Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(2): 289 – 304 (2023)
www.ejabf.journals.ekb.eg

Species Composition, Larval Habitats and Co-Occurrence of Mosquitoes (Diptera: Culicidae) in Damietta Region, Egypt

Mohammed M. Elbokl[1,*], Eman A. Hewait[1], Mosaad M. Hassan[2]
[1]Department of Zoology, Faculty of Science, Damietta University, Egypt
[2]Department of Entomology, Faculty of Science, Ain Shams University, Egypt
***Corresponding author: mmelbokl@du.edu.eg**

ARTICLE INFO

Article History:
Received: June 9, 2022
Accepted: Jan. 29, 2023
Online: March 21, 2023

Keywords:
Mosquitoes,
Breeding habitats,
Distribution,
Abundance,
Damietta,
Egypt

ABSTRACT

The patterns of mosquito species composition in Damietta governorate, northeast Egypt and ecological data are essential in mosquito vector control management. Thus, the current study provided comprehensive data on species composition, abundance and distribution of mosquito larvae in Damietta governorate in addition to the physicochemical properties of mosquito larval habitats, as well as exploring various aspects of mosquito ecology such as co-occurrence and association in the larval habitats. Via the dipping technique, water samples were collected during larval collection (March-December 2019) from seven localities. Water temperature (°C), acidity (pH), salinity (ppt), dissolved oxygen (mg/l), electrical conductivity (μS/cm) and turbidity (NTU) were assessed using standard methods. The Spearman correlation coefficient was used to analyze the relationship between mosquito larval density and habitat characteristics. In total, 35809 mosquito larvae were collected representing 9 species and five genera. The species composition was as follows: *Culex antennatus, Cx. pipiens, Anopheles pharoensis, Ochlerotatus caspius, Cx. pusillus, Cx. perexiguus, Culiseta longiareolata, An. Tenebrosus* and *Uranotaenia unguiculata.* The density of *Cx. pusillus* showed a significant negative correlation with salinity and electrical conductivity but the density of *Oc. caspius* recorded a highly significant positive correlation with dissolved oxygen. There was a highly significant negative correlation between the density of *Ur. unguiculata* and turbidity. The highest association was detected between a pair of species, *Cx. pusillus* and *Oc. caspius* (0.256).

INTRODUCTION

Mosquitoes are vectors for various pathogens that pose a threat to human and animal health around the world **(Franklinos *et al.*, 2019; Chandrasegaran *et al.*, 2020)**. Due to their epidemiological impacts on viral infections, several mosquito species are among the world's most serious arboviral vectors **(Tandina *et al.*, 2018; Jones *et al.*, 2020)**. Among these vectors, Zika **(Kauffman & Kramer, 2017)** and Chikungunya **(Prudhomme *et al.*, 2019)** are identified. In Egypt, additional outbreaks of rift valley fever took place in 1993, 1999 and most recently in 2003 **(Hanafi *et al.*, 2011)**. The principal mosquito vectors of the rift valley fever virus in the Nile Delta are *Cx. pipiens* L. and *Cx. antennatus* Becker **(Turell *et al.*, 2002; Hanafi *et al.*, 2011)**. *Cx. pipiens* is the major vector responsible for filariasis transmission in Egypt **(Gad *et al.*, 1987a)**. Bancroftian filariasis is primarily found in Egypt's Nile Delta **(Harb *et al.*, 1993)**. The composition and abundance patterns of mosquito species are affected by ecological parameters, such as temperature, humidity, and the availability of appropriate breeding sites **(Grillet, 2000)**, which differ seasonally. Seasonality impacts larval development and adult mosquito abundance, and subsequently indirectly affects disease transmission **(Preechaporn *et al.*, 2007)**. Generally, mosquitoes breed in different habitats with various types of water that are known to be particularly suitable for many species **(Seghal & Pillai, 1970)**. The parameters that influence mosquito oviposition site selection play a critical role in larval density and species composition **(Hanafi-Bojd *et al.*, 2012; Nikookar *et al.*, 2017)**. The optimal temperature and pH range for the existence and abundance of mosquito larvae species have been studied and identified **(Clements, 1992)**. Unfortunately, no more suitable information are found about mosquitoes in Damietta governorate. Remarkably, the survey of mosquito species associations and the calculation of related indices are essential. They can provide crucial information for a better understanding of their biology and significance in pathogen transmission. Co-occurrence and association between larval species may be due to similar breeding requirements or adult feeding habits **(Marcondes & Paterno, 2005)**. Mosquito abundance and their associated diseases necessitate a broad vector control strategy based on reliable biological and ecological information about mosquito vectors. Therefore, this study was conducted to provide details about the variation in species composition, abundance, distribution of mosquito larvae and the effect of some ecological factors on larval density.

