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Survey on mosquito larvae in different water bodies in Lithuania

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Abstract

The aim of this research was to evaluate mosquito species diversity and seasonality, and to determine relationships between investigated parameters of mosquito breeding environment and the presence and abundance of mosquito larvae of different species in water bodies. For that, large-scale mosquito larval collections were made in different water bodies in Lithuania using a standard dipper. In 2021 and 2022, samples were collected from the end of March to October and from April to July, respectively and were done once or twice a month. Physical and chemical parameters (water temperature, pH, amounts of nitrites and nitrates, the water body size, bottom coverage, exposure to the sunlight, and temporality of each water body) were evaluated. A total of 5,392 mosquito larvae were collected from 134 water bodies (606 samples as each water body was investigated several times during the season). 25 mosquito species have been identified. Results of multiple regression analysis revealed that the abundance of larvae of some mosquito species is related to the collection time, temporality of the water body, the amount of nitrates in water, pH level and bottom coverage. Redundancy analysis showed that mosquito species abundance and diversity are significantly influenced by water temperature, pH values, and amount of nitrogen.

Key words: ecology, seasonality, species diversity, physical parameters, chemical parameters, mosquitoes, Culicidae.

Introduction

Mosquitoes are known as vectors of arboviruses, bacteria, protozoan and other parasites which can infect wild and domestic animals, and can cause outbreaks of diseases (Ibanez-Justicia *et al*., 2015; Bernotienė and Valkiūnas, 2016). Different mosquito species can serve as vectors for specific parasite groups, species, or genetic lineages (Synek *et al*., 2012), for example, *Anopheles* mosquitoes can transmit human malaria (Sallum, 2000; Okech *et al*., 2007) and *Culex* mosquitoes are mainly responsible for the transmission of avian malaria parasites (Valkiūnas, 2005; Kazlauskienė *et al*., 2013). Mosquitoes of some species can also transmit avian trypanosomes, filarial worms, and various arboviruses (Lehane, 2005; Votypka *et al*., 2012; Svobodová *et al*., 2015). Worldwide 3,719 mosquito species are currently known (Harbach, 2023). Meanwhile, 37 mosquito species are recorded from Lithuania (Bernotienė and Lučiūnaitė, 2011). Mosquitoes, depending on species (Becker *et al*., 2003; Wegner, 2009b), can inhabit vast territories due to a wide range of mosquito larval habitats: permanent or temporary, polluted or clean water bodies, natural or artificial, etc. (Becker *et al*., 2003; Rueda, 2008). Because of their ability to spread various pathogens of birds and mammals, including humans, adult mosquitoes have been more investigated compared with immature stages, therefore, information about the peculiarities of development of mosquito immature stages is still lacking (Westbrook *et al*., 2010).

Relationships between mosquito diversity and seasonality, and the regularities determining these relationships are of great importance, especially those due to climate change. Rettich *et al*. (2007) investigated the seasonality of mosquito larvae of different species in flood plains in the Czech Republic. Species composition and seasonality of mosquito larvae were studied in Slovakia (Bocková *et al*., 2013), Turkey (Aldemir *et al*., 2009; Sengil *et al*., 2011), Serbia (Vujić *et al*., 2010), Poland (Wegner, 2009a; 2009b). Mosquito larval diversity of Lithuania was not investigated since the work of Podėnaitė (1962) during early sixties of last century. Back then water-body parameters were not measured. Therefore, knowledge of the types of water bodies in which mosquitoes of certain species choose to breed more often and more successfully is critical in understanding patterns of mosquito distribution. It is important to find out the most important water chemical properties that affect larval distribution. The detailed knowledge of different mosquito species preferences for the larval development habitats, and spatial distribution is of great importance in deciding vector control strategies (Mereta *et al*., 2013).

In this study, mosquito larvae were collected and identified in order to understand mosquito species diversity and seasonality in various water bodies and to get new information on the relationships between parameters of water bodies and the presence or absence of mosquito larvae of certain species in them.

Materials and methods

Study site

The study was conducted at 134 water bodies in Lithuania (in Vilnius, Alytus, Elektrėnai, Prienai, Šilutė, and Šiauliai municipalities) (figure 1). Water body types include natural wetlands, natural ponds, puddles and vehicle ruts. Habitats surrounding studied water bodies were parks, deciduous and mixed forests, meadows, urbanized and abandoned areas. Lithuania is located in temperate zone with the average annual air temperature (years 1991-2020) being 7.4 °C - the warmest month is July (18.3 °C), and the coldest in January (−2.9 °C). The average annual precipitation (years 1991-2020) in Lithuania is 695 mm - the highest rainfall occurs in July (84 mm) and the lowest in April (37 mm). Climate data

Figure 1. Map of Lithuania of mosquito larvae collecting sites.

is taken from the Lithuanian Hydrometeorological Service under the Ministry of Environment. Lithuania is located at the edge of the North European Plain with landscape formed by the glaciers of the last Ice Age. It makes Lithuania's terrain of alternating moderate lowlands and highlands with average altitude being 102 m and highest point 313 m (Vardys *et al*., 1996).

Larval survey

Mosquito larvae were collected in 2021 from March to October and in 2022 from April till July. Samples were taken every two weeks or once a month depending on the location of the water body. Mosquito larvae were collected using a standard dipper (⌀ 12.5 cm), each sample was repeated three times in order to measure the average number of caught mosquito larvae, which were placed in separate containers, water removed by pipette, and 96% alcohol put to preserve larvae.

