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Research of breeding sites capacity for oviposition of floodwater mosquitoes in the flooded area of Danube and Drava rivers in Croatia

Istraživanje kapaciteta staništa za ovipoziciju jaja poplavnih komaraca na poplavnom prostoru Dunava i Drave, Hrvatska

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Abstract

Floodwater mosquitoes lay their eggs on dry ground where water will eventually flood the area. By flooding the area and submerging the eggs of floodwater mosquitoes, the development of larvae begins. For a gravid female mosquito, the most important thing is choosing the place where to lay eggs. As part of this research, which was conducted in April and May 2022, soil and leaf samples were taken on an area of 50 x 50 cm from different altitudes in the area of Dunavac near Ilok (Danube) and Halaševo near Osijek (Drava) in order to determine at which altitude, the largest number of eggs are laid. Collected soil was submerged in the laboratory for the purpose of further mosquito development. Part of the larvae was preserved in alcohol, and part of larvae was left to develop into adults, after which species determination was carried out. A large difference was found in the potential of breeding sites in the floodplain of the Drava and Danube, and the number of collected eggs ranged from 0 to 66 per sample. The number of individuals sampled in the Danube area was 53, and in the Drava area 133. The individuals sampled in the researched areas were determined as *Aedes vexans* and *Aedes sticticus*. A large difference in oviposition was found in the floodplain of the Danube, while in the floodplain of the Drava, this level is significantly higher.

Keywords: mosquito eggs, oviposition, floodwater mosquitoes, inundation of Danube and Drava rivers, breeding site capacity

Sažetak

Poplavni komarci jaja polažu na suho tlo tamo gdje će u određenom trenutku voda poplaviti područje. Plavljenjem područja i potapanjem jaja poplavnih komaraca započinje razvoj ličinki. Za gravidnu ženku komarca najvažniji je odabir mjesta na koje će položiti jaja. U okviru ovog istraživanja, koje je provedeno u travnju i svibnju 2022. godine, uzeti su uzorci zemlje i lišća na površini 50 x 50 cm s različitih nadmorskih visina na području Dunavca kod Iloka (Dunav) i Halaševa kod Osijeka (Drava) da bi utvrdili na kojoj je nadmorskoj visini položen najveći broj jaja. Prikupljena je zemlja u laboratoriju potopljena vodom u svrhu praćenja razvoja komaraca. Dio ličinki sačuvan je u alkoholu, a dio ličinki ostavljen je da se razvije do odraslih jedinki nakon

čega je provedena determinacija do vrste. Utvrđena je velika razlika u potencijalu legla na poplavnom području Drave i Dunava, a broj sakupljenih jaja kretao se od 0 do 66 po uzorku. Broj uzorkovanih jedinki na području Dunava iznosio je 53, a na području Drave 133. Uzorkovane jedinke na istraživanim područjima determinirane su kao vrste *Aedes vexans* i *Aedes sticticus*. Utvrđena je velika razlika u ovipoziciji na istraživanim područjima i to na poplavnom prostoru Dunava evidentirano je manje komaraca nego na poplavnom prostoru Drave.

Ključne riječi: jaja komaraca, ovipozicija, poplavni komarci, poplavni prostor Dunava i Drave, kapacitet staništa

Introduction - *Uvod*

After ingestion of a blood meal, adult mosquito females usually lay between 50 and 500 eggs to a suitable breeding site, which can be pools of melted snow, floodplains, canals, tree-holes, and small water bodies (Becker et al. 2010). In lowland Croatia the most numerous are floodwater mosquitoes such as *Aedes vexans* (Meigen, 1830) or *Aedes sticticus* (Meigen 1838) which develop in huge numbers along the lowland rivers (Merdić and Lovaković, 2004).

Areas for developing floodwater mosquitoes are characterized by temporary water flow caused by fluctuating river levels after snowmelt (in spring) or heavy rains (early and mid-summer), while late summer and winter periods are usually periods of low water levels (Merdić et al. 2020).