MATERIALS AND METHODS

Study area

Damietta governorate is located on the Mediterranean Sea, northeast the Nile-Delta. It lies between the coordinates 31^o 28’ 29”, 32^o 03’ 32” E, and 31^o 09’ 28”, 31^o 31’ 45” N, as shown in Fig. (1). It covers about 1029 km^2 and represents about 4.7% of the Delta region and 1.22% of Egypt’s total land area. It comprises four districts, with ten major cities, 47 rural units, 85 villages and 722 Kafrs. It has a Mediterranean climate that is hot, dry in summer and cold, rainy in winter. The lowest air temperature ranges between 9°C in January and 21.8°C in July, while the highest temperature ranges between 17.9°C in January and 30.6°C in July (the general authority of meteorology, unpublished data, 2017). Water is the second most abundant land cover of Damietta. The average annual rainfall is approximately 125.4mm **(Elnaggar *et al.*, 2017)**. It occupies 223km^2 (25%) and comprises Lake Manzalah, the Nile and the Damietta seaport.

The study was conducted in 7 localities in Damietta (Fig. 1). Certain sites in each locality were chosen for mosquito sampling. From March to December 2019, each site was surveyed biweekly.

Identification of mosquito breeding places

Mosquito larvae were gathered from various water bodies for this study. Sampling sites included different mosquito breeding habitats, such as pools, sakia pits, rice fields and irrigation/drainage canals. Mosquitoes were biweekly gathered by the dipping method, using a small ladle (200ml). Ten dips (a survey unit) were taken from each habitat. Bottles or vials were used to transfer collected larvae. The bottles were labelled (date, sampling site name) and then taken to the laboratory. Larval numbers were counted and calculated. Some larvae (about 10%) were reared to adulthood to verify species identification, while others were kept in 70% alcohol and classified according to a key developed by **Harbach (1985, 1988)**. Along with mosquito collections, water temp., pH and relative humidity (RH) were measured.

Statistical analysis

Data analysis was done using SPSS software (version 22 for Windows). The Spearman correlation coefficient was used to analyze the relationship between mosquito larval density and habitat characteristics such as temperature, pH and turbidity. The distribution pattern of mosquito larvae in Damietta region (C%) was evaluated by using the following formula **(Rydzanicz & Lonc, 2003)**: C=n/N*100; Where, C = distribution; n = number of sites positive for the occurrence of mosquitoes, and N =the total number of investigated sites. Mosquito species were categorized into five groups based on their distribution value; if C= 0–20%, then the species' distribution pattern is sporadic; whereas, if C = 20.1–40%; the pattern would be infrequent, and in case C = 40.1–60%; a moderate pattern is verified, and if C = 60.1– 80%, then it is frequent, while, when C = 80.1–100%; it is constant. Density was estimated as the percentage of specimens for a specific species in the whole sample, utilizing the followig formula: D =I/L*100%; Where, D = density; l = number of specimens of each mosquito species, and L = total number of specimens. The following density classes were used: <u>Satellite species</u> (I think that this expression cannot be applied here)(D<1%)-Subdominant species (1<D<5%)-Dominant species (D>5%). The formula shown below was used to determine the association index: I = 2[J/(A + B) – 0.5], where J represents the number of individuals of both species in samples where they co-occur, and A and B represent the total number of individuals of both species in all samples.

RESULTS

Species composition, abundance and spatiotemporal distribution

Following our survey covering seven localities in Damietta governorate, 35809 mosquito larvae (Diptera-Culicidae) were collected and identified. Data recorded nine mosquito species belonging to five genera: *Anopheles, Culex, Culiseta, Ochlerotatus* and *Uranotaenia.* The genus *Culex* showed more diverse with four species, followed by *Anopheles* with two species, *Ochlerotatus, Culiseta* and *Uranotaenia,* with one species each. Among the culicines, *Culex pipiens* Linnaeus, *Cx. antennatus* Becker, *Cx. pusillus* Macquart, and *Cx. perexiguus* Theobald were collected. The genus *Anopheles* was mainly dominated by *An. pharoensis* Theobald, followed by *An. tenebrosus* Dönitz. Other species found were *Ochlerotatus caspius* Pallas, *Culiseta longiareolata* Macquart and *Uranotaenia unguiculata* Edwards (Table 1). *A. tenebrosus, Cx. pusillus, C. longiariolata, Oc. caspius, Ur. unguiculata* were reported for the first time in Damietta governorate.

The distribution was as follows: *Cx. antennatus* (60.87%) was recorded only as frequent in Damietta region. It was found in all localities. While, *Cx. pipiens* (52.17%) was moderate; *An. pharoensis* (36.96%), *Oc. caspius* (30.43%), *Cx. Pusillus* (28.26%) and *Cx. Perexiguus* (26.09%) were infrequent; and other species *Cs. Longiareolata* (17.39%), *An. tenebrosus* (15.22%) and *Ur. unguiculata* (6.52%) appeared sporadically (Table1). In terms of density, *Cx. pipiens* (44.16%), *Cx. antennatus* (24.12%), *Cx. pusillus* (15.48%) and *Oc. caspius*

(9.16%) were dominant species; *Cs. Longiareolata* (3.42%), *Cx. perexiguus* (1.66%) and *An. pharoensis* (1.51%) were subdominant; *An. tenebrosus* (0.32%) and *Ur. unguiculata* (0.17%) were satellite species.

