus *Aedes*, the only present genus in observed traps. From the data, the average number of eggs, larvae and adults in a trap of a particular colour were calculated. When analysing the data of the second research period (summer 2021), the data on the total number of eggs, larvae and adults of the genus *Aedes* were analysed, which is again the only one present in traps. In the summer research period, one trap for each colour was used. During the first period (spring 2021), the total number of occurrences (by summing the average numbers; of eggs, larvae and adults calculated for containers of specific colours) of all developmental stages of mosquitoes of the genus *Aedes* found in the research was calculated. For the second, summer, research period, the total number of occurrences of all stages of development (eggs, larvae and adults) of the mosquito's genus *Aedes* were calculated.

Results

Table 2. The average number of *Aedes* mosquito eggs, larvae and adults during April and May of 2021.

Colour of the trap	Average number of eggs	Average number of larvae	Average number of adults
Black	243	156	2
Brown	207	142	0
Dark blue	268	235	3
Green	186	139	4
Light blue	134	175	2
Purple	87	98	0
Orange	71	48	0
Pink	62	29	0
Yellow	25	14	0
White	18	7	0

The number of mosquitoes during the spring period is bigger in darker coloured traps. The same pattern is visible for eggs and for adult individuals. It is also seen that the number of individuals drops as the life stages advance so the most represented are the eggs and the least represented are the adult individuals.

Table 3. Total number of *Aedes* mosquito eggs, larvae and adults during August and September 2021.

Colour of the trap	Average number of eggs	Average number of larvae	Average number of adults
Black	374	269	12
Brown	328	175	13
Dark blue	291	257	9
Green	283	227	4
Light blue	210	148	0
Purple	156	102	1
Orange	105	55	1
Pink	94	73	2
Yellow	65	29	0
White	47	22	1

The number of mosquitoes of the *Aedes* genus during the summer period was bigger darker coloured traps (Table 3.). The same pattern is visible for eggs as well for adult individuals. It is also seen that the number of individuals drops as the life stages advance so the most represented are the eggs and the least represented are the adult individuals. Considering the data, the overall number of mosquitoes of all life stages in different coloured traps (Figure 4. and Figure 5.) is bigger during the summer period.

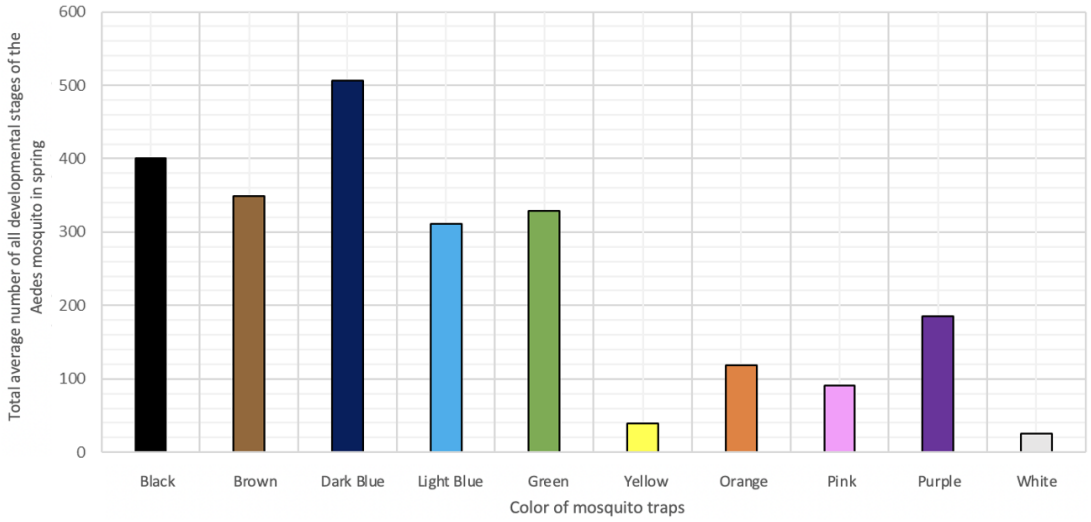

Figure 4. Dependence of the total average number of all *Aedes* mosquito developmental stages during April and May 2021. on the colour of the plastic traps