Diversity and density of larvae

Fourth-instar mosquito larvae were identified morphologically using Becker *et al*. (2003) and MosKeyTool mosquito identification keys (Gunay *et al*., 2018). Earlier instar larvae are unsuitable for identification and were only counted for the overall number.

The density (D) of mosquito species were counted as a percentage of the number of individuals of certain mosquito species on the total number of mosquitoes, as described by Bocková *et al*. (2013). Mosquito distribution (C) was assessed by calculating the percentage of water bodies where a certain species was found out of all investigated water bodies (Bocková *et al*., 2013).

Physicochemical characteristics

Water temperature (°C), pH value, amount of nitrites [NO_2 (mg/l)], and nitrates [NO_3 (mg/l)] were measured during the sampling. For pH measuring pH color-fixed indicator strips (Carl Roth, Germany) and for the amounts of NO_2 and NO_3, Nitrate and Nitrite indicator strips (Macherey - Nagel GmbH&Co, Germany) were used. The size of each water body was evaluated and assigned to one of three size categories: small (smaller than 4 m^2 in size), medium (in the range of 4-100 m^2), and big (bigger than 100 m^2). The sizes of water bodies were measured in springtime when they were full of water. The bottom coverage of the water body was evaluated in each case and described (covered with sand, mud, grass, organic matter -branches, leaves, etc.- or clay). Water bodies were also characterized according to the exposure to the sunlight (the water body was in an open area and was exposed to direct sunlight or it was in a closed shady area), and it was also noted if the water dries completely during the season or if the water body is permanent.

Statistical analysis

The average number of mosquito larvae per sample (one water body during one sampling) and the standard error (SE) have been calculated. For 11 mosquito species that occurred in more than five different samples, multiple regression analysis was applied using STATISTICA 12.5 software to determine which parameters were related to the abundance of mosquito larvae in different water bodies. Redundancy analysis (RDA) was applied to find associations between abundance of mosquito species and physicochemical variables using Brodgar software (Highland Statistics Ltd.). The material collected is deposited in the Laboratory of Entomology, Nature Research Centre, Vilnius.

Results

A total of 606 samples were taken (of which 225 samples positive for mosquito larvae). Each water body was investigated from one (in case the water body dries up) up to ten times during the study period and 134 water bodies were investigated for the presence of mosquito larvae, of which in 101 water bodies mosquito larvae were found at least once. Mosquito larvae were detected from the 31st of March (with water temperature being 7 °C) until the 30th of September (with water temperature being 9 °C). Out of 5,392 mosquito larvae that were collected, 2,141 were 4th instar larvae (154 samples out of 225) and have been identified to the species level. 25 mosquito species were identified belonging to five genera (table 1). The most numerous genera were *Ochlerotatus* comprising 67.6% of all collected mosquitoes, followed by *Aedes* (25.2%), while *Culiseta*, *Culex* and *Anopheles* formed only 3.4%, 3.3% and 0.5% respectively. On average 8.9 ± 1.5 mosquito larvae were caught per sample with the maximum number of mosquito larvae in a sample being 381 (beginning of April). April (2021) was the month when the majority of mosquito larvae were caught (50.6% of all collected mosquito larvae) (figure 2) even though sampling was done according to the same methodology during the season. The number of mosquito larvae was also high (28.4%) in May and fell down (2.8%) in June (during both years). The first encounter of mosquito larvae was in March (both in 2021 and 2022). It is worth noting that all collected specimens therein belong to one sampling site and reported as an average of three replicates (figure 2).

In 99 positive for mosquito larvae samples (64.3%), only one mosquito species was identified. *Anopheles maculipennis* Meigen and *Ochlerotatus riparius* (Dyar et Knab) are the only species that were found without other species, while others were found alone or with other species. In 44 samples (28.6%) two species coexisted at the same time (table 2). In nine samples (5.8%) three mosquito species were found, but all of them were found

Table 1. Mosquito species identified in investigated water bodies with their distribution (C) and density (D); n - number of water bodies where larvae of certain species have been found, l - number of individuals of certain species found throughout the season.

Species	n	C	l	D
Aedes cinereus Meigen	47	35.1	367	17.1
Aedes vexans (Meigen)	6	4.48	172	8.03
Anopheles claviger (Meigen)	2	1.49	2	0.09
Anopheles maculipennis Meigen	2	1.49	8	0.37
Culiseta alaskaensis (Ludlow)	4	2.99	16	0.75
Culiseta annulata (Schrank)	6	4.48	42	1.96
Culiseta morsitans (Theobald)	11	8.21	16	0.75
Culex pipiens L.	5	3.73	47	2.2
Culex territans Walker	6	4.48	9	0.42
Culex torrentium Martini	1	0.75	14	0.65
Ochlerotatus annulipes (Meigen)	5	4.48	41	1.91
Ochlerotatus behningi (Martini)	1	0.75	2	0.09
Ochlerotatus cantans (Meigen)	48	35.8	607	28.4
Ochlerotatus caspius (Pallas)	2	1.49	6	0.28
Ochlerotatus cataphylla (Dyar)	14	10.5	274	12.8
Ochlerotatus communis (De Geer)	3	2.24	30	1.4
Ochlerotatus diantaeus (Howard, Dyar et Knab)	1	0.75	4	0.19
Ochlerotatus euedes (Howard, Dyar et Knab)	2	1.49	30	1.4
Ochlerotatus flavescens (Muller)	13	9.7	53	2.48
Ochlerotatus geniculatus (Olivier)	1	0.75	2	0.09
Ochlerotatus intrudens (Dyar)	2	1.49	4	0.19
Ochlerotatus nigrinus (Eckstein)	2	1.49	12	0.56
Ochlerotatus punctor (Kirby)	11	8.21	29	1.35
Ochlerotatus riparius (Dyar et Knab)	1	0.75	6	0.28
Ochlerotatus sticticus (Meigen)	5	3.73	348	16.3