In **Ezbet El Borg** 31° 50' 28"N 31° 30' 11"E county located in northern Damietta province, there were four larval sites descripted as irrigation with sewage effluents (end of Alsharkawyia irrigation canal) and three pools of stagnant water; those were closed to human and animal houses. In these larval habitats harbored *Cx. pipiens* (8732/12468), *Cx. pusillus* (2323/12468), followed by *Cs. Longiareolata*, *Cx. antennatus, Oc. caspius, Cx. perexiguus* and *Ur. Unguiculata* (Table1). In **Kafr Saad** 31° 21′ 21″ N 31° 41′ 5″ E is in southeastern Damietta province, there were eight larval sites described as irrigation/drainage ditches, sewage pool and sewage tank. These sites contain *Cx. pipiens* (5454/8530), *Cx. antennatus* (2504/8530), *An. pharoensis, Cs. Longiareolata, Oc. caspius, Cx. perexiguus* and *An. tenebrosus.* **Kafr Al Battikh** 31° 24' 14" N 31 44' 16" E is a rural area closed to New Damietta city, including larval habitats such as small irrigation/drainage ditches around rice fields, sewage pools and footprints. *Cx. antennatus* (2910/4507), *Cx. pipiens* (808/4507), *Cs. longiareolata, Oc. caspius, Cx. perexiguus. An. pharoensis* and *An. tenebrosus* were gathered (Table 1). **New Damietta** 31° 26' 12.48" N 31° 40' 1.2" E (recent urban community city along of Mediterranean Sea) is found northeastern Damietta province, where larval sites are represented as wetlands from groundwater and rainfall swamps. *Cx. pusillus* (3222/6880), *Oc. caspius* (2643/6880), *Cx. pipiens, Cx. perexiguus, Cx. antennatus, An. pharoensis* were obtained in this locality. **Al Khayata** village 31°27'40" N 31°49'06" E lies along the Damietta branch of the Nile River. Larval sites are represented as irrigation/drainage ditches, rice fields and cement tanks. *Cx. antennatus* (585/731), *An. pharoensis, An. tenebrosus, Cx. pipiens, Cx. perexiguus, Ur. Unguiculata* and *Oc. caspius* were collected. **El-Sheikh Dorgham** village 31°29'31"N 31°50'18" E lies along the the Nile River (Damietta branch); the larval sites were irrigation/drainage ditches, cement tank, Sakia pit. *Cx. antennatus*, (2011/2431), *Cx. pipiens* (224/ 2431) *Cx. perexiguus, An. tenebrosus, Ur. Unguiculata, An. pharoensis, Cs. longiareolata* were detected. In **Faraskur** center 31°19'47"N 31°42'53" E, the larval sites were irrigation/drainage ditches, and only *An. pharoensis* and *Cx. antennatus* were recorded.

Physicochemical parameters of breeding water

Table 2 illustrates the means ± SD of various variables of larval habitats for nine species collected in the study. We found a highly significant difference in temperature, salinity, conductivity and turbidity among the different species ($P< 0.001$); however, the difference was significant for pH and dissolved oxygen ($P < 0.05$).

In Table (3), The Spearman correlation coefficient results indicate that densities of *An. Pharoensis* & *Cx. antennatus* were directly related to temperature i.e., they increase as temperature increases while those of *An. tenebrosus, Cx. perexiguus, Cx. pipiens, Cx. pusillus, Oc. caspius* and *Cs. longiareolata* were indirectly related to temperature. Larval densities of all species were directly related to pH, except *Cx. antennatus*. Only the abundance of *Cx. antennatus* and *Cx. pusillus* had negative correlation with salinity, while that of *Cx. pusillus* was statistically significant ($r = -0.34$, $P<0.05$). The density of *Cx. pusillus* had significant negative correlation with EC ($r = -0.34$, $P<0.05$). Larval densities of *Cx. perexiguus, Cx. pusillus*, *Oc. caspius*, *Ur. unguiculata* had positive relation with dissolved oxygen in breeding habitats, while those of other species were negatively correlated. The abundance of *Oc.*

caspius recorded highly significant direct correlation with dissolved oxygen (r = 0.42, *P*<0.01). It was noticed also that the density of *Ur. unguiculata* had highly significant indirect correlation with turbidity (r = -1, *P*<0.01).

Co-occurrence and association index between collected species

In Table (4), *Cx. pipiens* had the highest association with *Cs. longiareolata* (6394, 40.44%), followed by *Cx. antennatus* (3166, 20.02%). *Oc. caspius* showed the highest association with *Cx. pusillus* (1965, 59.93%). It demonstrated the lowest association of *An. pharoensis, Cx. Antennatus* and *Oc. caspius* (1, 0.01%).

Mosquito larvae were found in 291 co-occurrences in 46 breeding sites during the investigation, as shown in Table (6). The highest co-occurrence observed is related to *Cx. antennatus* (67 co-occurrences, 23.02% of the total) and *Cx. pipiens* (54 co-occurrences, 18.56% of the total), respectively. Of these, *An. pharoensis* was found in 27 occasions in association with *Cx. Antennatus*, and *Cx. pusillus* was found in 27 occasions with *Oc. Caspius*, which showed the highest co-occurrence for the species. *Ur. unguiculata* (4, 1.37% of the total) and *An. tenebrosus* (9, 3.09% of the total) showed the lowest co-occurrence in the study. The co-occurrence data of the rest of the species are displayed in Table (6). The positive association were observed only between the pair of species, *Cx. pusillus* and *Oc. caspius* (0.256).

