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ARTHROPODS AND MEDICAL ENTOMOLOGY - ORIGINAL PAPER

Composition, seasonal abundance, and public health importance of mosquito species in the regional unit of Thessaloniki, Northern Greece

Christos G. Spanoudis[1] · Christos S. Pappas[1] · Matilda Savopoulou-Soultani[1] · Stefanos S. Andreadis[2]

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Abstract

Mosquitoes (Diptera: Culicidae) are the largest group of blood-feeding insects that disturb not only humans but also other mammals and birds. This study reports the presence of native mosquito species in the regional unit of Thessaloniki and the monitoring of their population. In total, 13 mosquito species belonging to four genera were identified. The most dominant species was *Culex pipiens*, followed by *Aedes caspius*. In the present study, we report for the first time the presence of *Ae. vittatus* in Greece and of *Anopheles plumbeus* in the regional unit of Thessaloniki. Regarding the seasonal variation, species of the genus *Aedes* were the ones that first appeared in late March, followed by *Culex* species at the end of April and finally species of the genus *Anopheles* in July. Species of the *Aedes* genus were found to be the most abundant in the first quarter of the year (late March to early April). Population of *Cx. pipiens* remained at high levels from late April to late September. Species of the genus *Anopheles* were found in high densities from early August to October. The current study contributes to the knowledge of the mosquito species composition and their relative abundance in an area where West Nile virus caused severe epidemic outbreaks.

Keywords Mosquito species · Seasonal abundance · Public health importance · *Aedes vittatus* · *Culex pipiens* · Greece

Introduction

The scourge of mosquitoes exists in Greece from early times as illustrated by various historical references (Bruce-Chwatt and Zuluetta 1980). The first attempts to record native mosquito species in Greece were initiated at the beginning of the twentieth century right after the identification of the role of *Anopheles* mosquitoes in malaria transmission. Cardamatis (1931) was the first researcher who recorded mosquito species throughout Greece, and his catalogue included 19 species. Pandazis' catalogue (1935) presented 38 species belonging to 7 genera. A subsequent record was the one belonging to Sakelariou and Lane (1977) which included 38 species. In 1993, Samanidou-Voyadjoglou and Dorsie published a catalogue based mainly on previous bibliographical references which contained 7 genera, 15 subgenera, 53 species, and 2 subspecies. The most recent catalogue of the native mosquito species in Greece was published by Voyadjoglou-Samanidou (2011), including 64 species belonging to 8 genera.

The identification of mosquito species is a key factor for successful mosquito control program. Despite the wide range of taxonomic catalogues in Europe, the taxonomy of European mosquitoes still requires further investigation. Many questions arise concerning the formal status of various nominal species and subspecies, while the entire mosquito fauna is generally poorly examined. Although there are various studies on the mosquito fauna of certain countries, the vast majority of them are not up to date and lack a broader scope. Like most European countries, the catalogue of mosquito species in Greece is not investigated in depth. Studies on the mosquito fauna in different regions of Greece during the last four decades have primarily been focused on anopheline mosquitoes due to the severe malaria outbreak

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✉ Stefanos S. Andreadis
stefandr@ipgrb.gr

[1] Laboratory of Applied Zoology and Parasitology, Faculty of Agriculture, Forestry and Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

[2] Hellenic Agricultural Organization Dimitra, Institute of Plant Breeding and Genetic Resources, 57001 Thermi, Greece

recorded at that time (Samanidou-Voyadjoglou and Darsie 1993) and secondarily on new distribution of mosquito species in certain area (Kaiser et al. 2001; ECDC 2014; Kioulos et al. 2014; Lytra and Emmanouel 2014; Beleri et al. 2017). The main reason why the investigation of native mosquito fauna has regained substantial scientific interest in recent years is basically attributed to the epidemic incidents of mosquito-borne viruses, such as West Nile Virus, which have sporadically resulted in human casualties, as well as to the ongoing threat of malaria reintroduction (ECDC 2010; Patsoula et al. 2016; NPHO 2019). The aim of the present study was (1) to inventory the native mosquito fauna of the regional unit (RU) of Thessaloniki, (2) to report the seasonal variation of species, and (3) to determine species imposing risk to public health.