Floodwater mosquitoes such as *Ae. vexans* lay single eggs to the moist soil of their breeding places, which are going to be flooded when the water level rises (Barr and Azawi 1958) to ensure that the sensitive, and still unprotected freshly laid eggs do not dry out (Gillett 1955). Sufficient flooding is necessary to complete development. It is important that the water body have a low number of mosquito predators. An oviposition site is never in low-lying area with an almost permanent water flow, which can float away eggs, and generally, this kind of water body has a high number of natural predators. Another problem can be flooded areas with a very short period of water flow because they have an unsuitable wet and dry sequence. Also, these areas dry rapidly after a flood so the freshly laid eggs are at risk of desiccation (Becker et al. 2010).

It is not yet known how mosquitoes select the optimal oviposition place. Preferred egg-laying sites of the floodwater mosquitoes usually are areas of silty soil and dense vegetation. Floodwater mosquitoes are likely able to recognize the soil in floodplains, which consists of a high percentage of clay and a low percentage of humus or organic materials. It is also speculated that those areas produce pheromone-like odors from previously laid eggs or from particular associations of plants that indicate a specific moisture level in the soil, which the female mosquitoes recognize (Becker et al. 2010).

The optimal conditions for floodwater mosquito larvae development occur between April and September with a diapause during autumn, and early spring (Merdić et al. 2020; Telford 1963).

Two factors are important for egg hatching. First is the level of the oxygen when larval hatching is triggered by a decline of dissolved oxygen as a consequence of several factors like microbial-induced. The second abiotic factor is suitable adequate water temperature (Schäfer and Lundström 2006; Horsfall and Fowler 1961). However, not all larvae hatch uniformly and this so-called "hatching in installments" ensures the

survival of populations in case initial larval populations are killed by drying of breeding sites. The larval development is temperature-dependent and can be completed within very short time frames. In addition, floodwater mosquito species are known for egg mass-production and long-range adult dispersal.

A big challenge for female mosquitoes is where to lay eggs. Every year water level (flood) is different and a small difference in altitude can be significant for mosquito survival. The aim of this paper is to compare two breeding sites of floodwater mosquitoes (one in Danube River inundation and one in the Drava River) and assess which habitat and altitude are more suitable for egg laying of floodwater mosquitoes.

Materials and Methods - *Materijali i metode*

The research was conducted in the surrounding of the town of Ilok, at the shores of the Danube River, and in inundation area of the Drava River in the surrounding of the city of Osijek (Figure 1). This research was done before the regular spring flooding of lowland rivers. In both areas on uneven ground, a secluded place with developed vegetation (ass. *Populetum nigro-albae*) and high index of humidity (according to vegetation characteristics) near the water bodies was selected. In the Danube area six samples from six localities, on different altitudes with approximately 0,5 m difference starting from 75masl, were selected and sampled on April 9th, 2022. In the Drava area four localities on different altitudes with approximately 0,5 m difference starting from 84masl were selected and sampled on May 2nd, 2022.

Figure 1 Researched area. Yellow circle – flooded area on the Drava river in surroundings of the Osijek city; Blue circle – flooded area on the Danube River in surroundings of the town of Ilok

Slika 1. Istraživano područje. Žuta kružnica - poplavno područje na rijeci Dravi nedaleko od Osijeka; plava kružnica - poplavno područje na rijeci Dunav nedaleko Iloka

Method used for this research was the flooding method (Silver 2008), which implies taking standardized soil surface samples, so the number of eggs per surface unit can be counted. A metal frame (50x50) is used to take a soil sample. The sample of soil which consisted of 2-3 cm of surface soil and all dry leafage was put into big plastic containers (40x50x80 cm), labeled, and transported to the laboratory. In laboratory, the container was filled up with approximately 10 l of water and kept for eight days at an average temperature of 24°C to let the eggs hatch. Every two days, the containers were checked in to watch the progress of hatching the larvae, and the biofilm that appeared on the surface of the water, which may impede the correct development of the larvae, was removed. After hatching, without feeding, the larvae were allowed to develop until 4 instars then were removed from the container. Part of larvae was mounted on slides for determination and parts were removed in the insect cage to finish development to the adult stage. Adult mosquitoes were killed by cigarette smoke and mounted on entomological pins. Determination was done by using keys Becker et al. 2010 and Gutsevich et al. 1974. To calculate the population size estimate, we took the average number of eggs for the entire researched areas.

