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CONTRIBUTION TO THE MOSQUITO FAUNA (DIPTERA, CULICIDAE) OF LIKA, CENTRAL CROATIA, WITH SPECIAL REFERENCE TO INVASIVE SPECIES

Nataša Bušić, Matej Modrušan, Helena Vilc & Enrih Merdić*

Josip Juraj Strossmayer University of Osijek, Department of biology, Cara Hadrijana 8/A, 31000 Osijek, Croatia

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This study of mosquitoes in Lika, geographically, geologically and climatically a very specific region, was conducted to gain insight into the composition and relative abundance of the mosquito fauna, as well as to check for the possible presence of invasive species. Sampling took place from July to September 2020. Mosquito larvae were captured from medium and small breeding sites using a 25 cm diameter net and a plastic dipper. Adult specimens were sampled in both urban and rural areas using CO_2 baited CDC traps, CO_2 baited BG Sentinel traps with BG Lure and human landing catch. The study was conducted at 69 sites. We collected 5,126 specimens (100 adults and 5,026 larvae) and demonstrated the presence of 16 mosquito species. CDC traps were almost five times more effective (quantity and quality) than BG Sentinel traps. The most common mosquito taxa caught in Lika were *Culex pipiens* complex (78.36% of all larvae) and *Ae. geniculatus* (30.26% of all adults). Both invasive mosquito species present in Croatia, *Aedes albopictus* and *Ae. japonicus*, were recorded, with *Ae. albopictus* being detected in Lika for the first time. *Aedes japonicus* was widespread, recorded in 23 localities. According to molecular analysis, only *An. maculipennis* s.s. was confirmed in the *An. maculipennis* complex. Within the *Cx. pipiens* complex, both *Cx. pipiens* biotypes, *pipiens* and *molestus* were confirmed together with one single hybrid specimen. Analysis of mosquito occurrence with regard to altitude shows that most samples and species were collected between 601 and 700 m a.s.l., although the Shannon evenness index and Hill's index show the highest value in the range of 901 to 1140 m a.s.l. In this systematic study on the mosquito fauna in Lika, a significant species diversity, including invasive species, was found.

Key words: mosquitoes, fauna, invasive species, altitude dispersal, Lika

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Istraživanje komaraca u Lici, geografski, geološki i klimatski vrlo specifičnoj regiji, provedeno je kako bi se stekao uvid u faunu i brojnost komaraca, kao i da bi se utvrdila moguća prisutnost invazivnih vrsta. Uzorkovanje se odvijalo od srpnja do rujna 2020. godine. Ličinke komaraca uzorkovane su u srednjim i malim leglima mrežicom promjera 25 cm i plastičnim posudicama. Uzorkovanje odraslih komaraca u urbanim i ruralnim područjima obavljeno je koristeći CDC klopke uz suhi led kao atraktant, BG Sentinel klopke uz BG Lure i suhi led kao atraktant i metodom čovjek-aspirator. Istraživanje je provedeno na 69 postaja. Uzorkovano je 5126 jedinki (100 odraslih i 5026 ličinki) i utvrđena prisutnost 16 vrsta komaraca. CDC klopke bile su gotovo pet puta kvantitativno učinkovitije od BG Sentinel klopki. Najrasprostranjenija i najbrojnija vrsta komaraca uzorkovana u Lici bila

* corresponding author: enrih@biologija.unios.hr

je *Culex pipiens* kompleks (78,36% svih ličinki) i *Ae. geniculatus* (30,26% svih odraslih). Zabilježene su obje invazivne vrste komaraca prisutne u Hrvatskoj, *Aedes albopictus* i *Ae. japonicus*, s tim da je *Ae. albopictus* prvi put zabilježen u Lici. *Ae. japonicus* je široko rasprostranjen, zabilježen na 23 postaje. Prema molekularnoj analizi, unutar kompleksa *An. maculipennis* potvrđena je prisutnost samo *An. maculipennis* s. s. Unutar kompleksa *Cx. pipiens*, potvrđena su oba biotipa, *Cx. pipiens pipiens* i *Cx. pipiens molestus* te jedna hibridna jedinka. Analiza pojave komaraca s obzirom na nadmorsku visinu pokazuje da je većina uzoraka i vrsta prikupljena na nadmorskoj visini od 601 do 700 m, iako Shannonov indeks ravnomjernosti i Hillov indeks pokazuju najveću vrijednost na nadmorskoj visini 901 do 1140 m. Ovim sustavnim istraživanjem faune komaraca u Lici utvrđena je značajna raznolikost vrsta, uključujući i invazivne vrste.