Materials and methods

Study area

The RU of Thessaloniki is one of the biggest administrative areas of Greece, with a total area of 3,683 km^2 and belongs to the Central Macedonia Region. The RU is divided into 13 municipalities and its landscape is dominated by extensive rice fields and farmlands throughout the west and northwest and mountainous areas in the north and northeast. Climate is mainly Mediterranean in the southern part with hot summers and cool to mild winters, while in the northern part, the climate is more continental with colder winters. Thessaloniki, the capital city of the RU, is the second largest city in Greece, with over one million inhabitants in its metropolitan area, located on the Thermaic Gulf, at the northwest corner of the Aegean Sea.

The RU of Thessaloniki suffers from high mosquito populations every summer causing significant nuisance to inhabitants and visitors. It is one of the areas mostly affected by the recent outbreaks of West Nile Fever (WNF) in Greece (NPHO 2010; Patsoula et al. 2016; NPHO 2019). Larval collection took place from April to October through the years 2012 and 2013. In total, 11 sampling stations that covered most of the representative sites of the Thessaloniki RU were established (Metropolitan area, Kalamaria, Polichni, Thermi, Epanomi, Kalochori, Pentalofos, Chalastra, Axios, Lagadas, and Volvi) (Fig. 1). Various types of larval habitats were sampled, either permanent or temporary, e.g., rice fields, river margins, lake margins, ponds, rain water craters, marsh, and irrigation canals (Table 1).

Fig. 1 Satellite photo of the area under study. Sampling sites in the RU of Thessaloniki are pointed with a green icon (Google Earth © 2021 Maxar Technologies). In the smaller map of Greece, the RU of Thessaloniki is marked with red color (copyrights Wikipedia)

Table 1 Data of sampling sites in the RU of Thessaloniki

Location	Longitude	Latitude	Altitude (m)	Type of habitat	Habitat details
Axios	40.64533451	22.70017782	5	River margin	Slow running water/permanent
Chalastra	40.62512986	22.74377243	4	Rice field	Standing water/temporary
Epanomi	40.38897761	22.90313799	0	Marsh	Standing water/permanent
Kalamaria	40.56872186	22.95475178	1	Rain water crater	Standing water/temporary
Kalochori/Sindos	40.63427020	22.84847445	-6	Rain water crater	Standing water/temporary
Lagkadas	40.69900886	23.16702453	72	Lake margin	Slow running water/permanent
Pentalofos	40.73872974	22.83227256	28	River margin	Standing water/permanent
Polichni	40.66327223	22.95204706	117	Pond	Standing water/artificial
Thermi	40.53688651	22.98774062	4	Pond	Standing water/temporary
Metropolitan area	40.63179134	22.95840531	34	Pond	Standing water/temporary
Volvi	40.69221780	23.42149879	36	Lake margin	Slow running water/permanent

Specimen and data collection

In the present study, larval collection was carried out using the traditional dipping method (Silver 2007) with a dipper with extendable handle (volume: 300 mL) (BioQuip Products, CA, USA). Each sample consisted of the content of 5 consecutive dipper sinks in random spots, and a total of approximately 1,500 mL water volume was placed in plastic cups (8 cm in diameter and 14 cm in height), which were afterwards carried to the Laboratory of Applied Zoology and Parasitology of the Aristotle University of Thessaloniki within 2 h. To avoid exposure to high temperatures, the plastic cups were carried in a cooler. A layer of newspaper was used to prevent direct contact of the plastic cups with ice packs at the bottom of the cooler (Andreadis et al. 2014). Samplings were executed every 15 days in each region from early April until end of October for 2 consecutive years, 2012 and 2013, reaching a total of 196 samplings. As soon as the samples were transferred to the laboratory, the larvae and pupae were counted, and microscope slides of the larvae were prepared. The third- and fourth-instar larvae were identified using the keys of Snow (1990), Darsie and Samanidou-Voyadjoglou (1997), and Becker et al. (2010). In addition, adult mosquitoes were reared from the pupae in standard laboratory conditions (25 ± 1 °C, 65 ± 5% RH, and 16:8 h L:D). Emerging adults were kept alive for 24 h, so that all the taxonomic characteristics were complete, such as color pattern or the shape of scales. The adult mosquitoes were pinned and separately classified by the sampling date to identify them using appropriate keys (Samanidou-Voyadjoglou and Harbach 2001; Becker et al. 2010).