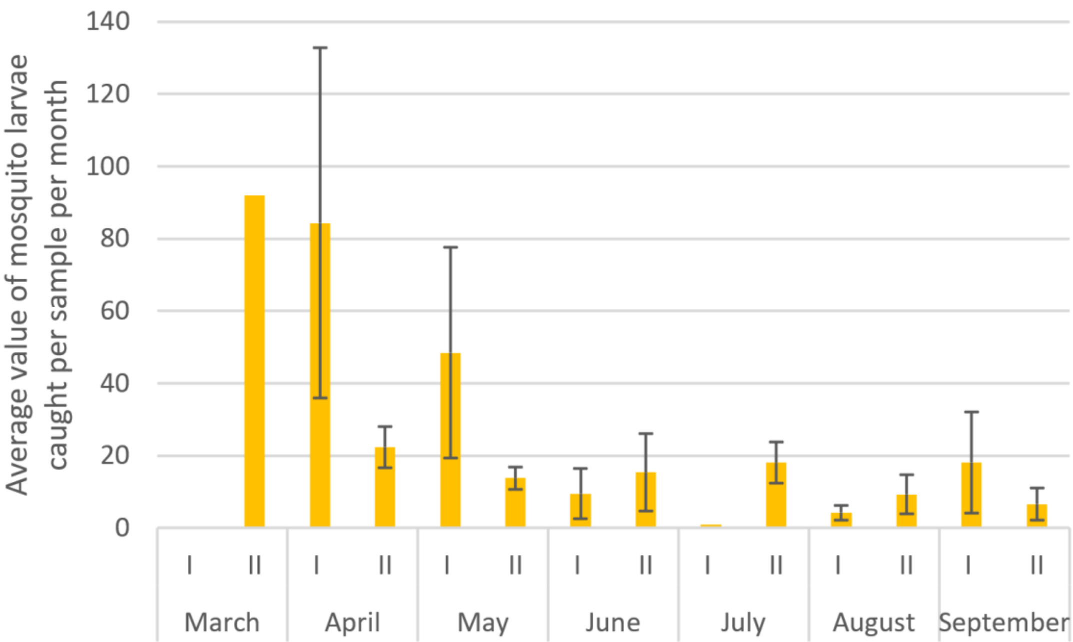

Figure 2. Average number of mosquito larvae caught per sampling each half of the month during this study (Average and SE values).

Table 2. Mosquito species that were found together in the same samples. The top right of the table shows samples where two species were found together (the number indicates how many samples two species were found together). The bottom left part of the table shows cases where 3 species were found together; the third species is indicated by the letter: a - *Cs. alaskaensis*; b - *Cx. pipiens*; c - *Oc. annulipes*; d - *Oc. cantans*; e - *Oc. caspius*; f - *Oc. diantaeus*; g - *Oc. flavescens*; h - *Oc. intrudens*. In all cases three species in one sample were found once.

Mosquito species	*Ae. cinereus*	*Ae. vexans*	*An. claviger*	*Cs. annulata*	*Cs. morsitans*	*Cx. territans*	*Cx. torrentium*	*Oc. annulipes*	*Oc. behningi*	*Oc. cantans*	*Oc. cataphylla*	*Oc. communis*	*Oc. euedes*	*Oc. flavescens*	*Oc. geniculatus*	*Oc. Intrudens*	*Oc. nigrinus*	*Oc. punctor*	*Oc. sticticus*
Ae. cinereus		1		1					1	7				5			1		2
Ae. vexans										1	1								1
An. claviger																			
Cs. annulata	d																		
Cs. morsitans								1		1									
Cx. territans								1											
Cx. torrentium			a																
Oc. annulipes											1								
Oc. behningi																			
Oc. cantans	b										7	1	1				1	6	
Oc. cataphylla	d				c					f				1					1
Oc. communis											g								
Oc. euedes																			
Oc. flavescens																			
Oc. geniculatus																		1	
Oc. intrudens																			
Oc. nigrinus																			
Oc. punctor										h						e			
Oc. sticticus																			

together only once (table 2) and in two cases four species were found in one sample [*Aedes cinereus* Meigen - *Culex pipiens* L. - *Culiseta annulata* (Schrank) - *Culiseta morsitans* (Theobald); *Ochlerotatus annulipes* (Meigen) - *Ochlerotatus cantans* (Meigen) - *Ochlerotatus cataphylla* (Dyar) - *Ochlerotatus punctor* (Kirby)].

The prevalent pH value was 7.5 (in 35.2% of the sites). The amount of NO_2 in water varied from 0 mg/l to 0.5 mg/l and NO_3 values varied from 0 mg/l to 50 mg/l with the majority (more than 91%) of water bodies having none of the nitrites and nitrates.