DISCUSSION

The species composition, relative abundance, seasonal variation and physicochemical characteristics of mosquito breeding sites in Damietta region were described in this study. Nine mosquito species from five genera were present: four *Culex* species, two *Anopheles* species, and one each of *Ochlerotatus*, *Culiseta* and *Uranotaenia*. These species were collected from 46 sites during the study period from March to December 2019. Commonly, it was noticed that mosquito breeding localities in Damietta governorate could be divided into rural areas (Ezbet El Borg, El-Sheikh Dorgham and Al Khayata) and urbanized areas (New Damietta city). In this survey, four species of the genus *Culex* were gathered, *Cx. pipiens*, *Cx. antennatus* and *Cx. pusillus* had the highest abundance, respectively, and *Cx. perexiguus* had the lowest abundance. Whereas, the prevalence of the genus *Culex* was *Cx. antennatus*, *Cx. pipiens*, *Cx. pusillus,* and *Cx. perexiguus,* respectively. From the genus *Anopheles*, two species were collected (*An. pharoensis* and *An. Tenebrosus*). *An. pharoensis* was more abundant and prevalent than *An. tenebrosus* and its breeding sites were spatially and temporally associated with rice cultivation. *An. pharoensis* was recorded previously in Damietta by **El-Said and Kenawy (1983)**. *An. pharoensis* is unique as it prefers a rice field habitat for breeding **(Gad *et al.*, 1982)**. The association of *An. pharoensis* with rice plantation has been fully documented **(Kirkpatrick, 1925; Halawani & Shawarby, 1957; Zahar, 1974)**, and the impact of such association on species distribution in Egypt was discussed in detail in the study of **Kenawy and El-Said (1990)**. *Cs. longiareolata* and *Ur. unguiculata* were discovered for the first time in Damietta, whereas *Cs. longiareolata* was found at most breeding sites in Egypt, including most the Egyptian governorates as Ismailia; Sharkia (**Kenawy *et al.*, 1996; Abdel-Hamid *et al.*, 2011a**); Menoufia **(Abdel-Hamid *et al.*, 2011b)**;

Qaluobia **(Ibrahim *et al*., 2011)** and Cairo **(Ammar *et al.*, 2012)**. *Ur. unguiculata* was recorded only in the Suez Canal zone in Egypt **(Sowilem *et al.*, 2017)**.

In Egypt, *Oc. caspius* is one of the most frequent mosquito species throughout various geographical regions **(Gad *et al.*, 1987b; Harbach *et al.*, 1988)**. In our study, *Oc. caspius* was encountered with the highest percentage in New Damietta city (urbanized area) (*ca.* 80%, n=2643), where breeding sites were represented by swamps of groundwater. The density and distribution of *Oc. caspius* in Damietta need to be investigated further regarding this species incriminated to transmit Rift Valley Fever virus in Egypt **(Gad *et al.*, 1987b; Turell *et al.*, 1996)**. This study found that environmental factors may have affected the abundance of mosquitoes across life stages at the breeding sites. From the Spearman correlation coefficient, all culicine species, except for *Cx. Antennatus*, decrease in density when temperature increases and pH decreases. The exact correlation was detected with *Cs. longiareolata* and *Oc. caspius*. The density of *An. pharoensis* increases as the temperature and pH increase. The observed mean pH at the breeding sites ranged from 7.6 to 8.3 for all species encountered.

Based on water salinity, the breeding habitats of nine species detected in the study had (1.4-38.5 ppt) brackish to saline water. The larval densities of *Cx. antennatus* and *Cx. pusillus* were indirectly related to salinity, with a significant difference for *Cx. pusillus* ($P < 0.05$), while the other species were directly related to salinity (r = 0.01 to 0.5). From the species sampled, *Oc. caspius* breeding habitats were characterized by high salt content (mean 38.5 ppt ± 23.02). In a previous study, **Gad *et al.* (1987c)** found that *Oc. caspius* inhabited breeding pools with high salt content. **Ibrahim *et al.* (2011)** stated that *A. caspius* in Akrasha village (Qalyubia governorate) was detected in a foul-smelling pool with a salinity up to 1.67 ppt. **Kenawy *et al.* (2013)** found that the larval habitats of the collected five species (*Cx. perexiguus*, *Cx. pipiens*, *Cx. pusillus*, *Cs. longiareolata*, and *Oc. caspius*) contained fresh/brackish water (0.05-1.2%) in two urban areas of the Cairo governorate. *Cx. pusillus* larvae were common in salt marches, salt lacks, and brackish pools in Egypt, according to **Wasim (1993)**.

The Spearman correlation with conductivity revealed that the larval densities of *Cx. antennatus*, *Cx. pusillus,* and *Ur. unguiculata* increase as conductivity decreases with a significant difference with *Cx. pusillus,* while the rest of species were directly related to conductivity. From previous studies, **Emidi *et al.* (2017)** concluded that electrical conductivity had a significant positive correlation with the abundance of *Anopheles* larvae in Tanzania. **Musonda and Sichilima (2019)** also revealed a significant positive relationship between electrical conductivity and larval density of *Anopheles* mosquitoes in Zambia.