Results

During this 2-year study, 6,227 third- and fourth-instar larvae and pupae were collected and identified to the species level based on morphological characteristics. Thirteen species within 4 genera were identified in the studied areas (Table 2). In the majority of the areas that were studied the most common species was *Cx. pipiens* (45.5% of total samples), followed by *Ae. caspius* (23.2%) (Table 2). In specific regions, *Anopheles* species occupied a significant percentage of the mosquito population (*An. hyrcanus* and *An. maculipennis s.l.*, 8.6 and 8.5%, respectively) (Table 2). The other species were not found in large numbers and formed < 5% of the total samples (Table 2).

Regarding the seasonal variation of species, it was observed that mosquitoes of the genus *Aedes* appear first (late March) in 2012 and 2013 compared to the other species, followed by the genus *Culex* (April to May) (Fig. 2). Individuals of the genus *Anopheles* were the last to occur in the region in mid-July in both years. *Culiseta* mosquitoes were only recorded in 2013 in low populations in June. At the beginning of the period, from early April to mid-May, the most abundant species was *Ae. caspius* (Table 3). *Culex pipiens* appeared in mid-May, and its population remained high until September (Table 3). Dominant species from early August to October were species of the genus *Culex* and *Anopheles* in both years, 2012 and 2013 (Fig. 2).

Discussion

According to the hitherto known facts, in Greece (mainland and islands), 64 mosquito species exist (Voyadjoglou-Samanidou 2011). In the present study, 13 of these species have been identified in the RU of Thessaloniki. The sampling method applied in this research is focused on the inspection of the larval breeding habitats. The above-mentioned sampling method apart from the fact that it provides basic information about the mosquito species composition of the study areas, it also offers specific information on the locations where larvicides could be applied and thus contributing to the quality control of mosquito control

Table 2 Composition and relative abundance (%) of mosquito species per sampling site of the RU of Thessaloniki

Localities	Relative abundance of mosquito species (%) per sampling site													Total number per site
	An. hyrcanus	*An. maculipennis*	*An. plumbeus*	*Ae. caspius*	*Ae. vexans*	*Ae. vittatus*	*Cx. mimeticus*	*Cx. pipiens*	*Cx. territans*	*Cx. theileri*	*Cx. torrentium*	*Cs. annulata*	*Cs. longiareolata*	
Axios					15.4	34.9		43.4	6.3					332
Chalastra				44.5	16.9			38.6						319
Epanomi				58.2				41.8						196
Kalamaria				54	13.2			32.8						189
Kalochori/Sindos				70.6	19.1	10.3								1078
Lagkadas								88.2	11.8					102
Pentalofos	18.4	16.2	1.1				0.6	57.1	1.3	0.9	4.4			2318
Polichni				16.9				83.1						65
Thermi				47.2				52.8						661
Metropolitan area					25.1			53.9				10.8	10.2	167
Volvi	13.5	19.2						64.8	2.5					800
Total number per species	**534**	**530**	**26**	**1442**	**348**	**257**	**13**	**2836**	**82**	**21**	**103**	**18**	**17**	**6,227**