Bottom of 31.3% of investigated water bodies were covered with various organic matter, 28.1% was covered exclusively with tree leaves, 20.3% with grass, 17.2% with mud, and 3.1% was covered with sand. The majority (52.3%) of water bodies were of medium size, 25.0% were big, and the remaining (22.7%) were small. 58.6% of water bodies completely dried at some point during the season and the remaining 41.4% stayed with water during all the season. 64.7% of water bodies were in shade and the remaining 35.3% were exposed to the sun at least at some point of the day.

Multiple regression analysis showed that the abundance of *Aedes* mosquitoes was statistically significantly higher in water bodies with the bottom covered by leaves ($p = 0.000$, $\beta = 0.340$). The abundance of *Anopheles* mosquitoes showed relationships with the collection time ($p = 0.026$, $\beta = 0.098$) as *Anopheles* mosquitoes were mainly caught in June and September (2021); *Culex* mosquitoes were found mainly in temporary water bodies ($p = 0.041$, $\beta = 0.090$). The abundance of *Culiseta* mosquitoes showed relationships with the collection time ($p = 0.019$, $\beta = 0.103$) and with drying up of the water body ($p = 0.025$, $\beta = 0.097$) as 85.7% of *Culiseta* mosquitoes were found in temporary water bodies. *Ochlerotatus* did not show any significant dependencies to any of the investigated parameters.

Multiple regression analysis revealed that some mosquito species are seasonally dependent, for example, *Culex territans* Walker (found in August, 2021), *Oc. cantans* (mostly found in April, during both years) and *Oc. punctor* (April) (table 3, supplemental material table S1). Larvae of *Ae. cinereus* and *Cs. annulata* were found statistically significantly more abundant in temporary water bodies (table 3, supplemental material figure S1). The amount of nitrates in the water had a negative influence on the abundance of *Cx. pipiens* larvae and positive influence to the abundance of *Oc. cantans* larvae (table 3). The abundance of *Oc. cantans* was also related to certain pH levels (table 3) and *Cs. annulata* were mostly collected in water bodies covered by organic matter (table 3, supplemental material figure S1).

Redundancy analysis confirmed the results of multiple regression showing that *Oc. cantans*, as well as *Aedes vexans* (Meigen) and *Ae. cinereus* can be found more abundant in water bodies with higher amounts of NO_2 and NO_3 (figure 3, supplemental material figure S1). Redundancy analysis showed positive relationships between the abundance of *Cs. annulata*, *Cx. pipiens* and *Cx. territans* and pH value, water temperature and the collection time. Mosquitoes of these species were more abundant in the second part of the summer when water temperature was higher. On the contrary, the abundance of *Oc. punctor*, *Ochlerotatus flavescens* (Muller) and *Oc. annulipes* was higher in cold water and abundance of *Oc. cataphylla* was related negatively with pH values (figure 3), as larvae of this species were found in water bodies with the lowest pH values (supplemental material figure S2).

Discussion

One of the aims of this study was to evaluate mosquito species diversity in Lithuania and during this research 25 mosquito species were found, making up 69.4% of all Culicidae species that have been found in Lithuania (Pakalniškis *et al*., 2006; Bernotienė and Lučiūnaitė, 2011). Podėnaitė (1959; 1962) investigated mosquito larvae in various regions of Lithuania and found mosquito larvae of 26 species in 1959 and 22 species in 1962. Further surveillance and long-term mosquito larvae monitoring that would cover more diverse habitats might increase the number of mosquito species that can be found in Lithuania. The second aim of the study was to get knowledge on the seasonality of mosquitoes at our study sites. Data shows that the highest abundance of mosquito larvae in water bodies can be detected from April through May, but mosquito larvae of different species can be found from the end of March till the end of September. The final aim of this study was to determine relationships between some parameters and the presence and the abundance of mosquito larvae of different species in investigated water

Table 3. Multiple regression analysis data showing the significant correlations between most abundant species and the prevalent water parameters of the collecting sites.

	Ae. cinereus	*Cx. pipiens*	*Cx. territans*	*Culiseta annulata*		*Ochlerotatus cantans*			*Oc. cataphylla*	*Oc. punctor*
	Permanent/ temporary water bodies	NO3	Sampling time	Permanent/ temporary water bodies	Bottom cover	Sampling time	pH	NO3	Sampling time	Sampling time
b*	0.09	0.11	0.12	0.11	0.11	-0.11	-0.09	0.11	-0.09	-0.10
Std.Err of b*	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
b	0.63	0.04	0.01	0.18	0.05	-0.44	-0.91	0.26	-0.16	-0.03
Std.Err of b	0.32	0.02	0.00	0.07	0.02	0.18	0.46	0.09	0.08	0.01
t (591)	2.00	2.70	2.63	2.48	2.60	-2.46	-1.98	2.83	-1.95	-2.34
p-value	≥ 0.05	≥ 0.01	≥ 0.01	≥ 0.01	≥ 0.01	≥ 0.01	≥ 0.05	≥ 0.00	≥ 0.05	≥ 0.02