Ključne riječi: komarci, fauna, invazivne vrste, rasprostranjenost na nadmorskim visinama, Lika

INTRODUCTION

Lika is a geographical region in southwestern Croatia. It forms a plateau between Velebit Mountain to the west and south, Lička Plješivica Mountain to the east and Kapela Mountain to the northwest. The northern border is rather vague because the Ogulin-Plašćanska valley forms a transitional area between Lika and Gorski Kotar. Lika is specific for its karst relief, formed of permeable rocks mainly composed of limestone and dolomite. The climate is continental to mountainous, resultant upon Velebit being a barrier.

Because of the permeable substrate (karstic), water does not stay on the surface for long, and there are no significant stagnant water bodies. Nevertheless, due to the high amount of precipitation, there are many potential habitats for the aquatic development of mosquitoes, since water may accumulate in plenty of man-made constructions and containers such as canals, pools, rainwater barrels, buckets, machinery, tyres, etc.

Information on the geographical distribution of mosquitoes is very important for mosquito control and the management of mosquito-borne diseases (Gimnig *et al.*, 2005; Palaniyandi, 2014). The abundance of mosquitoes depends primarily on biotic factors, but abiotic factors related to climate and landscape also play a significant role (Honghoh *et al.*, 2012). To better understand all changes occurring globally, data on the occurrence of mosquito species are needed, especially of invasive species. Although mosquitoes are vectors of pathogens of various diseases, not a single case of vector-borne disease has recently been reported from the Lika area. This could be a reason why this area has not been of particular interest to researchers.

Mosquitoes are well studied in many areas of Croatia (Merdić *et al.*, 2020a). So far, 52 mosquito species have been recorded in Croatia (Merdić *et al.*, 2020b), two of which are the invasive species *Aedes albopictus* and *Aedes japonicus*. Since their first detection (Klobučar *et al.*, 2006, 2014), these two species have spread to most parts of Croatia (Capak *et al.*, 2017; Janssen *et al.*, 2020). Due to the small population and relatively low numbers, the mosquitoes of Lika have been discussed in only a few papers (Adamović & Paulus, 1985.; Vignjević, 2014.; Merdić *et al.*, 2018; Janssen *et al.*, 2020; Bušić *et al.*, 2021), in which 11 species were recorded.

Accurate identification of mosquitoes is critical to establish effective mosquito control programmes and strategies. Identification is commonly based on morphological determination of females and fourth-stage larvae, but this is not possible if parts of the mosquito needed for determination are damaged or missing (Hebert *et al.*, 2003). Moreover, mosquitoes very often occur as complexes of species, the members of

which are difficult to distinguish morphologically, or the differences required for determination are limited to a certain sex or life stage of a particular species (Khrabrova *et al.*, 2013). Therefore, morphological determination must often be supplemented by molecular determination.

Despite the low health-related impact of mosquitoes in Lika and the relatively low abundance, mosquito research in this area may be interesting from the point of view of biodiversity in a karst area and the spread of invasive species.

STUDY AREA

According to the climate classification of Koppen, the area of Lika belongs to the climate class "Cfsbx" (C – temperate rainy climate, fs – no dry periods and the highest monthly precipitation in the cold part of the year, b – the warmest months of the year have an average temperature of less than 22°C, x – two maxima in the annual course of precipitation in autumn and winter/spring), while the mountain peaks (above 1200 m a.s.l.) belong to the class "Dfsbx" (D – snow-forest climate). Due to the strong orographic indentation of the Lika area, mean annual air temperatures range from 5°C to 9°C (in winter (January) from –4°C to 0°C and in summer (July) from 15°C to 20°C). In terms of the annual pattern of monthly precipitation, the Lika region belongs to the maritime type. The average precipitation ranges from 1200 to 1800 mm, with more precipitation in the cold part of the year. The maximum occurs in November and the minimum in July. A snow cover of at least 30 cm remains on the ground on the Lika plateau for 18 days in January.

MATERIAL AND METHODS

The sampling took place from July to September 2020 in the Lika region. In Fig. 1, all sites covered by this survey are presented according to sampling method: CDC traps (ten sites), BG-Sentinel traps (five sites), larval sampling (53 sites) and human landing catch (one site). All sampled sites were georeferenced using the mobile application GPS Essentials. Altitude of sampling sites ranged from 413 to 1139 m.