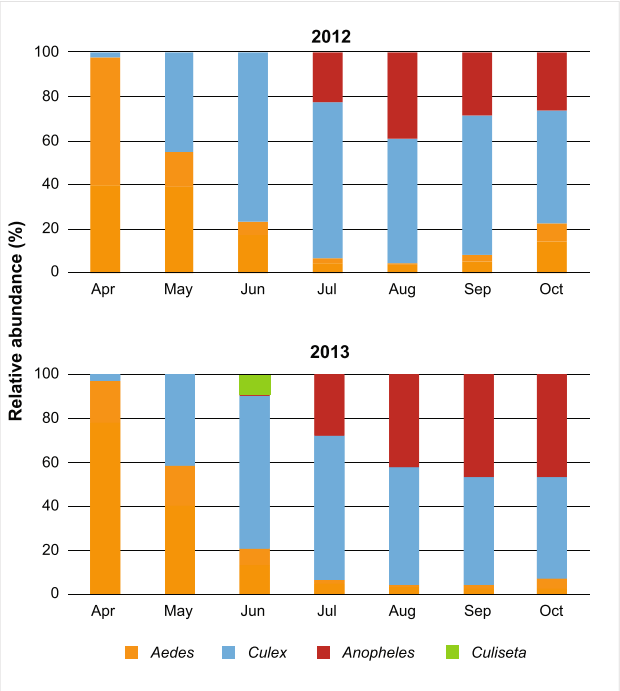

Fig. 2 Monthly abundance of mosquito species in the regional unit of Thessaloniki during the years 2012 and 2013, categorized by genus

programs that are implemented over temperate areas (Michaelakis et al. 2021). One of the most significant factors of a rationally designed mosquito control program is to be mainly focused on the species which cause particular discomfort or those species which are competent vectors, posing a potential danger for transmitting disease agents (Govella et al. 2013; Majeed 2013). On the contrary, non-targeted control of mosquito and other Diptera species of no particular interest for public health could possibly disrupt the ecological balance (Majeed 2013).

The following species have been identified in the present study: *Cx. pipiens*, *Cx. territans*, *Cx. theileri*, *Cx. mimeticus*, *Cx. torrentium*, *Ae. caspius*, *Ae. vexans*, *Ae. vittatus*, *An. maculipennis s.l.*, *An. hyrcanus*, *An. plumbeus*, *Cs. annulata*, *and Cs. longiareolata*. It is the first time that *Ae. vittatus* has been recorded in Greece and the first time that *An. plumbeus* has been found in the RU of Thessaloniki. All the other species recorded in this study have been previously reported to be native in the region (Samanidou-Voyadjoglou and Darsie 1993; Voyadjoglou-Samanidou 2011; Lytra 2015). Interestingly, although *Ae. albopictus* is confirmed in the RU of Thessaloniki (Badieritakis et al. 2018), it was not possible to be collected during this study due to the fact that the breeding sites that we surveyed were not suitable for its development. This species is mainly established in urban areas and therefore is very difficult to identify their breeding sites with the selected sampling method (Stefopoulou et al. 2018; Bellini et al. 2020).

Concerning the public health significance of the identified species, *Ae. caspius* has been reported positive to WNV, Tahyna virus, and bacterium *Francisella tularensis* (Milankov et al. 2009; Vázquez et al 2012). *Culex pipiens* mosquitoes are the main vectors of WNV causing severe epidemic outbreaks of West Nile Fever in Romania and New York in 1996 and 1999, respectively (Nicolescu 1998; Bernard et al. 2001). It is also considered responsible for the re-emergence of West Nile Fever in Greece in 2010 onwards (Gomes et al. 2013). West Nile Virus provoked serious outbreaks in the Balkan area, including Greece, Romania, Croatia, the Former Yugoslav Republic of Macedonia, Kosovo, Montenegro, and Serbia during the 2010s (Sambri et al. 2013). Climatic conditions, temperature, and humidity favor the presence and the multiplication of *Culex* spp. from May to October in the affected zones (ECDC 2010). *Culex pipiens* biotype *pipiens* seems to play a minor role as an arbovirus vector in Europe, whereas *Cx pipiens* biotype *molestus* presents greater medical significance (Voyadjoglou-Samanidou 2011). *Culiseta annulata* is known to be a potential vector of Tahina virus (Danielová 1972) and various *Plasmodium* sp. on birds and humans (Valkiunas 2005; Inci et al. 2012). *Culex territans*, *Cx. theileri*, *Cx. mimeticus*, *Ae. vexans*, *Ae. vittatus*, and *Cs. longiareolata* species recorded in this study are not considered of great medical importance.

When it comes to Anophelinae species, *An. hyrcanus* due to its exophilic nature is not considered to be an important malaria vector in the Mediterranean region (Piperaki and Daikos 2016; Tagliapietra et al. 2019). On the other hand, members of the *Anopheles maculipennis* complex had been reported to be responsible for the transmission of malaria to humans in Greece (Betzios 1989). Contrary to the previous scientific assumption, Papadakis (1956) claims that its sporozoic index was low, thus was considered to be less important in transmitting malaria. Nonetheless, in certain local outbreaks of the disease, *An. maculipennis* complex was the only *Anopheles* species to be found active in the region (Voyadjoglou-Samanidou 2011). Regarding *An. plumbeus*, even though laboratory studies have shown that it can carry *Plasmodium vivax* (Marchant et al. 1998), because of its feeding behavior, it is not considered epidemiologically hazardous (Becker et al. 2010).