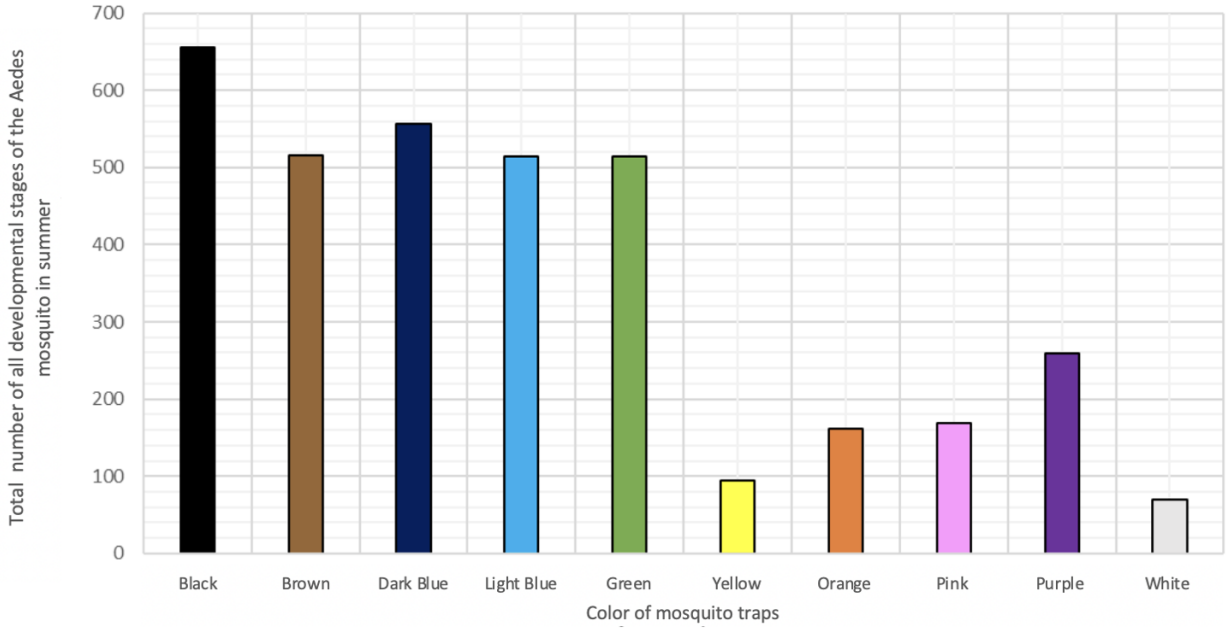

Figure 5. Dependence of the total number of all *Aedes* mosquito developmental stages during August and September 2021. on the colour of the plastic trap

Date/Time (UTC): **05/17/2021 12:11:00**	Date/Time (UTC): **09/04/2021 12:46:00**
Data Source: **GLOBE Observer App**	Data Source: **GLOBE Observer App**
Latitude/Longitude: **45.8187, 16.0066 (45° 49' 7.32", 16° 0' 23.76")**	Latitude/Longitude: **45.8601, 15.7794 (45° 51' 36.36", 15° 46' 45.84")**
Organization: **Croatia Citizen Science**	Organization: **Croatia Citizen Science**
Site: **33TWL782744**	Site: **33TWL605788**
Water Source Type: **Container: Artificial**	Water Source Type: **Container: Artificial**
Water Source: **Adult Mosquito Trap**	Water Source: **Adult Mosquito Trap**
Larvae Count: **235**	Larvae Count: **22**
Mosquito Eggs: **Yes**	Mosquito Eggs: **Yes**
Mosquito Pupae: **No**	Mosquito Pupae: **No**
Mosquito Adults: **Yes**	Mosquito Adults: **Yes**
Genus/Species: **Aedes incerta**	Genus/Species: **Aedes incerta**
Breeding Ground Eliminated: **No**	Breeding Ground Eliminated: **No**

Figure 6. Print screen of entries to GLOBE visualisation page

Discussion and Conclusions

Results of the research match with hypothesis and research questions set in this work.

The first research question set in this work tackles the question of the influence of colour of the habitat (the trap) on oviposition and abundance of mosquitoes in all life stages. Hypothesis that adds onto this research question assumes that oviposition and abundance of mosquitoes will be bigger in traps of darker colour. With this research the given hypothesis was confirmed because during both periods of research in 2021. a bigger number of all life stages of the *Aedes* genus were observed while *Anopheles* and *Culex* genera were not found. For example, in the spring period in the black trap there was found 93,7% more individuals of all life stages of the *Aedes* genus then in a trap of a brighter, white colour. The same is visible in other darker traps; in the brown trap there is 92,8%, dark blue 95,1%, green 92,4%, light blue 91,9%, purple 86,5&, orange 78,9%, pink 72,5% and yellow 35,9% more *Aedes* genus individuals of all life stages than in the control, white trap. During the summer period in the black trap there was found 89,3% more individuals of the *Aedes* genus of all life stages than in the control, white trap. The same is again visible in other darker traps, in the brown trap there was found 86,4%, in dark blue 87,4%, in green 86,4%, in light blue 80,4%, in purple 73,0%, in orange 56,5%, in pink 58,6% and in yellow 25,5% more individuals of the *Aedes* genus of all life stages than in the control, white trap. It is also proven that the season does not impact preference for colours of the traps since the abundance of all life stages in both periods follow a similar pattern, in which mosquitoes are most represented in traps of black, brown and dark blue colour while they are least represented in traps of white and yellow colour.