There was a highly significant positive correlation between the density of *Oc. caspius* and dissolved oxygen ($P < 0.01$), whereas positive correlation with *Cx. perexiguus, Cx. pusillus, and Ur. unguiculata* and a negative correlation with the rest of species. **Sehgal and Pillai (1970)** reported that breeding waters of *Aedes* showed higher oxygen content than those of *Culex* and *Anopheles*. **Kenawy *et al.* (2013)** observed that *Oc. caspius* needs higher dissolved oxygen than those of *Culex* species.

Larval densities of *An. pharoensis, Cx. antennatus, Cx. pusillus, Cx. pipiens, and Cs. longiareolata* were directly related to the turbidity of the breeding habitats. Whereas densities of *Oc. caspius, Cx. perexiguus,* and *An. tenebrosus* were indirectly related to water turbidity.

Ur. unguiculata had a highly significant negative correlation with the turbidity of breeding habitats (P<0.01). Culicine sp. and *An. pharoensis* had a high tolerance to highly turbid water. The lowest mean of water turbidity was observed in this study for *Oc. caspius.* Sattler, *et al.*, (2005) reported that in turbid breeding sites culicine larvae were much more likely to be present, whereas *Anopheles* larvae were much more likely to be absent.

As far as we know from the available literature this is the first study on co-occurrence and association indices of mosquito larvae in this region. A positive association was observed between the species *Oc. caspius* and *Cx. pusillus,* whereas negative associations were observed for the rest of the species. The association of mosquito species identifies them as controphic species, implying that they have similar habitat requirements.

In the present investigation many vectors of human and domestic animal pathogens such as *Cx. pipiens, Cx. antennatus, Oc. caspius,* and *An. pharoensis* were collectd fron Damietta governorate. Various ecological factors, such as active season, host preference, and population dynamics of these species require extensive research.

CONCLUSION

The prevalence and abundance of mosquito species mainly the disease vectors such as *Cx. pipiens, Cx. antennatus, Oc. caspius* and *An. pharoensis* in Damietta that generated during the present investigation will provide the vector control authorities with more information that would assist in their control activities and prioritization.

ACKNOWLEDGEMENT

The authors are grateful to the Ministry of Health, Staff in the Department of Vector Control in Damietta Governorate for kind allowing and facilitating this field study. Thanks, are also due to our colleague Dr. Wael Salah El-Tohamy Assistant Professor of marine invertebrates, faculty of Science, Damietta University for his assistance in statistical analysis of the obtained data.

REFERENCES

Abdel-Hamid, Y.M.; Soliman, M.I. and Kenawy, M.A. (2011a). Mosquitoes (Diptera: Culicidae) in relation to the risk of disease transmission in El Ismailia Governorate, Egypt. J. Egypt. Soc. Parasitol., 41(2): 347-356.

Abdel-Hamid, Y.M.; Soliman, M.I. and Kenawy, M.A. (2011b). Geographical distribution and relative abundance of culicine mosquitoes in relation to transmission of lymphatic filariasis in El Menoufia Governorate, Egypt. J. Egypt. Soc. Parasitol., 41(1): 109-118.

Ammar, S.E.; Kenawy, M.A.; Abdel-Rahman, H.A.; Gad, A.M. and Hamed, A.F. (2012). Ecology of the mosquito larvae in urban environments of Cairo Governorate, Egypt. J. Egypt. Soc. Parasitol., 42(1): 191–202.

Chandrasegaran, K.; Lahondère, C.; Escobar, L.E. and Vinauger, C. (2020). Linking mosquito ecology, traits, behavior, and disease transmission. Trends in Parasitology, 36(4): 393–403.

Clements, A.N. (1992). The biology of mosquitoes. Volume 1: development, nutrition and reproduction: Chapman & Hall.

Elnaggar, A. A.; El-Hamdi, Kh. H. and Daibes, T. Y. (2017) Fertility Evaluation of Some Soils in Damietta Governorate, Egypt Using GIS. J. Soil Sci. and Agric. Eng., Mansoura Univ., Vol. 8(2): 85 – 92.

El-Said, S. and Kenawy, M. (1983). Anopheline and culicine mosquito species and their abundance in Egypt. J. Egypt. Publ. Hlth. Assoc., 58: 108-42.

Emidi, B.; Kisinza, W.N.; Mmbando, B.P.; Malima, R. and Mosha, F.W. (2017). Effect of physicochemical parameters on *Anopheles* and *Culex* mosquito larvae abundance in different breeding sites in a rural setting of Muheza, Tanzania. Parasites & Vectors, 10: 304.

Franklinos, L.H.; Jones, K.E.; Redding, D.W. and Abubakar, I. (2019). The effect of global change on mosquito-borne disease. Lancet Infect Dis., 19(9): e302–e312.

Gad, A.M.; El-Said, S.; Soliman, B.A.; Hassan, A.N. and Shoukry, A. (1987a). Distribution and bionomics of Egyptian *Culex univittatus* (Theobald). J. Egypt. Soc. Parasitol., 17(1):17-31.

Gad, A.; Hassan, M.; EI-Said, S.; Moussa, M. and Wood, O. (1987b). Rift Valley fever transmission by different Egyptian mosquito species. Trans. Roy. Soc. Trop. Med. Hyg., 81(4): 694-8.