Mosquito collection

During this study, both larval and adult mosquitoes were collected. Larvae were sampled from medium-sized breeding sites (1 – 100 m^2) with a 25 cm diameter net and from smaller water bodies ($\leq$ 1 m^2), such as used car tyres, barrels, buckets, vases, cans, tubs, and tree holes with plastic dippers. Adult specimens were caught in both urban and forested areas using a variety of methods: CDC traps baited with CO_2, BG-Sentinel traps (BGS) baited with CO_2 and BG Lure, and human landing catch. CDC and BGS traps were operated three times during the season (Tab. 1). Traps were set at dusk, collected in the morning and run for approximately 12 hours. The areas where the CDC traps were set were carefully selected for microclimatic conditions suitable for mosquitoes, such as shaded places with vegetation. BGS traps were set attyre repair shops . Human landing catch was performed only once at dusk during the high activity period of mosquitoes. The sampling methods are described in detail in Merdić *et al.* (2020a).

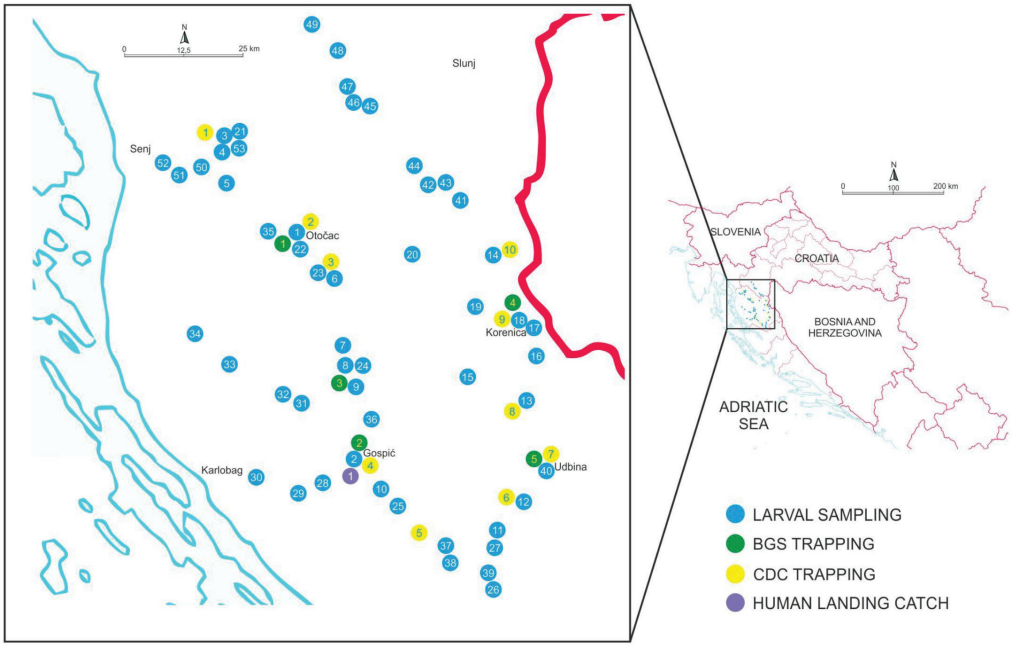


Fig. 1. Mosquito sampling sites in Lika

Legend: Larval sampling: 1. Otočac, 2. Gospić, 3. Brinje, 4. Brinje 2, 5 jezero Gušić, 6 Ličko Lešće, 7. Ličko Lešće, 8. Kvarte, 9. Perušić, 10. Bilaj, 11. Gornja ploča, 12. Kurjak, 13. Pećane, 14. Jezerce, 15. Bunić, 16. Bijelo Polje, 17. Korenica, 18. Korenica, 19. Humoljac, 20. Donji Babin Potok, 21. Brinje, 22. Otočac, 23. Ličko Lešće, 24. Kvarte, 25. Lički Ribnik, 26. Sveti Rok, 27. Lovinac, 28. Trnovačko Novoselo, 29. Brušane, 30. Baške Oštarije, 31. Vranovine, 32. Aleksinica, 33. Pazariški Bakovac, 34. Sj. Velebit, 35. Otočac, 36. Lički Osik, 37. Papuča, 38. Raduč, 39. Sv Rok (ili Jurjevići?), 40. Udbina, 41. Poljanak, 42. Kuselj, 43. Kuselj, 44. Saborsko, 45. Lapat, 46. Lapat, 47. Plaški, 48. Vojnovac, 49. Josipdol, 50. Žuta Lokva, 51. Melnice, 52. Vratnik, 53. Brinje