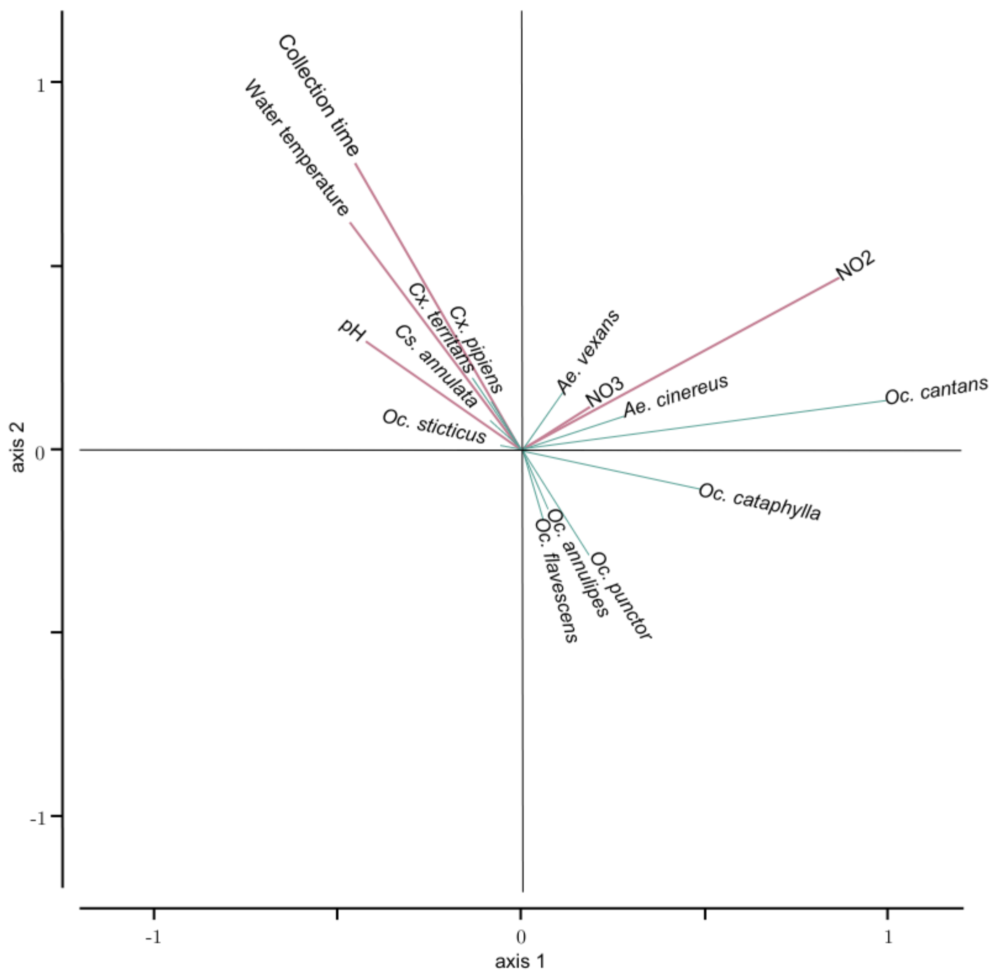

Figure 3. Redundancy analysis showing mosquito species relationships with investigated parameters. The two-dimensional approximation explained 94.3% of this (76.9% on axis 1 and 17.4% on axis 2).

bodies. Statistical analysis in this study showed that collection time, temporality of the water bodies, the amount of nitrates in water, pH levels and bottom coverage have an impact on the presence of mosquito larva of some species, but the abundance of neither species was associated with exposure to the sun or water body size.

Species distribution by breeding site characteristics

Some mosquito species were found in a wide range of investigated parameters: for example, *Ae. vexans*, *Oc. annulipes*, *Oc. flavescens*, *Ochlerotatus sticticus* (Meigen) (both water temperature and pH values) (supplemental material figure S2), while some other species were found in a narrow range, such as *Oc. cantans*, *Oc. cataphylla*, *Ae. cinereus* (supplemental material figure S2). Interestingly, according to Becker *et al*. (2003) mosquito larvae of these later three species can often be found developing together. Certain mosquito species abundance had a statistically significant relationship with investigated parameters: for example, *Oc. cantans* showed a narrow range of water temperature and pH values (supplemental material figure S2). Interestingly *Oc. cataphylla* and *Oc. cantans* were commonly found together (15.9% of species pairs). Becker *et al*. (2003) noted that *Oc. cataphylla* develops more abundantly in neutral and slightly alkaline water, but our data showed that larvae of this species were found in slightly acidic (pH = 6) waters. We found larvae of *Oc. cantans* in water bodies with pH values on average 6.9. *Oc. cataphylla* and *Oc. cantans* species are also associated with low water temperature, which lets them develop early in spring when water is cold in North European countries, and emerge early in May (Becker *et al*., 2003; Wegner, 2009b), which is consistent with the data of our study as the majority of individuals were found in April.

Conversely to *Oc. cantans* and *Oc. cataphylla*, higher water temperatures were preferred by species belonging to *Culex* and *Culiseta* genera. These species develop during summertime with the higher water temperatures (except *Cs. morsitans*). The fact that *Culiseta* prefers to develop at high water temperatures is also indicated by other authors (Becker *et al*., 2003), who also state that, for example, *Cs. annulata* in Europe is a widespread species and covered quite a long season (Becker *et al*., 2003; Wegner, 2009b). Though in our study they were found in June-August, at low density. *Cs. morsitans* larvae can develop in various water bodies, they overwinter in the larval stage and pupation only occurs after spring (Becker *et al*., 2003), yet Oliver and Howard (2011) reveal that northern *Cs. morsitans* overwinter in the egg stage and larvae of this species need high water temperatures to develop. In our research they were present in April (more than a third of all found) and then re-appeared from June through September. *Cx. territans* and *Oc. punctor* both showed statistically significant relationships with sampling time, but these relationships were the opposite. While *Oc. punctor* was found only in April, with average water temperature being 8.2 °C, *Cx. territans* was found in July-August with the average water temperature being 15.8 °C. Becker *et al*. (2003) noted that *Oc. punctor* was a snow-melt mosquito species that starts development early in spring. While *Cx. territans* may occur from early spring until September with its peak being in late summer.