Results - *Rezultati*

On both locations in all samples a total of 186 mosquitoes were hatched and determined. A much higher number of mosquito eggs, in total 133, were noted in the Drava area, and 53 mosquitoes were noted in locations in the Danube area (Figure 2). As we had a different number of samples from two areas of research, we calculated the average number per sample per area. The average number in the Drava area is 33,25 mosquitoes per sample and in Danube area 10,60 mosquitoes.

All sampling sites were at different altitudes. The difference between sites is approximately 0.5 m. The number of eggs differs from location to location depending on altitude. The highest number of eggs (59), was noted in the Drava area at the highest altitude approximately 86masl. No mosquitoes were noted at the lowest altitude in the Danube area (Table 1).

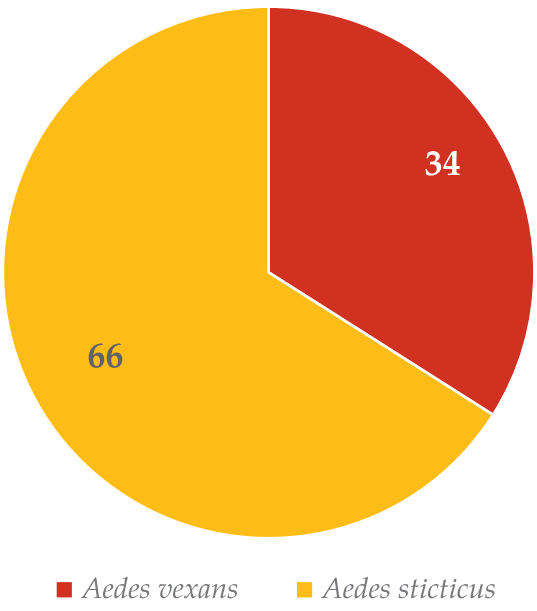

Figure 2 Composition of mosquito species from samples from the Danube area
Slika 2. Sastav vrsta komaraca iz uzoraka s područja Dunava

Table 1 Noted number of mosquitoes in different altitudes
Tablica 1. Broj komaraca po postajama s različitim nadmorskim visinama

Area *Područje*	Sampling date *Datum uzimanja uzorka*	Location *Lokacija*	Coordinates *Koordinate*	Altitude, masl *Nadmorska visina, m*	Number of mosquitoes *Broj komaraca*
Danube / *Dunav*	9.04.2022.	1	45°13'39'' N 19°21'50'' E	75	0
Danube / *Dunav*	9.04.2022.	2	45°13'40'' N 19°21'48'' E	75,5	9
Danube / *Dunav*	9.04.2022.	3	45°13'42'' N 19°21'46'' E	76	7
Danube/ *Dunav*	9.04.2022.	4	45°13'44'' N 19°21'49'' E	76,5	2
Danube / *Dunav*	9.04.2022.	5	45°13'43'' N 19°22'11'' E	74	25
Danube / *Dunav*	9.04.2022.	6	45°13'35'' N 19°22'39'' E	77	10
Drava	2.05.2022.	1	45°35'24'' N 18°38'40'' E	84	25
Drava	2.05.2022.	2	45°35'24'' N 18°38'40'' E	84,5	41
Drava	2.05.2022.	3	45°35'25'' N 18°38'41'' E	85	8
Drava	2.05.2022.	4	45°35'25'' N 18°38'41'' E	85,5	59

All 186 hatched mosquitoes were determined to species level. All mosquitoes belong to the ecological group of floodwater mosquitoes which lay eggs on the soil surface. Two species noted were *Ae. vexans* and *Ae. sticticus*. Out of all mosquitoes, a total of 135 specimens belong to the species of *Ae. vexans*. There is a big difference in species composition in different areas of research. On the Danube area, a significantly larger share belongs to the species *Ae. sticticus* (Figure 2 and Figure 3).

As floodwater mosquitoes make big populations, according to our data we calculated number of mosquitoes per ha. The calculated number for the Danube area is 2.1 million and for the Drava area is 5,3 million specimens on researched area per ha.