BG Sentinel trapping: 1. Otočac, 2. Perušić, 3. Gospić, 4. Udbina, 5. Korenica

CDC trapping: 1. Brinje, 2. Otočac 3. Ličko Lešće, 4. Gospić, 5. Medak, 6. Kurjak, 7. Udbina 8. Pećane, 9. Korenica, 10. Jezerce

Human landing catch: 1. Gospić

All sampled adult mosquitoes were killed by freezing at temperatures below 0 °C or with cigarette smoke and mounted on entomological pins. The sampled larvae were preserved in 96% alcohol for molecular analysis.

Mosquito identification

All mosquitoes were morphologically identified according to Gutshevich *et al.* (1974) and Becker *et al.* (2010). Cryptic species within the *Anopheles maculipennis* complex were identified using molecular methods based on polymerase chain reaction of ribosomal DNA according to Di Luca *et al.* (2014). Following Smith & Fonseca (2004) and Bahnk & Fonseca (2006), the *Culex pipiens* complex was analysed for occurrence of *Cx. torrentium* and the *pipiens/molestus* biotypes of *Cx. pipiens* s.s. Three specimens of that complex were taken from each locality for molecular analysis, if there was more than one. All mounted mosquitoes are kept in the entomological collection of the Department of Biology, Josip Juraj Strossmayer University in Osijek.

Tab. 1. Total number of individuals collected per locality by different methods during three samplings

date	8–10 July	22–25 July	2–5 Sept.	
locality	number of individuals			method
Brinje	0	0	1	CDC trap*
Otočac	1	2	0	CDC trap
Ličko Lešće	0	4	1	CDC trap
Medak	8	0	2	CDC trap
Gospić	1	19	6	CDC trap
Pećane	1	2	0	CDC trap
Korenica	1	2	0	CDC trap
Jezerce	1	8	11	CDC trap
Kurjak	0	0	1	CDC trap
Udbina	2	0	2	CDC trap
Gospić	-	-	9	HLC**
Otočac	1	0	4	BGS trap***
Gospić	0	0	0	BGS trap
Perušić	4	1	5	BGS trap
Korenica	0	0	0	BGS trap
Udbina	0	0	0	BGS trap

***CDC –** CDC trap baited with CO_2

****BGS –** BG-Sentinel trap baited with CO_2 and BG lure

*****HLC –** Human lending catch

Statistical analysis

The analysis was performed using ComEcoPaC (version 1, Drozd, 2010). Species diversity was analysed using the Shannon evenness index (E) and Hill's index (N2). The Shannon evenness index (E=H'/Hmax) takes values between 0 and 1, where a value closer to '0' represents lower evenness (the dominance of one species) and a value closer to '1' represents full evenness (an even abundance of species). The value of t Hill's index (inverse Simpson index) starts with 1 as the lowest possible value. The higher the value of this index, the greater the diversity. To compare the similarity of the samples, Jaccard's similarity index (Ja) was used.

RESULTS

The total number of mosquitoes sampled in the study was 5,126. One hundred of these were caught as adults, using CDC traps (76 specimens), BGS traps (15 specimens) and human landing catch (9 specimens). All other individuals were collected from different water bodies at the larval stage. A total of 16 species within 5 genera were recorded (*Culex* – 4 species; *Aedes* – 6 species; *Anopheles* – 3 species; *Culiseta* – 2 species; *Coquillettidia* – 1 species). The collected taxa are: *Cx. pipiens* s.s. (biotype *pipiens* and biotype *molestus*), *Cx. torrentium*, *Cx. hortensis*, *Cx. territans*, *Ae. japonicus*, *Ae. albopictus*, *Ae. vexans*, *Ae. geniculatus*, *Ae. sticticus*, *Ae. cantans*, *An. maculipennis* s.s., *An. claviger*, *An. plumbeus*, *Cs. annulata*, *Cs. longiareolata* and *Cq. richiardii*.