If the usual habitats of mosquitoes in nature are considered, it is possible to concluded that mosquitoes are found in the immediate vicinity of standing water, which is commonly darker in colour (Gilbert and Gouck, 1957). For this reason, it is intuitive to assume that mosquitoes will be more attracted to traps that are of similar colour as their natural habitats.

The second research question posed in this paper investigated the influence of season on oviposition and the abundance of mosquitoes in all stages of the life cycle. It was assumed that during the summer months the total number of mosquitoes in all stages of its life cycle will be higher. The previously mentioned assumption was confirmed by this research, considering that the total number of mosquitoes in all stages of its life cycle found in traps of all colours was higher during the summer than during the spring, for the genus *Aedes*, while the genera *Anopheles* and *Culex* were not found in any observed containers in either period.

During the summer months, Zagreb has the highest temperatures and the highest amount of precipitation. Hence, during said months, the rain falls rarely, but in large quantities, so it is more common for it to stay in certain depressions for a long time. During spring, the rains do not fall in equal amounts and the temperatures are significantly lower. Considering that mosquitoes, especially *Aedes* genus, prefer areas where it is humid and warm, it is understandable that their numbers increased significantly during the summer months when their traps were wetter and warmer than those during the spring months. Due to lower spring temperatures (compared to those in summer), the water in containers and other mosquito habitats warms up slower, and

mosquitoes need approximately three or four weeks to develop, while during hot summer temperatures, it takes them less than a week to develop (MacMillian, 2018).

The third and final research question asked in this study examined the change in abundance, genus and preference of mosquitoes according to the colour of the trap in two separate studies conducted 5 years apart. Looking at the results obtained by the research of the MIOC GLOBE group from 2016, which are shown in Table 4, several observations can be made.

Table 4. The number of *Culex* mosquito eggs found in mosquito traps in 2016. project

Date	18.4.2016.	25.4.2016.
Mosquito trap materials	**The number of *Culex* mosquito eggs found in mosquito traps**	
Black glass	27	36
Black plastic	/	2
Blue glass	1	0
Green glass	2	0

If the results of the number of mosquitoes of the genus *Culex* in April 2016. are compared with this year's results, it is evident that the mosquitoes from both studies prefer habitats, i.e. traps of darker colours. However, research from 2016 is done on a small sample with a narrow range of colours of traps consisting of different materials, and the research was conducted during only one season, while in the newer one it was conducted during two. Likewise, it should be considered that only the *Culex* species was recorded in the 2016 research, while only the *Aedes* species was recorded in this research. This could potentially indicate that the appearance of the mentioned individuals can be influenced by the material of the container (this year's research uses plastic hunting containers, traps) or that the dominant genus of mosquitoes present in these areas has changed in the last 5 years (Klobučar, 2022). However, one should contemplate the differences in the method of this year's research and the one in 2016. At that time, the research was conducted on a much smaller sample, and not only plastic, but also glass and rubber hunting vessels/traps were used.

Taking into the account that the measurements were made at different times of the year, the data could have also been affected by weather conditions, i.e., different amounts of precipitation (during the period of the first research, it rained twice as much as during the second period). The research method is limited by the colour of the traps and the time period in which the research is carried out, thereby leaving out the possibility of other factors that could have influenced the number of mosquitoes in the traps. Likewise, to improve the precision of the results, it would be necessary to conduct research in all four seasons, with an even greater number of traps in different locations, in order to determine the influence of the location on the number of mosquitoes. Furthermore, by manually counting individuals of mosquitoes viewed with a microscope in different stages of the life cycle, it is not possible to say with absolute certainty a perfectly accurate number of individuals due to their large number. Additionally, it would be desirable to determine the physical and chemical characteristics of the water after the end of the experiment, which was not possible to do in this research due to limitations caused by the epidemiological situation and

the combination of online and live classes.

Ultimately, the main research questions were successfully answered in this project. The colour of the traps was established as an extremely important factor that influenced the oviposition and the number of individuals of the *Aedes* mosquito in different stages of their development cycle. Namely, the eggs, larvae, and adults of the mentioned genus of mosquito appeared the most in the black traps in both seasons, and then also in the other darker coloured traps, while they appeared the least in the white trap. The preference of mosquitoes for colour was not affected by the season, because in both research periods it was established that the number of individuals in tanks was higher in dark tanks (black, blue, green, brown) and lower in light tanks (pink, white, orange, purple, yellow). Although the season did not affect the colour preference of *Aedes* mosquitoes, the overall abundance of the above was significantly higher in summer than in spring. In the same way, we come to the observation that mosquitoes of the genera *Anopheles* and *Culex* were not found in the traps, therefore we conclude that they are not so widespread as genus *Aedes* in the vicinity of the XV. gimnazija in Zagreb. The hypothesis about increased oviposition and number of mosquitoes in darker coloured traps was confirmed, just like the hypothesis about increased appearance of mosquitoes in the summer months. In addition to the mentioned two, the third hypothesis about the increase in the number of mosquito larvae found in traps over a period of 5 years was also confirmed. The results of this year's work confirm that more mosquitoes were caught this year than during the 2016. project, although one should consider the fact that in 2016. study, the traps were exposed for a slightly shorter time. The hypothesis about the continuity of the appearance of certain genera was challenged since not a single individual of the *Culex* mosquito genus appeared in this year's survey, while in the 2016 survey, the *Culex* genus was the only and most represented one.

