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The Monthly Oviposition Characteristics of the Mosquito Species *Anopheles maculipennis sensu stricto*, *Anopheles claviger*, and *Culiseta longiareolata* Observed at Some Artificial Containers under Natural Circumstances in a Selected Area of Thrace, Türkiye

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ABSTRACT

This study was carried out in order to determine the monthly oviposition characteristics of *Anopheles maculipennis Meigen sensu lato, Anopheles claviger Meigen sensu stricto*, and *Culiseta longiareolata Macquart* at the artificial containers in a selected area under the influence of "hot dry summer sub-climate type (Csa)" in Thrace, Turkiye. For the study performed between June 2015 and May 2016 on monthly basis, four artificial containers were placed on different sites in a selected periurban location, and the year-round breeding characteristics of the mosquitoes were monitored from egg-laying to adult emergence. The number of oviposited eggs and emerged adults recorded during the study were as it follows: five egg batches and eight adults of *An. maculipennis*, two egg batches and 55 adults of *An. claviger*, and five egg rafts and 185 adults of *Cs. longiareolata*. Our data indicates that *An. maculipennis* is a typical hot summer species, and *An. claviger* is active in spring. Although the number of eggs that can be observed is insufficient for precise inference, the results reveal that *Cs. longiareolata* may have a biphasic seasonal breeding pattern.

1. Introduction

Mosquitoes (Diptera: Culicidae), represented by more than 3500 species that have been identified to the present, can be seen across six continents, especially in tropical and subtropical climatic zones. Due to their importance as vectors for a wide range of debilitating pathogens, they are considered to be some of the most dangerous animals confronted by the mankind [1]. Moreover, environmental changes such as urbanization and deforestation are implicated in the expansion and increased incidence of mosquito-borne diseases in recent years, and this trend is expected to continue in the coming decades most likely in an increasing manner [2].

It has been always emphasized that the dynamics of mosquito and mosquito-borne diseases are quite complex, possibly due to the varying degree of influence of many factors [3, 4] whose severity of the influence may vary significantly regionally and from laboratory to nature [5, 6]. It was indicated that modellers may commonly ignore seasonality in favor of population dynamics in poorly studied and data-sparse systems, and many model systems neglect density-dependence due to the complexities associated with multiple abiotic and biotic population drivers [4]. Comprehending the population dynamics of the vectors is known to be crucial for understanding accurately the current dynamics and predicting the future occurrence of the vector-borne diseases, as well as determining the control strategies [4,7]. In relevant studies, surveillances usually focus on monitoring only the adult stage mostly during the peak transmission season; however, understanding the relative importance of density-dependent and -independent factors on mosquito abundance is crucial because different life stages inhabit different ecological niches [4].

This study was carried out with the following purposes: to determine the monthly breeding characteristics of *Anopheles maculipennis (An. maculipennis)*, *Anopheles claviger (An. claviger)*, and *Culiseta longiareolata (Cs. longiareolata)* in Turkish Thrace as well as to reveal the ability of An. maculipennis to use in periurban with no characteristic/priority breeding grounds for the species [1].

2. Materials and Methods

2.1. Geographic and climatic characters of the study region

Thrace (41°58' N, 27°22' E, at the center) is bordered by Greece, Bulgaria, Bosphorus Strait, Black Sea, Marmara Sea, and Aegean Sea. Tekirdag province, our main study area, is located in the south, on the coast of the Sea of Marmara. The northern part of Thrace has elevated mountains that run parallel to the Black Sea and are covered by dense, rainy, deciduous

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forests. There is also a certain number of mountainous and forested areas on the south-west coast of the region, extending from the province of Tekirdag to the west. Geographically, large plains and hilly surfaces occupy the interior and south parts which predominantly contain cultivable areas and comprise a rare belt of bush with short and relatively dry vegetation, maquis in places, and very rare woodlands in some highlands. In the coastal zone of Thrace, especially from the province of Tekirdag to the Bosphorus, urbanization is quite high; however, due to the afforestation, the proportion of wooded areas is mostly higher compared to the inner parts of Thrace, which is mostly composed of agricultural lands.