Seasonal abundance of mosquitoes is affected greatly by climate variables such as temperature and rainfall that alter the quantity and quality of mosquito breeding habitats (Bashar and Tuno 2014; Roiz et al. 2014). Drakou et al. (2020) reported that monthly relative humidity showed positive correlation with the numbers of the species that were sampled, i.e., *Cx. pipiens*, *Ae. detritus*, and *Ae. caspius*. Moreover, mosquito abundance of *Cx. pipiens* and *Ae. detritus* was strongly correlated to seasonal precipitation

Table 3 Number of mosquito species sampled per month in the RU of Thessaloniki during the years 2012 and 2013

Year	Month	Number of mosquito species													Total number per month
		An. hyrcanus	*An. maculipennis*	*An. plumbeus*	*Ae. caspius*	*Ae. vexans*	*Ae. vittatus*	*Cx. mimeticus*	*Cx. pipiens*	*Cx. territans*	*Cx. theileri*	*Cx. torrentium*	*Cs. annulata*	*Cs. longiareolata*	
2012	April	0	0	0	220	53	65	0	5	0	0	0	0	0	343
	May	0	0	0	301	65	62	0	317	32	0	0	0	0	777
	June	0	0	0	70	25	0	6	263	19	0	31	0	0	414
	July	47	47	0	18	10	0	7	241	18	3	19	0	0	410
	August	70	69	15	14	3	0	0	216	0	0	7	0	0	394
	September	44	39	11	15	11	0	0	211	0	0	0	0	0	331
	October	47	47	0	18	10	0	7	241	18	3	19	0	0	366
2013	April	0	0	0	323	37	41	0	12	0	0	0	0	0	413
	May	0	0	0	316	64	76	0	297	0	0	23	0	0	776
	June	0	3	0	54	16	13	0	250	13	0	15	18	17	399
	July	60	62	0	22	7	0	0	274	0	7	8	0	0	440
	August	86	84	0	15	2	0	0	216	0	0	0	0	0	403
	September	84	81	0	11	5	0	0	166	0	6	0	0	0	353
	October	93	97	0	12	19	0	0	182	0	5	0	0	0	408

(Drakou et al. 2020). The relationship between environmental factors and mosquito populations provides crucial information on parasite activity levels; thus, it contributes towards prediction of mosquito-borne disease outbreaks (Bashar and Tuno 2014; Roiz et al. 2014; Meuti and Short 2019; Drakou et al. 2020). Other factors influencing mosquito populations include the presence of potential predators or competitors in larval habitats and human activities such as cultural practices in rice fields (Lytra and Emmanouel 2014). In the present study, the seasonal distributions of the most abundant species reveal population fluctuations in different months. *Culex pipiens* was the dominant species present almost throughout the year with peaks in June and August and smaller peaks in September. In contrast, *Anopheles* species were found during July to September only, with a peak observed in August to September. *Aedes* species were the first to appear in the season with a peak of their population in April to May. Our findings are in accordance with a previous study conducted in various geographical areas of Central and Southern Greece during the years 2009–2011 (Lytra 2015).

Thorough notes of the composition and seasonal abundance of mosquitoes in a specific region is fundamental for the development of efficient mosquito control programs (Alten et al. 2000). Moreover, detailed studies of bionomics, surveillance, and disease transmission dynamics of mosquitoes are required for predicting disease outbreaks and vector control in a region (Bashar and Tuno 2014). Taking this into consideration, recording the mosquito fauna in the RU of Thessaloniki as well as their seasonal abundance is essential for designing an integrated control program especially against species with medical importance. Such catalogues should be created in all prefectures of Greece, and mosquito populations should be monitored in a regular basis which will eventually contribute to the most effective control of the mosquitoes.

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Declarations

Conflict of interest The authors declare no competing interests.

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