Gad, A.M.; El-Said, S.; Hamed, M.S.; Soliman, B.A. and Abdel-Mohsen, A. (1987c). Distribution and bionomics of Egyptian *Cx. antennatus* (Becker). J. Egypt. Soc. Parasitol., 17(2): 591-608.

Gad, A.M.; Kenawy, M.A.; El-Said, S. and Merdan, A. (1982). Field studies on Anopheline mosquito larvae in Egypt (Diptera: Culicidae). 1. Different types and characteristics of the breeding places in relation of the abundance of Anopheline species in Egypt. J. Egypt. Pub. Hlth. Ass., 57: 541-561.

Grillet, M.E. (2000). Factors associated with distribution of *Anopheles aquasalis* and *Anopheles oswaldoi* (Diptera: Culicidae) in a malarious area, northeastern Venezuela. J. Med. Entomol., 37(2): 231–238.

Halawani, A. and Shawarby, A.A. (1957). Malaria in Egypt. J. Egypt. Med. Assoc., 40: 753-92.

Hanafi, H.A.; Fryauff, D.J.; Saad, M.D.; Soliman, A.K.; Mohareb, E.W.; Medhat, I.; Zayed, A.B.; Szumlas, D.E. and Earhart, K.C. (2011). Virus isolations and high population density implicate *Culex antennatus* (Becker) (Diptera: Culicidae) as a vector of Rift Valley fever virus during an outbreak in the Nile Delta of Egypt. Acta Tropica, 119(2-3): 119–124.

Hanafi-Bojd, A.; Vatandoost, H.; Oshaghi, M.; Charrahy, Z.; Haghdoost, A.; Sedaghat, M.; Abedi, F.; Soltani, M. and Raeisi, A. (2012). Larval habitats and biodiversity of anopheline mosquitoes (Diptera: Culicidae) in a malarious area of southern Iran. J. Vector Borne Diseases, 49(2): 91-100.

Harb, M.; Faris, R.; Gad, A.M.; Hafez, O.N.; Ramzy, R.M. and Buck, A.A. (1993). The resurgence of lymphatic filariasis in the Nile Delta. Bull. World Health Organ., 71(1): 49–54.

Harbach, R.E. (1985). Pictorial keys to the genera of mosquitoes, subgenera of *Culex* and the species of *Culex* occurring in southwestern Asia and Egypt, with a note on the subgeneric placement of *Culex deserticola* (Diptera: Culicidae). Mosq. Syst., 17(2): 83-107.

Harbach, R.E. (1988). The mosquitoes of the subgenus *Culex* in southwestern Asia and Egypt (Diptera: Culicidae). Contributions of the American Entomological Institute, 24(1): 1-240.

Harbach, R.E.; Harrison, B.A.; Gad, A.M.; Kenawy, M.A. and El-Said, S. (1988). Records and notes on mosquitoes (Diptera: Culicidae) collected in Egypt. Mosq. Syst., 20: 317-342.

Ibrahim, A.A.; El-Monairy, O.M.; El-Sayed, Y.A. and Baz, M.M. (2011). Mosquito breeding sources in Qalyubiya Governorate, Egypt. Egypt. Acad. J. Biolog. Sci., 3(1): 25-39.

Jones, R.; Kulkarni, M.A.; Davidson, T.M.V.; RADAMLAC Research Team and Talbot, B. (2020). Arbovirus vectors of epidemiological concern in the Americas: A scoping review of entomological studies on Zika, dengue and chikungunya virus vectors. PLOS ONE, 15(2): e0220753.

Kauffman, E.B. and Kramer, L.D. (2017). Zika virus mosquito vectors: competence, biology, and vector control. *The Journal of Infectious Diseases,* 216 (suppl_10): S976–S990.

Kenawy, M.A.; Ammar, S.E. and Abdel-Rahman, H.A. (2013). Physico-chemical characteristics of the mosquito breeding water in two urban areas of Cairo Governorate, Egypt. Journal of Entomological and Acarological Research, 45(3): 96-100.

Kenawy, M.A. and El-Said, S. (1990). Factors affecting breeding of Culicine mosquitoes and their associations in the Canal Zone, Egypt. Proc. Int. Conf. St. Comp. Sc. Soc. Res. Demog., 1: 215-233.

Kenawy MA.; Rashed SS and Teleb SS (1996). Population ecology of mosquito larvae (Diptera: Culicidae) in Sharkiya Governorate, Egypt. J. Egypt. Ger. Soc. Zool., 21(E):121-142.

Kirkpatrick, T.W. (1925). Mosquitoes of Egypt. Cairo Government Press. 224 pp.

Marcondes, C.B. and Paterno, U. (2005). Preliminary evidence of association between species of mosquitoes in Atlantic Forest of Santa Catarina State, (Diptera: Culicidae). Rev. Soc. Bras. Med. Trop., 38(1): 75–76.

Musonda, M.. and Sichilima, A. (2019). The effect of total dissolved solids, salinity and electrical conductivity parameters of water on abundance of *Anopheles* mosquito Larvae in different breeding sites of Kapiri Mposhi district of Zambia. Int. J. Sci. Technol. Res., 8(4): 70-75.