According to the records of the Turkish State Meteorological Service (https://mgm.gov.tr/eng/forecast-cities.aspx), Thrace has diverse and transitional weather conditions due to being surrounded by different climatic patterns such as the Black Sea, the Balkans, and the Mediterranean. The summer season is generally hot and moderately rainy, except the Black Sea coast receiving relatively higher precipitation than the inlands. The winters are cold and rainy, especially inland experiencing heavy snow and freezing temperatures below 0°C in places. During the study period, monthly meteorological parameters from January to December were as follows: average temperature (ºC) was 5.5, 6.5, 10.3, 15.9, 17.9, 21.3, 24.9, 26.1, 22.8, 16.4, 13.8, and 7.5, average relative humidity (%) was 80.3, 79.4, 81.3, 72.9, 75.3, 73.9, 71.3, 69.5, 77.9, 80.7, 81.4, and 80.6, total precipitation (mm= kg/m2) was 70.4, 90.6, 30.8, 18.3, 28.4, 58.4, 0.5, 0.0, 34.9, 19.8, 48.5, and 0.0, and average wind (m/sec) was 3.1, 2.7, 3.0, 2.2, 2.7, 2.8, 3.0, 3.4, 2.8, 3.2, 2.9, and 2.5, respectively.

2.2. Study area

The study was conducted in the agricultural study area of Tekirdag Namik Kemal University, Thrace, Türkiye (40º59'30"N, 27º34'41"E, altitude 14 m). This area is located on the coast of the Marmara Sea (almost 825 m to the seaside), on the outer margin of an urbanized area of the central district of Tekirdag province (Fig. 1). The area consists of woodland, fields, greenhouses, a pen complex containing 70 sheep, 6 goats, 30 hens, and 10 ducks, and ranging areas for animals.