Bibliography/Citations

Balenghien, T., Fouque, F., Sabatier, P., & Bicout, D. J. (2006). Horse-, bird-, and human-seeking behaviour and seasonal abundance of mosquitoes in a West Nile virus focus of southern France. Journal of medical entomology, 43(5), 936–946. https://doi.org/10.1603/0022- Accessed 18.1.2022.

Bogojević, M.S., Merdić, E., Turić, N. *et al.* Seasonal dynamics of mosquitoes (Diptera: Culicidae) in Osijek (Croatia) for the period 1995–2004. *Biologia 64***,** 760–767 (2009). https://doi.org/10.2478/s11756-009-0138-z, Accessed 18.1.2022.

Gilbert, I. H., & Gouck, H. K. (1957). Influence of surface colour on mosquito landing rates. Journal of Economic Entomology, 50(5), 678-680., https://doi.org/10.1093/jee/50.5.678, Accessed 14.1.2022.

GLOBE.gov. (2014). Dissolved Oxygen protocol. https://www.globe.gov/documents/11865/354449/Dissolved+Oxygen+protocol/f9946a8c-7aa9-4634-a39d-d0f876b00c35, Accessed 10.1.2022.

GLOBE.gov. (2014). Temperature protocol. https://www.globe.gov/documents/11865/354449/Temperature+protocol/f3841172-0e17-

4e45-b52f-e68674a276e5, Accessed 10.1.2022.
GLOBE.gov. (2014). pH Protocol. Https://www.globe.gov/documents/11865/354449/pH+Protocol/42e3b8fe-847c-429a-a105-d18691d99e32. Accessed 10.1.2022.
GLOBE.gov.(2021), Navigation, https://observer.globe.gov/toolkit/mosquito-habitat-mapper-toolkit/printables-and-promotional-materials. Accessed 10.1.2022.
Klobučar. A. (2021). Razvoj i staništa komaraca. Nastavni zavod za javno zdravstvo Andrija Štampar. https://www.stampar.hr/hr/komarci , Accessed 5.4.2022.
MacMillan H. (2018). Why your summer might be full of mosquitoes. Scientific American. https://www.scientificamerican.com/article/why-your-summer-might-be-full-of-mosquitoes/ ps://www.stampar.hr/hr/komarci , Accessed 5.4.2022.
Ugarković, D., Popić, K., Tikvić, I., & Matijević, M. (2021). Neka obilježja klime i klimatskih elemenata na području grada Zagreba. *Šumarski List*, *145*(9-10), 479–488. https://doi.org/10.31298/sl.145.9-10.6 , Accessed 6.4.2022.
Vrućina, I., Merdić, E, Kata, K. & Ankica, I. (2020). Povećanje brojnosti i širenje azijskog tigrastog komarca *Aedes albopictus* na području Vukovarsko-srijemske županije od 2016. do 2019. https://www.bib.irb.hr/1092035 , Accessed 5.4.2022.

Badge Descriptions/Justifications

We believe we deserve to be awarded the “I am a collaborator” badge because students participating in this research project have been named as authors. During the course of our research project, we clearly defined our roles (all three of us were doing the experiments, but each one of us had a specific job we had to do e.g. counting mosquito individuals, data analysis, literature review, etc.) The fact that we collaborated during the making of this research project has enabled us to conduct it much better than we would have done it if only one person was doing it. During the project our class was involved in the design of experimental equipment.

We believe we deserve to be awarded the “I make an impact” badge because our research investigates a local problem of the increased number of mosquitoes in our area and what are the factors that affect it. Mosquito-borne diseases are a big global problem that we are trying to investigate and solve in our local community. In order to raise awareness about the problem of increasing mosquito populations in Zagreb region, we’ve organised meetings and presented our research work to fellow students and citizen scientists.

We believe we deserve to be awarded the “I am a STEM storyteller” badge because in order to raise awareness about the growing problem of increasing mosquito populations in Zagreb region to people with sight disabilities, members of our school’s GLOBE group created an app that teaches blind people to recognize different mosquito genera based on the sounds they make while flying. We are also conducting mosquito themed workshops in kindergarten to raise awareness about the problem of mosquitoes with young children.