In all three CDC trap samplings, the number of mosquitoes ranged from 0 to 19 per site. The highest number of mosquitoes was caught in July. Two localities stand out for the large number of mosquitoes: Gospić with 26 and Jezerce with 20 mosquitoes. At other localities, significantly fewer or no mosquitoes were caught (Tab. 1). Eudominant species were *Ae. geniculatus* (30.26%), collected at localities 4, 5, 7 and 10 (below, localities are marked with numbers according to the map in Fig. 1) and *Ae. sticticus* (27.63%), caught at localities 2, 3, 4, 5 and 8. The dominant species were *Ae. vexans* (14.47%), sampled at localities 2, 3, 4, 6 and 10 and *An. plumbeus* (10.52%), sampled at localities 4 and 10. Other species accounted for 17.10% of the mosquitoes collected. Among them, members of the *Cx. pipiens* complex were sampled at localities 1, 7 and 9, members of the *An. maculipennis* complex at localities 3, 5 and 8, two individuals of *Cs. longiareolata* at locality 9 and one individual of *Cq. richiardii* and *Oc. cantans*, each, at locality 4, and one individual of *Cs. annulata* at locality 10.

Significantly fewer mosquitoes were caught with BGS traps, almost five times fewer than with CDC traps. BGS traps also captured a lower number of species (Tab. 1). In these traps, the *Cx. pipiens* complex was eudominant with a proportion of 86.66% sampled at localities 1 and 3, further individuals were *Ae. geniculatus* sampled at location 1. Using human landing catch in Gospić (Fig. 1, locality 1 – purple dot), seven species were recorded: *An. plumbeus*, *Ae. japonicus*, *Ae. vexans*, *Ae. geniculatus*, *Ae. sticticus*, *Ae. cantans* and *Cq. richiardii*.

Most mosquito samples were collected as larvae in urban (backyards) and rural areas. The largest number of mosquitoes was caught in late July. Overall, the largest proportion belongs to the *Cx. pipiens* complex, which was eudominant here with a proportion of 78.36%, and breeding sites of this species were also the most numerous. Dominant species were the invasive *Ae. japonicus* (9.74%) and *Cs. longiareolata* (5.73%). All other species together accounted for 5.15% of the larvae collected.

The overview of the recorded species by localities is as follows: species of the *An. maculipennis* complex at localities 6, 12, 24, 25, 36, 47; *An. claviger* at localities 6, 23 ,25; *An. plumbeus* at localities 18, 34, 48, 52; *Ae. japonicus* at localities 1, 4, 6, 7, 9, 14, 22, 35, 37, 38, 40–45, 47–52; *Ae. albopictus* at locality 48, *Ae. geniculatus* at localities 22, 34. *Cx. torrentium* at localities 24–26, 28, 30, 34, 37, 40, 41, 43, 45 and 52; *Cx. hortensis* at localities 9, 29, 30, 42, 46 and 52; *Cx. territans* at localities 9, 23, 25, 29 and 4, *Cs. longiareolata* at localities 2, 4, 6, 9, 12, 13, 16, 22, 23, 27, 28, 33, 39, 40, 43, 44, 49, 51 and 52; *Cs. annulata* at locality 13. The most numerous *Cx. pipiens* complex was sampled at all localities except 24–26, 28, 30, 34, 37, 40, 41, 43, 45, and 52 (details below).

Within the *An. maculipennis* complex, only *An. maculipennis* s.s. individuals were confirmed, all of them occurring in the altitudinal range from 413 to 746 m a.s.l. (as adults at localities 3, 5, 8 and as larvae at localities 6, 23, 25). Within the *Cx. pipiens* complex, both biotypes, *pipiens* (n = 22) and *molestus* (n=1), were identified together with one hybrid individual. *Cx. pipiens* biotype *pipiens* was detected at elevations from 401 to 500 m a.s.l. with eight larvae (sites 6, 7, 23, 46), from 501 to 600 m a.s.l. with four larvae (sites 10, 21,36), and from 601 to 700 m a.s.l. with nine larvae (sites 11, 13, 16, 31, 32, 38) and at 783 m a.s.l. with one larva (site 19). The single *Cx. pipiens* biotype *molestus* larva was detected at an altitude of 758 m a.s.l. (site 39) and the hybrid larva at an altitude of 613 m a.s.l. (site 20).