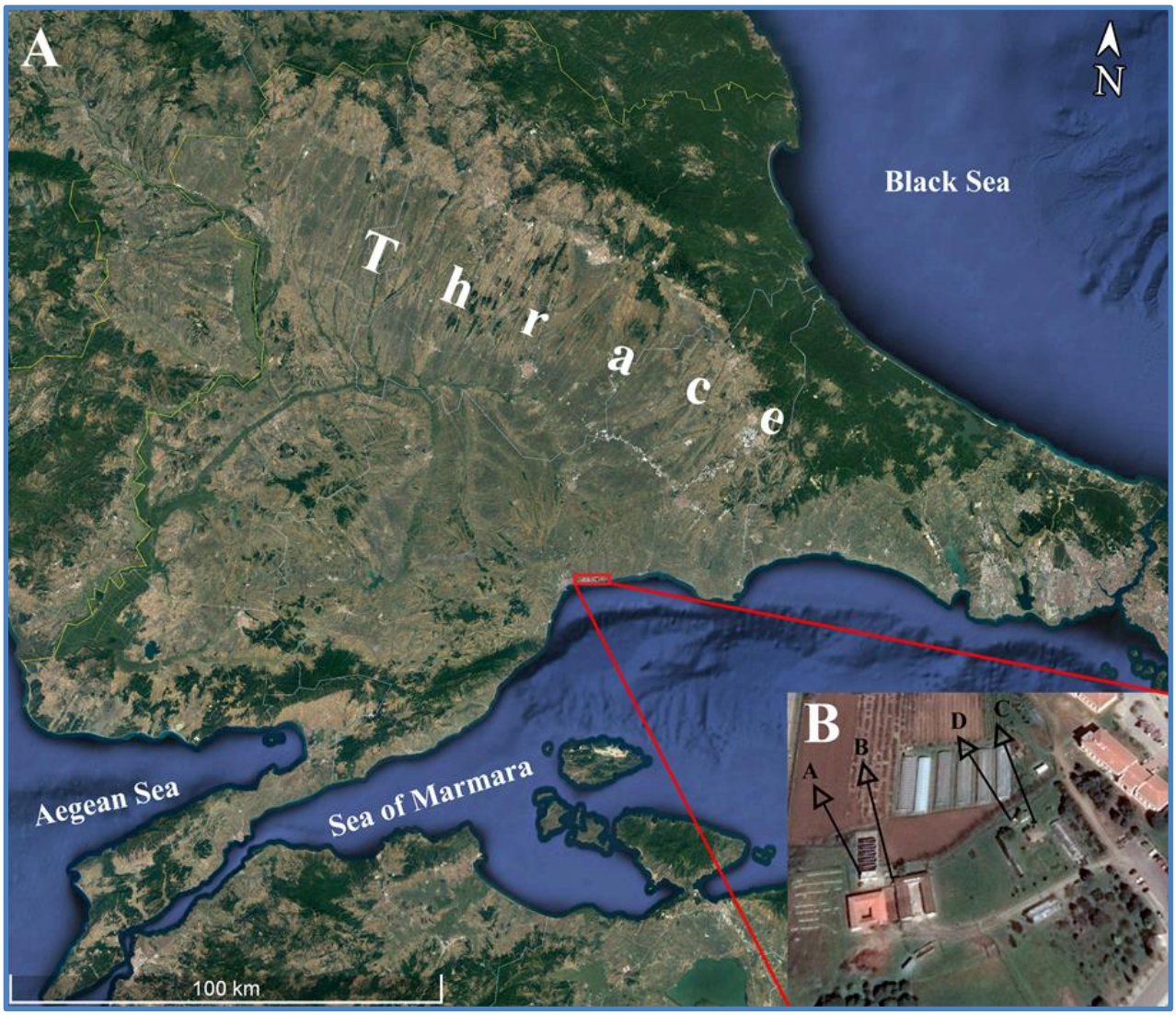

Fig. 1 Geographical location of the study area (A) and locations of gravitation containers (B).

2.3. Study units and gravitation conteiners

In order to make observations in a controlled manner, to eliminate the possible factors connected to the container which can be effective on the breeding characteristics of the mosquitoes [1], and to more accurately observe the effects of other parameters in this context, four artificial mosquito gravitation containers with same property were placed in the study area at the four locations (units A, B, C, and D) with some different characteristics (Fig. 1). By using units of different characters, we aimed to mimic the varying breeding containers found naturally in the habitat and therefore determine the seasonal mosquito breeding characteristics properly in the area. Container A was placed in a wastewater treatment plant (3.5x6x3.5 m), which is 9 m away from the pen complex. This concrete unit has a window (1x2 m) and a door (1x2 m), which are always open. Container B was placed on the floor of a cabin (1x1x1.8 m) next to the pen complex. This cabin, mostly made of wood, was open entirely on one side. Container C was placed on the floor of a similar type of wooden cabin located between the greenhouses with 85 m distance to the pen complex. Container D was placed on the floor of the concrete store (1.1x2x1.45 m), which has its door continuously open, and the distance between containers C and D was of 12 m [1].

The mosquito gravitation containers used in the study were made from black, a hard plastic material with dimensions 36x56 cm, h 15 cm. Some 8 lt of tap water with ~6 cm depth was added to each container. Because most of the mosquito species are attracted by organic matter in the water to gravitate [1], fry food (Mikromin, Pelagos, Türkiye; crude protein 44%, crude fiber 0.5%, ash 4%, vitamin A 2,700 Ul/kg, vitamin D3 3,500 Ul/kg, vitamin C 100 mg/kg, vitamin E 350 mg/kg) was sprinkled (8 mg per container) into the water.

2.4. Larval rearing and adult emergences

This study was conducted between June 2015 and May 2016. The gravitation containers were checked regularly (no more than 7 days apart) for the mosquito eggs and other aquatic forms. If any aquatic form was found in the controls, the relevant data was recorded and all water content of each container was transferred to a separate aquarium (26x29 cm, 2 cm wider at the top; h: 28 cm; transparent hard plastic). Following each unloading process, the containers were immediately washed, re-prepared with new water and fry food and placed back into the cabins.

During larval rearing, fry food was added to the water to feed mosquito larva considering the approximate number of eggs and larva, and notifications and warnings were followed [8-10]. At this point, possible adverse effects of excessive food on larval development in some mosquito species [11] were also considered.

In order to ensure that the larval development takes place directly in their breeding area, the aquariums, which were closed with the net, were placed on a desk in the concrete unit wherein container A was placed. No additional heating or humidifying was applied. Emerged mosquitoes were regularly collected from aquariums using an aspirator for live mosquitoes or using forceps for dead ones in the water, and stored at -20°C. Morphological identification of adults and selected samples from aquatic stages was made under a stereomicroscope using morphological keys described [1, 12].

3. Results

During the study period, four mosquito species were identified morphologically using adults and aquatic forms: *Culiseta longiareolata* (Macquart 1838), *Anopheles maculipennis* s.s. (Meigen 1818), *Anopheles claviger* s.s. (Meigen 1804), and *Culex pipiens* L. (detailed data not presented). The number of oviposited eggs and emerged adults recorded during the study were as follows: five egg batches and eight adults (1♂–7♀) of *An. maculipennis*, two egg batches and 55 (23♂–32♀) adults of *An. claviger*, and five egg rafts, and 185 (80♂–105♀) adults of *Cs. longiareolata* (Table 1).