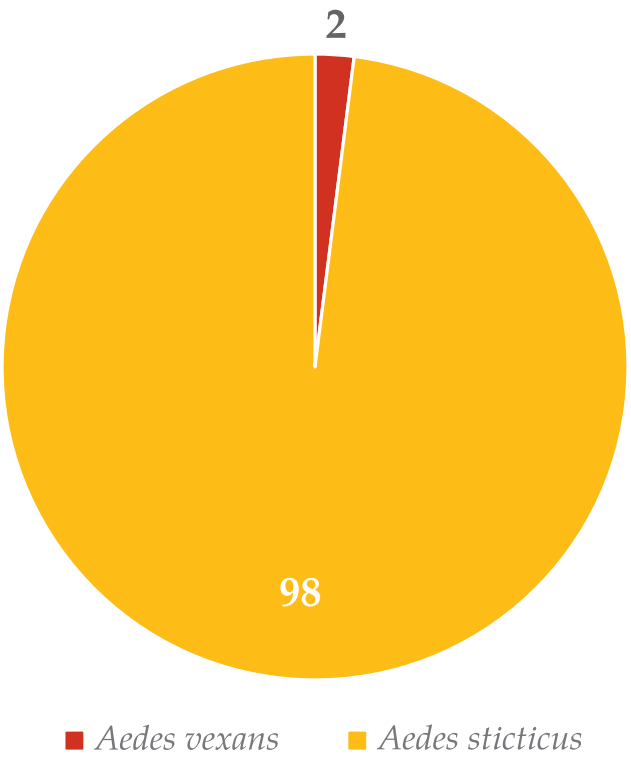

Figure 3 Composition of mosquito species from samples from Drava area
Slika 3. Sastav vrsta komaraca iz uzoraka s područja Drave

Discussion - *Rasprava*

The biggest challenge for female floodwater mosquitoes is the ability to find appropriate egg-laying places which guarantees to breed. However, these tiny insects adapted their behavior to overcome the various conditions and find appropriate breeding sites.

It was a challenge for us to find those areas and according to some experience we chose rough terrain not far from a riverbed in the Danube area and close to the dike on the Drava area. Our plan was to take soil samples before the usual water level rise in spring. As there was no rise of water level during 2021 we took samples in April and May. More mosquitoes were developed in the Drava area. A possible reason for more eggs and consequently more mosquitoes in the Drava area is the place where we took samples. There was a place close to the dike where a one-meter bigger depression occurs. This is a place where water comes first when entering inundation. Out of all 4 localities in the Drava area the best one was the highest one, where 59 mosquitoes hatched. Unlike the Drava area, the samples from the Danube were obviously taken too low, which is confirmed with the first sample, which is also the lowest, where not a single egg was sampled.

Although the idea of this work was to determine which altitude is best for laying eggs in the area of the Danube and Drava rivers, based on the results obtained, that conclusion could not be given.

In previous research, the following species were recorded among floodwater mosquitoes in the Drava area: *Ae. vexans*, *Ae. sticticus*, *Ae. cinereus* and *Ae. rossicus*. The eudominant species was *Ae. vexans* with a different proportion from year to year, ranging from 75.59% in 2004 to 92.97% in 2016 (Merdić et al. 2016; Merdić et al. 2010). Therefore, it is not surprising that in this study *Ae. vexans* was determined with a share of 98% in the Drava region.

On the other side, in the Danube area, a large proportion of *Ae. sticticus*, which is a companion species of *Ae. vexans* in the whole of Europe (Mihaly 1963), with a larger share in the northern areas (Becker et al. 2010; Schäfer and Lundström 2006), was noted. Another possible reason is that in this area the locations were slightly lower than the altitudes of the Drava River and floods at slightly lower water levels.

Both study areas are known as areas with a lot of mosquitoes. The main reason for this is the extensive area suitable for oviposition in the surroundings of Osijek city and the town of Ilok. Generally, the bigger oviposition area is around Osijek, but the capacity of breeding sites along the Drava River seems to be more suitable for oviposition for floodwater mosquitoes. This is the reason why Osijek is the city with the biggest abundance of mosquitoes in Croatia.

Conclusion - *Zaključak*

Based on this research it can be concluded that we found suitable places for the oviposition of floodwater mosquitoes. The appropriate place for oviposition is the area not far from the river basin where water appears when the higher water level of lowland rivers Danube and Drava occurs. The inundation area of the river of Drava is more appropriate for the oviposition of floodwater mosquitoes than the researched area on the Danube River. Based on the results it seems that the main reason is that the altitude differences are too small to demonstrate variations.

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