Nikookar, S.; Fazeli-Dinan, M.; Azari-Hamidian, S.; Mousavinasab, S.; Arabi, M.; Ziapour, S.; Shojaee, J. and Enayati, A. (2017). Species composition and abundance of mosquito larvae in relation with their habitat characteristics in Mazandaran Province, northern Iran. Bull. Entomol. Res., 107(5): 598–610.

Preechaporn, W.; Jaroensutasinee, M. and Jaroensutasinee, K. (2007). Seasonal prevalence of *Aedes aegypti* and *Ae. albopictus* in three topographical areas of southern Thailand. World Acad. Sci. Eng. Technol., 36:23–27.

Prudhomme, J.; Fontaine, A.; Lacour, G.; Gantier, J.C.; Diancourt, L.; Velo, E.; Bino, S.; Reiter, P. and Mercier, A. (2019). The native European *Aedes geniculatus* mosquito species can transmit chikungunya virus. Emerging Microbes & Infections, 8(1): 962–972.

Rydzanicz, K. and Lonc, E. (2003). Species composition and seasonal dynamics of mosquito larvae in the Wroclaw, Poland area. J. Vector Ecol., 28(2): 255–266.

Sattler M.A., Mtasiwa D., Kiama M., Premji Z., Tanner M., Killeen G.F., *et al.*, (2005) - Habitat characterization and spatial distribution of *Anopheles* sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. - Malaria J. 4: 4.

Seghal, S. and Pillai, M.K. (1970). Preliminary studies on the chemical nature of mosquito breeding waters in Delhi. Bull. World Health Organ., 42(4): 647-650.

Sowilem, M.; Elshaier, M.; Atwa, W.; El-Zeiny, A. and El-Hefni, A. (2017). Species composition and relative abundance of mosquito larvae in Suez Canal Zone, Egypt. Asian Journal of Biology, 3(3): 1-12.

Tandina, F.; Doumbo, O.; Yaro, A.S.; Traoré, S.F.; Parola, P. and Robert, V. (2018). Mosquitoes (Diptera: Culicidae) and mosquito-borne diseases in Mali, West Africa. Parasites & Vectors, 11:467.

Turell, M.J.; Morrill, J.C.; Rossi, C.A.; Gad, A.M.; Cope, S.E.; Clements, T.L.; Arthur, R.R.; Wasieloski, L.P.; Dohm, D.J.; Nash, D.; Hassan, M.M.; Hassan, A.N.; Morsy, Z.S. and Presley, S.M. (2002). Isolation of West Nile and sindbis viruses from mosquitoes collected in the Nile Valley of Egypt during an outbreak of Rift Valley fever. J. Med. Entomol., 39(1): 248–250.

Turell, M.J.; Presley, S.M.; Gad, A.M.; Cope, S.E.; Dohm, D.J.; Morrill, J.C. and Arthur, R. (1996). Vector competence of Egyptian mosquitoes for Rift Valley fever virus. Am. J. Trop. Med. Hyg., 54(2): 136-9.

Wasim, N.M. (1993). Ecological studies of saltwater mosquitoes in certain areas of Egypt. M. Sc. Thesis, Ain Shams University, Egypt.

Zahar, A.R. (1974). Review of the ecology of malaria vectors in the Eastern Mediterranean Region. Bull. World Health Organ., 50(5): 427-440.

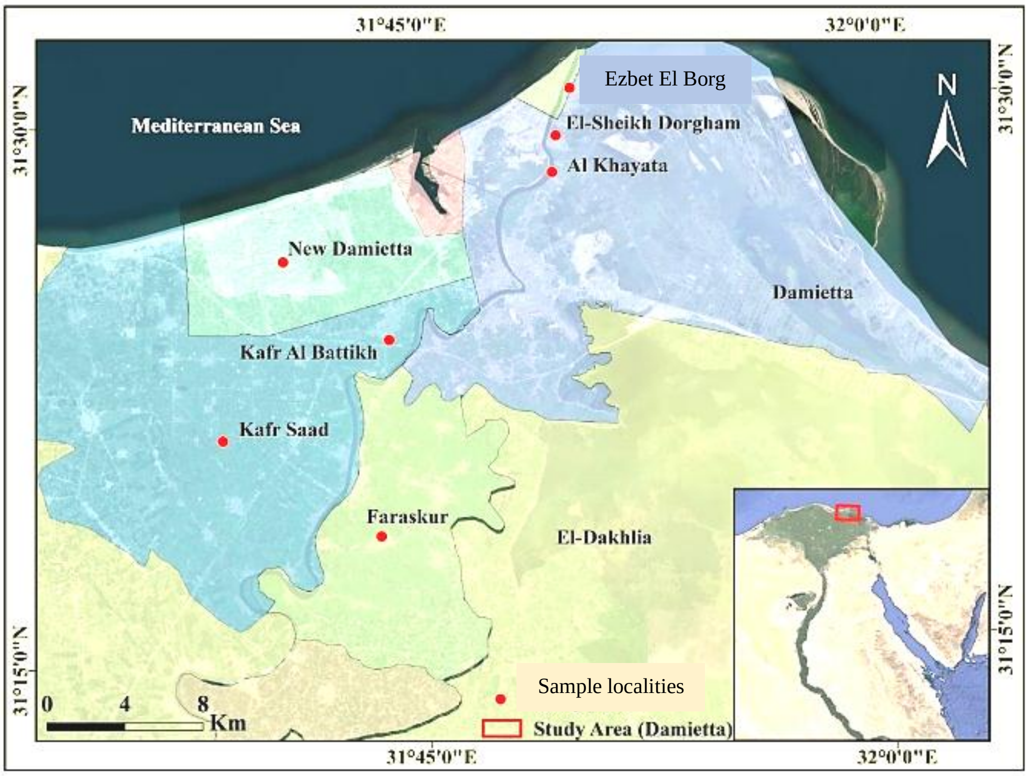

Fig. 1. Map of the study area and locations of sampling sites in Damietta Governorate.