The study area, in particular the interiors of buildings, was inspected for adult mosquitoes during the study period to obtain some qualitative data on the mosquito community naturally present in the environment. In this survey, *Cx. pipiens* was determined as obviously predominant species, and this species was followed by *An. maculipennis* (especially in the pen complex), and *Cs. longiaerolata*. However, adults of *An. claviger* were not encountered. *Culex pipiens* was encountered at the highest level in the daytime as resting mostly at walls of dwellings, predominantly concrete buildings mostly with high humidity. *Anopheles maculipennis* s.s. was seen particularly in the second half of summer and in September, which was seen predominantly in livestock shelters, especially in the pens of sheep and goats. It was revealed that *An. maculipennis* s.s. prefers mostly ceilings for resting in the daytime, which is bare or covered with the spider web. Interestingly, no hesitation was noticed in this species for resting at the spider web.

Table 1. Monthly distribution of eggs and emerged adults of the mosquito species.

Species	Units	Months	Counts of egg batches/rafts	Counts of emerged adults (male-female)
An. maculipennis	D	August	1	-
	A	August	1	-
	A	August	1	6 (1-5)
	B	August	1	1 (0-1)
	B	September	1	1 (0-1)
An. claviger	B	May	1	2 (1-1)
	D	May	1	53 (22-31)
Cs. longiareolata	B	April	2	11 (0-11)
	A	June	1	86 (33-53)
	A	October	1	86 (47-39)
	B	November	1	2 (0-2)
Total	Seven months		12	248 (104-144)

Culex pipiens, having been studied within the scope of the project, was not included in this study package. However, to determine the tendency of the mosquito species to use the same container simultaneously, all the aquatic forms of the mosquitoes encountered in the containers were transferred to the aquariums regardless of the species. Thus, it is aimed to obtain some preliminary data on the conditions of possible interferences and competitions in the process from ovulation to the adult emergences. As a result, it was found that *Cx. pipiens* can use the same container for oviposition as all the other three mosquito species, and in the same aquarium, larval development and adult emergence can continue simultaneously (Table 1).

4. Discussion

The egg-laying containers set up during the study were located in the near of the sheep and goat pens, and in the observations, many *An. maculipennis* eggs were found in the barn. The fact that *Cx. pipiens* lays a lot of eggs in the established units and a certain degree of organic content was created with the added fish food probably caused the species in question not to prefer the area. This is an expected result but surprisingly an extremely small number of adult flies developed from the groups of eggs laid. Although numerical records were not taken, it was observed that the larvae hatched from the eggs, but died before reaching the adulthood. This situation was associated with the larval density of other species in the environment.

In our study, a compatible correlation was determined in the seasonality of adults and oviposition of *An. maculipennis* s.s.. However, while it was possible to encounter a high adult population at any time in hot months in the pen complex in the study area, very few eggs and adult emergence were inspected during the study (Table 1). This may be due to the deterrent effect of the presence of *Cx. pipiens* aquatic forms in the containers on the oviposition, or that the prepared container conditions for the study were not suitable for oviposition, or both. The precise proof of this issue requires a more detailed and focused study.

Anopheles claviger is a zooanthropophile. It is mainly exophilic, but can often be found in confined spaces such as animal barns. Their feeding tendency changes depending on the availability of suitable animals, and they tend to suck blood from humans when these are not sufficient. Although it is accepted as an important malaria vector in the Mediterranean Region, it is not accepted as a malaria vector in other regions or it is thought that the effect on the transmission of malaria may be very small. Although no infected or infective specimens of *An. claviger* has been found in our country until today; due to the presence of sporozoite-positive females in Cyprus and Syria, it is thought that it may have an effect on the transmission of malaria in rural areas of Türkiye [13]. It has been stated that *An. claviger* can be vector for the agents such as malaria agents, *Setaria labiatopapillosa*, *Anaplasma spp.*, *Borrelia spp*., tularemia agents, Tahyna virus, Batai virus, and myxomatosis [12, 13]. Among the breeding areas determined in Türkiye for *Anopheles claviger*, there are areas such as streams [14], ponds [14], swamps [15], lakeside [16], canals [16], and artificial water container [17].