Both invasive mosquito species present in Croatia, *Ae. albopictus* and *Ae. japonicus*, were recorded in this study, *Ae. albopictus* for the first time in Lika. The only individ-

ual was found in the village of Vojnovac (Fig. 2), in a used tyre at an altitude of 432 m a.s.l. By contrast, *Ae. japonicus* was recorded with numerous specimens at an altitudinal range from 493 to 806 m a.s.l. Its numbers were highest in the northern part of Lika, along the border with Gorski Kotar (Fig. 2). *Ae. japonicus* was found at 22 out of 52 sites, representing 42.3% of all sites surveyed. The breeding site with the highest numbers of collected specimens (n=77) was located in Žuta lokva (site 50) at an altitude of 533 m a.s.l. Depending on the type of habitat, most of the sampled breeding sites were tyres, followed by barrels, buckets and others. Mostly, individuals of *Ae. japonicus* were found together with species of the genus *Culex*, less frequently with species of the genera *Culiseta, Anopheles* and *Aedes* (Tab. 2). In 20 of 23 localities, *Ae. japonicus* was found together with other species, while it was detected alone at only three localities.

Analysis of elevation shows that the largest numbers of specimens and species (n=14) were collected in an altitude range from 601 to 700 m a.s.l., and the largest number of sampling sites was also located in this altitudinal range (most of the plateau). The fewest specimens were collected in an altitude range of 901 to 1140 m a.s.l., which was where the fewest sampling sites were located (Tab. 3). By contrast, the Shannon evenness index and Hill's index show the highest value in this altitudinal

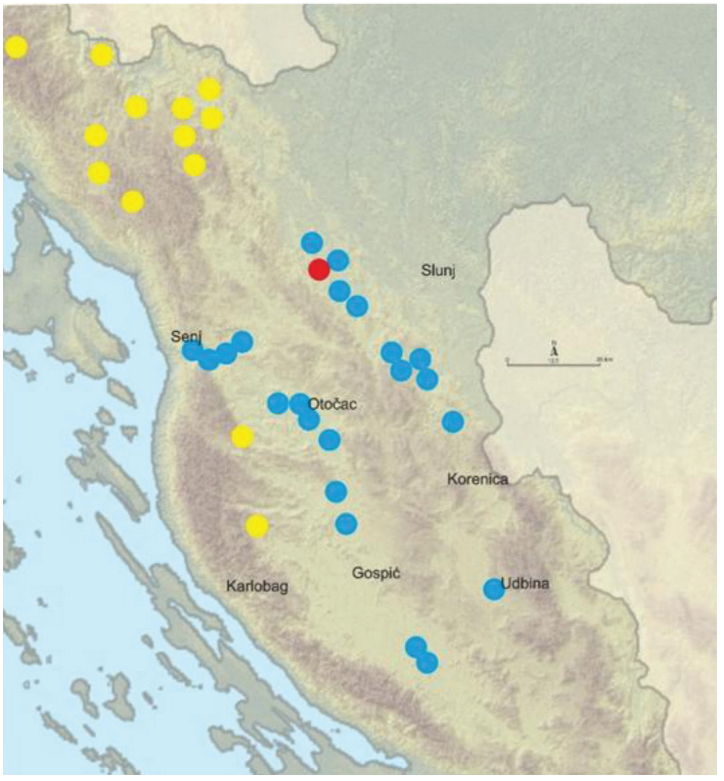


Fig. 2. Distribution of invasive mosquito species of *Ae. albopictus* (red dot) and *Ae. japonicus* (blue dot) in Lika. Yellow dots show the distribution of *Ae. japonicus* according to a previous study (Janssen *et al*., 2020).

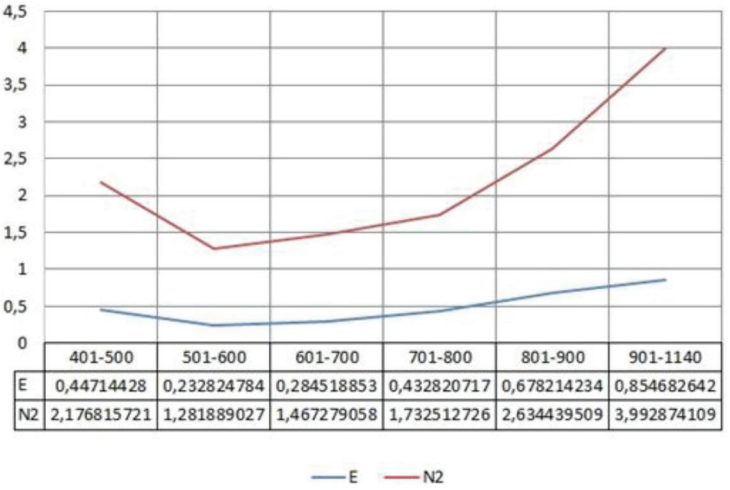
Tab. 2. Number of sites and different habitat types cohabitated by *Aedes japonicus* and other mosquito species

habitat type	barrel	tyre	bathtub	cemetery vase	bucket
species	number of sites				
An. maculipennis s.s.	1				
An. claviger	1				
An. plumbeus		1			
Ae. albopictus		1			
Ae. geniculatus		1			
Cx. pipiens complex	3	7	1	1	4
Cx. torrentium	2	1	1		2
Cx. hortensis	1	1			1
Cx. territans		1			
Cs. longiareolata	5	4	1		