There were some mosquito species (*Cx. territans*, *Cs. annulata*, *Oc. flavescens*, and *Oc. sticticus*) that were found only in water bodies with no nitrates and nitrites detected. The same was observed for *Cx. pipiens* as Dowling *et al*. (2013) revealed that *Cx. pipiens* preferred to oviposit in containers with lower nitrate content. *Oc. cantans* were found more abundantly in water bodies with higher amount of nitrates as was shown both by the multiple regression analysis, and the RDA, and this was new information about the development of this mosquito species.

Species seasonality

According to Becker *et al*. (2003) and Wegner (2009b) some mosquito species are univoltine, for instance the early spring species *Oc. cataphylla*. This is in agreement with our results, as the majority of *Oc. cataphylla* larvae were found in April and then some were found in May. Similarly, *Oc. annulipes* is known to be an univoltine species (Becker *et al*., 2003), which coincides with our observations, as this species was detected in April-May, but at low density, differently from what was expected since this species has been recorded as the most abundant in central Europe (Becker *et al*., 2003). It is known that *Oc. cantans* has one generation in the northern part of Europe, but there can be two generations in other parts (Becker *et al*., 2003). Our results showed two clear generations of *Oc. cantans*: the first one in April-May and the second one in August-September (supplemental material table S1). It is known that in northern parts of Europe *Cx. territans* have only one generation per season (Becker *et al*., 2003), but in Lithuania, it seems to have quite a long season with multiple generations as larvae of this species were found from June to August. According to Wegner (2009b), *Oc. sticticus* is considered to be univoltine, while Becker *et al*. (2003) stated that it is a multivoltine species. We found this species in April-May and in July, while no individuals were found in June, suggesting that at least two generations developed during the season. Wegner (2009b) noted that *Oc. punctor* was a multivoltine species, but in our study, it was found exclusively in April.

Ae. cinereus is a widespread species in the Holarctic region, that can have several generations in Lithuania (supplemental material table S1). In the northern part of Europe it has one generation, but further south it can have multiple generations a year (Wegner, 2009b). They hatch a little later because their larval development requires a higher temperature than typical snowmelt species (Becker *et al*., 2003), maybe because of this they were found in April, but they peaked only in May and that also lines up with larval stage findings in our research, where the average temperature for *Ae. cinereus* larvae were higher (12.0 °C) than that of *Oc. cantans* (8.7 °C) and *Oc. punctor* (8.2 °C).

Ae. vexans is multivoltine species, that is considered to be a "summer species", because its optimal development temperature is known to be around 30 °C (Becker *et al*., 2003; Wegner, 2009b). This coincides with our results as these mosquitoes were found in small numbers in April-May and they reached the peak in July when the average temperature was the highest (supplemental material table S1).

Species associations

Mosquito species found together in the same samples (table 2) have been also mentioned in other studies (Becker *et al*., 2003; Khalin and Aibulatov, 2020). *Ae. cinereus* can be found together with *Oc. cantans*, *Ae. vexans*, *Ochlerotatus behningi* (Martini), *Oc. cataphylla*; *Oc. cantans* with *Ochlerotatus communis* (De Geer), *Ochlerotatus diantaeus* (Howard, Dyar et Knab), *Oc. punctor*; *Oc. cataphylla* was found together with *Oc. annulipes* and *Oc. communis*; *Culex torrentium* Martini can be found together with *Anopheles claviger* (Meigen) and *Culiseta alaskaensis* (Ludlow); *Ochlerotatus intrudens* (Dyar) was found together with *Oc. punctor*.

Vectorial capacity

Many of the investigated mosquito species are known to be competent vectors of various pathogens. *Ae. cinereus* females are considered to be mammophilic and bite at low heights, but there are records of them feeding on birds (Service, 1971; Vujić *et al*., 2010). This species is known as a competent vector for some arboviruses like Ockelbo, Tahyna and West Nile (Wegner, 2009b). Despite the fact that this species is quite abundant, arboviruses that can be transmitted by *Ae. cinereus* were never investigated in Lithuania. It is known that *Ae. vexans* can migrate long distances (up to 15 km) in search of blood meals, which means that they can spread to new habitats. It is also known that *Ae. vexans* can act as vectors for viruses (West Nile, Eastern Equine Encephalomyelitis, Tahyna) and dog filarial worms (Becker *et al*., 2003; Wegner, 2009b). *Oc. cantans* can feed on any available hosts and since they are big mosquitoes, for egg laying they can take multiple blood meals and can lay eggs multiple times, so this would make them good vectors of various pathogens (Flavivirus, Bunyavirus, West Nile Virus, Tahyna virus) (Becker *et al*., 2003; Wegner, 2009b).

Two anopheline species were found during our study (table 1). *An. claviger* can transmit human malaria (Sallum, 2000; Okech *et al*., 2007). Even though in this study anophelines were not very numerous, it is still very important to monitor their distribution and density, because they tend to fly into buildings, spend their daytime in shady areas and feed during the night. They are active from May until October, sometimes until November, depending on the season (Wegner, 2009b). In Lithuania, since 1956 there was no local malaria transmission recorded (Žygutienė *et al*., 1999), but imported cases are being registered (data from Lithuania's National Public Health Centre Under the Ministry of Health).