In the observations made at the station located in the near of the sheep and goat pens and the spawning containers established during the study period, it was observed that the number of *Cs. longiareolata* was not sufficient in the barn. This was associated with the predominance of larval density of other species in the environment. In the observations made at the reinforced concrete water treatment station, it was observed that the species reproduced more adequately. This situation is associated with the fact that its adaptation to polluted water in the area is more dominant than the other species. In addition, *Cs. longiareolata* was most active in June in summer and most active in October in autumn. Only female species were seen in April and November, which are the transition months in terms of weather conditions. This can be explained by the fact that females are more adaptable to these weather conditions. The reason why it is not seen in other months can be explained by the dominance of other species and other species being more dominant in the environment. Although numerical records are not taken, the first and other generations of the hatched larvae are also found. This situation is associated with the adaptation of the species to the environment.

Due to the small number of species other than *Cx. pipiens*, significant data about the inter-specific competition at the level of larvae or adults could not be reached in this study. However, in terms of *Cx. pipiens*, since late autumn is essential for winterizing adult females, early spring means the beginning of the breeding season, it is understood that any competition in these two periods when the population is low, may affect the population much more than in other periods of the year. Thus, a possible competition, especially between *Cx. pipiens* and *Cs. longiareolata* that can be encountered in these periods, seem to be logical and worthy of research in respect of the natural population dynamics of these species.

Competition between the mosquito species is a well-known issue that occurs especially among the larval stages of container species. Many different factors affecting the characteristics of this competition are determined such as populations, species, instar, stages and sex of competitors, larval density, availability and quality of food, and temperature [18-20]. The related studies have shown the interference of the 4th instar larva of *Cs. longiaerolata* upon larva of *Cx. pipiens*, *Ae. caspius* and *An. multicolor* [20, 21], larval interference of *Ae. aegypti* on *Cx. pipiens* [22] and *Ae. albopictus* [18], and *Ae. albopictus* on *Ae. triseriatus* [23] and *Cx. pipiens* [19, 24]. In this concept, predation capacity which could be seen at the larval stages of some mosquito species such as *Culiseta spp*., *Aedes spp*., and *Culex spp*., is expected one of the most important instruments [1]. In addition to this direct impact, the interference could come into existence by decreasing larval survival, adult size, wing lengths, female emergence [22, 25, 26], and possibly as a result of these stressed conditions, an increase of the viral infection, body titer, and dissemination rates [18].

5. Conclusion

The results of our study indicate the competition superiority of *Cx. pipiens* upon *An. maculipennis* and *An. claviger* rearing in the same aquariums. However, no obvious competition was seen between *Cx. pipiens* and *Cs. longiaerolata,* at least under our rearing circumstances. The data shows that there is more than one factor affecting the characteristics of this phenomenon as indicated above [18-20]. Actually, the main interference inspected in our study suppressed egg-laying performance of *An. maculipennis*, at least less than our expectation. Our investigation shows that it was common in the barn in the study area, particularly in summer. But surprisingly, just 5 *An. maculipennis* egg batches were detected in the containers. Indeed, it has been stated that this species could benefit from relatively dirty water due to organic substance, and they could be found with *Culex spp*. in the same water content [1]. Although our study area did not contain plenty of watery areas that could be benefited from the species, *An. maculipennis* did not exhibit an obvious tendency to lay eggs in the containers which were inhabited mostly by larval forms of *Cx. pipiens*. Although this interference style is not a well-described issue among the mosquito species in the natural environment [27], it was reported that controphic species could alter oviposition habits as a result of competition on the rearing side [28]. Furthermore, there are many inspections on the avoidance of oviposition into waters containing possible kairomones belonging to some predators [23, 28, 29]. In general, the intra- and inter-specific competition interactions among the mosquitoes have been assumed to be important ecologically and medically due to their effects on the prevalence and incidence of mosquito species and related infections [19, 24], and, because of these features, mosquito larvae have been prompted as a model system for testing some ecological theories [27].

Declaration

Author Contribution: Conceive-S.K., A.G.K.; Design-Y.A., S.K., E.I., D.S.; Supervision-S.K.; Experimental Performance, Data Collection and/or Processing Y.A., S.K., E.I., Z.S., S.T.; Analysis and/or Interpretation Y.A., S.K., D.S., G.A., A.G.K.; Literature Review- S.K., G.A., Z.S.A., Z.S.; Writer- S.K., G.A., Z.S Critical Reviews - D.S., A.G.K.

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