Tab. 3. Total number of specimens and species collected by altitude along with sampling site

species/altitudinal range (m a.s.l.)	401–500	501–600	601–700	701–800	801–900	901–1140	total
An. maculipennis complex	16	33	6	25	0	0	80
An. maculipennis s.s.	9	10	7	5	0	0	31
An. claviger	20	1	0	0	0	0	21
An. plumbeus	1	0	11	4	0	2	18
Ae. japonicus	170	111	65	67	78	0	491
Ae. albopictus	1	0	0	0	0	0	1
Ae. vexans	2	1	6	3	0	0	12
Ae. geniculatus	2	4	6	13	2	2	29
Ae. sticticus	1	9	14	0	0	0	24
Ae. cantans	0	0	2	0	0	0	2
Cx. pipiens complex	474	1547	1537	420	16	12	4006
Cx. torrentium	5	4	13	8	2	3	35
Cx. hortensis	3	0	16	0	9	8	36
Cx. territans	29	15	2	0	0	0	46
Cs. annulata	0	0	1	1	0	0	2
Cs. longiareolata	13	22	190	16	35	14	290
Cq. richiardii	0	0	2	0	0	0	2
number of sampling sites	16	14	23	9	4	3	69
number of specimens	746	1757	1878	562	142	41	5126
number of species	13	10	15	10	6	6	16

range (Fig. 3), where the number of eudominant species was three, while the total number of species was six. The lowest values of the diversity index were obtained in an altitudinal range from 501 to 600 m a.s.l. Accordingly the number of eudominant species was equal to one, and the total number of species was ten. With altitude increasing, the diversity indices also increased, i.e. the dominance of a single species



	401-500	501-600	601-700	701-800	801-900	901-1140
E	0,44714428	0,232824784	0,284518853	0,432820717	0,678214234	0,854682642
N2	2,176815721	1,281889027	1,467279058	1,732512726	2,634439509	3,992874109

Fig. 3. Shannon evenness index (E-blue line) and Hill's index (N2-red line) as a function of altitude

decreased. The only exception was at heights from 401 to 500 m a.s.l. (Fig. 3). According to Jaccard's similarity index, the highest similarity between localities could be found in the altitudinal ranges from 401 to 500 and 501 to 600 m a.s.l. (Ja = 0.7692), and in the altitude ranges from 801 to 900 and 901 to 1140 m a.s.l. (Ja= 0.7142). The least similarity was found in the elevation range of 501 to 600 and 901 to 1140 m a.s.l. (Ja = 0.3333).

DISCUSSION

Due to the geomorphological and hydrological characteristics, the abundance of mosquitoes in Lika is low. Only a very small number of adult mosquitoes were sampled as compared to larvae (100 out of 5,126 individuals). The larvae were found generally in small to medium sized breeding sites. In addition, these breeding sites were usually located near houses and of were of an artificial nature: barrels, buckets, old tyres dumped in yards, plus gutters and various kinds of debris that retained water and became suitable habitats for mosquito development. In such breeding sites, the house mosquito *Cx. pipiens* s. l. was primarily sampled, in addition to other species that tolerate extremely cold winters, low water levels and higher elevations.

Sixteen mosquito species were recorded in this study. Another two species (*An. messeae* and *An. daciae*) had been recorded in previous studies (Adamović & Paulus, 1985; Vignjević, 2014; Bušić *et al*., 2021), making a total of 18 species recorded in Lika. This number represents 34.6% of the species known for the Croatian mosquito fauna. This is not a big ratio for such a large area, but considering the particular characteristics and altitude of the Lika region, the number of species is significant.