Even though *Cx. pipiens* is considered to be one of the most common mosquito species distributed throughout the Holarctic region and a main vector of West Nile virus (Wegner, 2009b; Weitzel *et al*., 2011), in our study they were rare, comprising 2.3% of all collected and identified mosquito larvae. More widespread and detailed research is needed to see if this species is not as common in Lithuania as in other European countries. It cannot be ruled out that during this study *Cx. pipiens* most favourable larval habitats were not found since in other studies adult *Cx. pipiens* are quite common in Lithuania (Valavičiūtė-Pocienė *et al*., 2024).

Since mostly mosquito females are studied, because of their feeding behaviour and vectorial capacity for various pathogens, it is also important to study larval stages, because it helps us to better understand the diversity of mosquitoes and can give new insight for mosquito control strategies (Westbrook *et al*., 2010; Vantaux *et al*., 2016; Gunathilaka *et al*., 2019).

Conclusions

Mosquito larvae were detected from March to September with the highest abundance determined in April-May. Various statistical analysis revealed that temporality of water bodies, amount of nitrates, pH level, bottom coverage, water temperature and collection time had a significant impact on abundance of mosquito larvae in water bodies. *Ae. cinereus* and *Cs. annulata* prefer temporary water bodies. Abundance of the later species also depends on bottom coverage of water bodies. Abundances of *Cx. territans*, *Oc. cantans*, *Oc. cataphylla* and *Oc. punctor* statistically significantly depend on the collecting time and the abundance of *Oc. cantans* depends also on pH and NO_3 levels. The abundance of *Cx. pipiens* was low but it showed relation to NO_3 amount in water.

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References

ALDEMIR A., DEMIRCI B., KIRPIK M. A., ALTEN B., BAYSAL A., 2009.- Species composition and seasonal dynamics of mosquito larvae (Diptera: Culicidae) in Igdir Plain, Turkey.- *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 15 (1): 103-110.

BECKER N., PETRIC D., BOASE C., LANE J., ZGOMBA M., DAHL C., KAISER A., 2003.- *Mosquitoes and their control*.- Kluwer Academic/Plenum Publishers, New York, USA.

BERNOTIENĖ R., LUČIŪNAITĖ V., 2011.- Mosquito (Diptera: Culicidae) species new for Lithuanian fauna.- *New and Rare for Lithuania Insect Species*, 23: 99-100.

BERNOTIENĖ R., VALKIŪNAS G., 2016.- PCR detection of malaria parasites and related haemosporidians: the sensitive methodology in determining bird-biting insects.- *Malaria Journal*, 15: 283.

BOCKOVÁ E., KOČIŠOVÁ A., HLAVATÁ H., 2013.- Evaluation of species composition and seasonal dynamics of mosquito larvae in the Košice Basin during 2010 and 2011.- *Biologia*, 68 (2): 337-344.

DOWLING Z., LADEAU S. L., ARMBRUSTER P., BIEHLER D., 2013.- Socioeconomic status affects mosquito (Diptera: Culicidae) larval habitat type availability and infestation level.- *Journal of Medical Entomology*, 50 (4): 764-772.

GUNATHILAKA N., UPULIKA H., UDAYANGA L., AMARASINGHE D., 2019.- Effect of larval nutritional regimes on morphometry and vectorial capacity of *Aedes aegypti* for dengue transmission.- *BioMed Research International*, 2019: 3607342.

GUNAY F., PICARD M., ROBERT V., 2018.- *MosKeyTool, an interactive identification key for mosquitoes of Euro-Mediterranean*.- Version 2.1 [online] URL: https://www.medilabsecure.com/moskeytool. Last update: 01/08/2018.

HARBACH R. E., 2023.- *Mosquito taxonomic inventory*.- [online] URL: http://mosquito-taxonomic-inventory.info/ (accessed November 20, 2023).

IBANEZ-JUSTICIA A., STROO A., DIK M., BEEUWKES J., SCHOLTE E. J., 2015.- National mosquito (Diptera : Culicidae) survey in The Netherlands 2010-2013.- *Journal of Medical Entomology*, 52 (2): 185-198.

KAZLAUSKIENĖ R., BERNOTIENĖ R., PALINAUSKAS V., IEZHOVA T. A., VALKIŪNAS G., 2013.- *Plasmodium relictum* (lineages pSGS1 and pGRW11): complete synchronous sporogony in mosquitoes *Culex pipiens pipiens*.- *Experimental Parasitology*, 133 (4): 454-461.

KHALIN A. V, AIBULATOV S. V., 2020.- Fauna of blood-sucking insects of the gnus complex in the northwestern region of Russia. III. Mosquitoes (Culicidae).- *Entomological Review*, 100 (1): 58-82.

LEHANE M. J., 2005.- *The biology of blood-sucking in insects* (2nd edition).- Cambridge University Press, New York, USA.

MERETA S. T., YEWHALAW D., BOETS P., AHMED A., DUCHATEAU L., SPEYBROECK N., GOETHALS P. L., 2013.- Physico-Chemical and biological characterization of anopheline mosquito larval habitats Diptera: Culicidae: implications for malaria control.- *Parasites and Vectors*, 6: 320.

OKECH B. A., GOUAGNA L. C., YAN G., GITHURE J. I., BEIER J. C., 2007.- Larval habitats of *Anopheles gambiae s.s.* (Diptera: Culicidae) influences vector competence to *Plasmodium falciparum* parasites.- *Malaria Journal*, 6: 50.