The fauna of the subfamily *Anophelinae* had previously been studied in this area, with four species of the genus *Anopheles* recorded: *An. claviger*, *An. maculipennis* s.s., *An. messeae* and *An. daciae* (Adamović & Paulus, 1985; Vignjević, 2014; Bušić *et al*., 2021). *An. claviger* had been found in an altitudinal range from 401 to 500 m a.s.l. (Adamović & Paulus, 1985), as in our study, with the exception of one individual

recorded at an altitude of 564 m a.s.l. It is interesting that larvae of this species were sampled on the margins of the Gacka and Lika rivers, i.e. in slowly flowing water. In our study, the most numerous species within the genus *Anopheles* was *An. maculipennis* s.s. A possible reason for not finding *An. messeae* is that this species prefers larger aquatic habitats, especially floodplains of rivers (Becker *et al.*, 2010), of which there are very few in the Lika region. By contrast, *An. maculipennis* s.s. tends to prefer smaller water bodies, which are more frequently represented in the study area. In addition, the elevation of the study area was above 400 m a.s.l., indicating that the species *An. maculipennis* s.s. is better adapted to higher altitudes than *An. messeae*. These data agree with the data of previous studies from other areas, where *An. messeae* was not found above 200 m a.s.l., in contrast to individuals of *An. maculipennis* s.s. and individuals of *An. daciae* (Kronefeld *et al.*, 2014; Vignjević, 2014; Bušić *et al.*, 2021).

Another species within the subfamily *Anophelina*e not previously recorded in Lika is *An. plumbeus*, which was sampled mainly in tyres together with larvae of the *Cx. pipiens* complex, *Ae. geniculatus* and once with *Ae. albopictus*, as well as in barrels with several other species (*Cx. torrentium, Cx. hortensis, Cs. longiareolata*). It should be emphasized that all habitats were close to the forest, which *An. plumbeus* prefers (Gutsevich et al., 1974; Becker et al., 2010).

So far, eight species (*Cx. pipiens* s.s., *Cx. torrentium*, *Cx. hortensis*, *Ae. japonicus*, *Ae. geniculatus*, *Ae. cantans*, *Cs. annulata, Cs. longiareolata*) have been recorded as belonging to the fauna of the *Culicinae* of Lika (Bušić *et al.*, 2021), which were also recorded in this study, and another four species (*Cx. territans*, *Ae. sticticus*, *Ae. vexans*, *Ae. albopictus*) have been recorded for the first time in Lika in this study.

In the last few years, the invasive Asian tiger mosquito *Ae. albopictus* and the Asian rock pool mosquito *Ae. japonicus* have spread throughout Europe, including Croatia (Caminade *et al.*, 2012; Medlock *et al.*, 2012; Klobučar *et al.*, 2019; Koban *et al.*, 2019; Janssen *et al.*, 2020). *Ae. albopictus* has been detected in all counties of Croatia (Capak *et al.*, 2017), while this is the first finding for the geographical region of Lika. During a study conducted in this area in 2017, not a single individual of *Ae. albopictus* was recorded (Bušić *et al.*, 2021). On the other hand, two individuals of *Ae. japonicus* were detected in this area in the locality of Baške Oštarije and Kuterevo (Janssen *et al.*, 2020), while the present study recorded a notable increase in the area occupied by this species. It can be assumed that *Ae. japonicus* has spread from the area of Gorski Kotar to the area of Lika since 2017, as indicated by the largest number of sampled individuals found directly on the border with Gorski Kotar. As in previous studies (Kaufman & Fonseca, 2014; Cunze *et al.*, 2016; Zielke *et al.*, 2016; Montarsi *et al.*, 2019; Bušić *et al.*, 2021), our results confirm the good adaptation of this species to higher altitudes and colder temperatures as well as the possibility of cohabitation with other species, especially those of the genus *Culex*.

The study area extends mainly at higher altitudes (around 600 m a.s.l.), which could be one of the reasons why we recorded the largest number of localities and species, as well as a large number of different habitats, in these altitudinal range from 601 to 700 m a.s.l. Although altitude is a natural barrier, mosquitoes were also found in a range from 901 to 1140 m a.s.l., as in other recent studies (Muja-Bajraktari *et al.*, 2019; Bušić *et al.*, 2021). The Shannon evenness index and Hill's index showed a high value in the altitudinal range from 901 to 1140 m a.s.l., indicating a more uniform abundance of species.

Since the fauna of Lika has only been sporadically examined so far, this is the first systematic study of the fauna of mosquitoes of Lika. Of course, it should be noted that this study probably did not cover all species inhabiting this area, which gives space for further research. Based on the observed spread of the invasive species *Ae. japonicus* in this area since 2017 and the recording of the invasive species *Ae. albopictus*, it is reasonable to assume that the spread will continue and to suggest that it should be monitored.

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