OLIVER J., HOWARD J. J., 2011.- Fecundity of wild-caught gravid *Culiseta morsitans* (Diptera: Culicidae).- *Journal of Medical Entomology*, 48 (2): 196-201.

PAKALNIŠKIS S., BERNOTIENĖ R., LUTOVINOVAS E., PETRAŠIŪNAS A., PODĖNAS S., RIMŠAITĖ J., SPUNGIS V., 2006.- Checklist of Lithuanian Diptera.- *New and Rare for Lithuania Insect Species*, 18: 16-154.

PODĖNAITĖ V., 1959.- The mosquito fauna of Lithuanian SSR [in Russian].- *Acta Parasitologica Lithuanica*, 2: 89-96.

PODĖNAITĖ V., 1962.- Breeding places of mosquitoes in the Lithuanian SSR [in Russian].- *Acta Parasitologica Lithuanica*, 4: 103-109.

RETTICH F., IMRICHOVA K., ŠEBESTA O., 2007.- Seasonal comparisons of the mosquito fauna in the flood plains of Bohemia and Moravia, Czech Republic.- *European Mosquito Bulletin*, 23: 10-16.

RUEDA L. M., 2008.- Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in freshwater.- *Hydrobiologia*, 595 (1): 477-487.

SALLUM M. A. M., SCHULTZ T. R., WILKERSON R. C., 2000.- Phylogeny of Anophelinae (Diptera Culicidae) based on morphological characters.- *Annals of the Entomological Society of America*, 93 (4): 745-775.
SENGIL A. Z., AKKAYA H., GONENC M., GONENC D., OZKAN D., 2011.- Species composition and monthly distribution of mosquito (Culicidae) larvae in the Istanbul metropolitan area, Turkey.- *International Journal of Biological & Medical Research*, 2 (1): 415-424.
SERVICE M. W., 1971.- Feeding behaviour and host preferences of British mosquitoes.- *Bulletin of Entomological Research*, 60 (4): 653-661.
SVOBODOVÁ M., VOLF P., VOTÝPKA J., 2015.- Trypanosomatids in ornithophilic bloodsucking Diptera.- *Medical and Veterinary Entomology*, 29 (4): 444-447.
SYNEK P., MUNCLINGER P., ALBRECHT T., VOTÝPKA J., 2012.- Avian haemosporidians in haematophagous insects in the Czech Republic.- *Parasitology Research*, 112: 839-845.
VALAVIČIŪTĖ-POCIENĖ K., KALINAUSKAITĖ G., CHAGAS C. F. R., BERNOTIENĖ R., 2024.- Avian haemosporidian parasites from wild-caught mosquitoes with new evidence on vectors of *Plasmodium matutinum*.- *Acta Tropica*, 256: 107260.
VALKIŪNAS G., 2005.- *Avian malaria parasites and other haemosporidia*.- CRC Press, Boca Raton, USA.
VANTAUX A., LEFÈVRE T., COHUET A., DABIRÉ K. R., ROCHE B., ROUX O., 2016.- Larval nutritional stress affects vector life history traits and human malaria transmission.- *Scientific Reports*, 6: 36778.
VARDYS V. S., SLOVEN W. A., 1996.- Lithuania: physical environment, pp. 186-189. In: *Estonia, Latvia and Lithuania: country studies. 1st ed* (IWASKIW W. R., Ed.).- Federal Research Division, Library of Congress, Washington DC, USA.
VOTYPKA J., SZABOVA J., RADROVA J., ZIDKOVA L., SVOBODOVA M., 2012.- *Trypanosoma culicavium* sp. nov., an avian trypanosome transmitted by *Culex* mosquitoes.- *International Journal of Systematic and Evolutionary Microbiology*, 62: 745-754.
VUJIĆ A., STEFANOVIĆ A., DRAGIČEVIĆ I., MATIJEVIĆ T., PEJČIĆ L., KNEŽEVIĆ M., VESELIĆ S., 2010.- Species composition and seasonal dynamics of mosquitoes (Diptera: Culicidae) in flooded areas of Vojvodina, Serbia.- *Archives of Biological Sciences*, 62 (4): 1193-1206.
WEGNER E., 2009a.- A study of mosquito fauna (Diptera: Culicidae) and the phenology of the species recorded in Wilanów (Warsaw, Poland).- *European Mosquito Bulletin*, 27: 23-32.
WEGNER E., 2009b.- The characteristics of the most troublesome mosquito species (Diptera: Culicidae) in Poland.- *Fragmenta Faunistica*, 52 (2): 157-179.
WEITZEL T., BRAUN K., COLLADO A., BECKER N., 2011.- Distribution and frequency of *Culex pipiens* and *Culex torrentium* (Culicidae) in Europe and diagnostic allozyme markers.- *European Mosquito Bulletin*, 29: 22-37.
WESTBROOK C. J., REISKIND M. H., PESKO K. N., GREENE K. E., LOUNIBOS L. P., 2010.- Larval environmental temperature and the susceptibility of *Aedes albopictus* Skuse (Diptera: Culicidae) to chikungunya virus.- *Vector-Borne and Zoonotic Diseases*, 10 (3): 241-247.
ŽYGUTIENĖ M., VIRBALIENĖ R., VEITIENĖ D., 1999.- Malaria problems in Lithuania.- *European Mosquito Bulletin*, 4: 14-15.

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