Table 1: The composition, density, and distribution of the mosquito larvae in seven localities in Damietta, Egypt, March-December 2019.

Species	Locality							n	Density %	Distribution (C%)	Density, Distribution status[a]
	New Damietta	Ezbet Al Borg	Al Khayata	El-Sheikh Dorgham	Kafr Al Batikh	Kafr Saad	Faraskur				
Anopheles pharoensis	3	-	56	15	28	250	189	541	1.51	36.96	Subdominant, infrequent
Anopheles tenebrosus	-	-	43	48	7	17	-	115	0.32	15.22	Satellite, sporadic
Culex antennatus	168	386	585	2011	2910	2504	73	8637	24.12	60.87	Dominant, frequent
Culex perexiguus	231	180	18	87	50	29	-	595	1.66	26.09	Subdominant, infrequent
Culex pipiens	575	8732	19	224	808	5454	-	15812	44.16	52.17	Dominant, moderate
Culex pusillus	3222	2323	-	-	-	-	-	5545	15.48	28.26	Dominant, infrequent
Culiseta longiareolata	38	580	-	4	404	198	-	1224	3.42	17.39	Subdominant, sporadic
Ochlerotatus caspius	2643	256	2	-	300	78	-	3279	9.16	30.43	Dominant, infrequent
Uranotaenia unguiculata	-	11	8	42	-	-	-	61	0.17	6.52	Satellite, sporadic
Total	6880	12468	731	2431	4507	8530	262	35809	100		

[a] C= occurrence 0-20% sporadic, C=20.1-40% infrequent, C=40.1-60% moderate, C=60.1-80% the frequent and C=80.1-100% is constant.

D= density, Satellite species (D<1%), Subdominant species (1<D<5), Dominant species (D>5%).

Table 2: Means ± SD of physicochemical parameters of mosquito larval habitats in Damietta, March- December 2019.

Species / Parameters	*An. Pharoensis* *(31)*	*An. Tenebrosus* *(9)*	*Cx. Antennatus* (67)	*Cx. Perexiguus* (19)	*Cx. Pipiens* (54)	*Cx. Pusillus* (47)	*Cs. Longiareolata* (14)	*Oc. Caspius* (46)	*Ur. Unguiculata* (4)	F	*P value*
Temperature (°C)	(29.87±2.29)[d]	(23.44±5.36)[c]	(27.63±5.25)[abc]	(23.53±4.83)[c]	(25.37±5.38)[abc]	(28.94±4.37)[ab]	(24.29±3.83)[bc]	(28.07±4.7)[abc]	(18±0.00)[d]	8.060	<0.001
Acidity (pH)	(8.26±0.27)[ab]	(7.89±0.37)[bc]	(8.12±0.42)[ab]	(7.86±0.46)[bc]	(8.01±0.43)[abc]	(8.09±0.43)[ab]	(8.42±0.26)[a]	(8.07±0.4)[ab]	(7.61±0.36)[c]	3.536	0.001
Salinity (ppt)	(1.44±1.22)[c]	(1.69±0.85)[c]	(2.31±3.89)[c]	(4.98±6.55)[bc]	(9.30±1.27)[bc]	(31.98±20.2)[a]	(5.10±6.76)[bc]	(38.47±23.02)[a]	(11.96±12.64)[b]	54.592	<0.001
EC (µS/cm)	(2.16±1.84)[b]	(2.55±1.28)[b]	(2.9±3.21)[b]	(5.45±4.79)[b]	(12.83±17.84)[b]	(47.92±30.27)[a]	(7.47±10.15)[b]	(57.35±34.32)[a]	(8.31±9.48)[b]	60.923	<0.001
Dissolved oxygen (mg/l)	5.38±3.86	5.65±2.43	5.09±3.6	5.21±4.82	4.2±4.46	7.5±6.6	3.74±2.4	9.31±6.06	2.6±1.99	2.748	0.001
Turbidity (NTU)	96.55±139.08	73.18±143.06	66.94±117.07	21.04±20.65	69.04±134.82	20.3±23.12	21.37±21.5	19.18±19.74	22.6±11.52	4.998	<0.001

Different letters denote significance

Table 3. Spearman correlation coefficient between Larval density and physicochemical characteristics of larval habitat.

Species	Spearman's Correlation (r)					
	Temp	PH	Salinity	EC	DO	Turbidity
An .pharoensis	0.3	0.02	0.01	0.01	-0.13	0.21
An. tenebrosus	-0.14	0.34	0.5	0.5	-0.03	-0.02
Cx. antennatus	0.01	-0.03	-0.04	-0.03	-0.2	0.1
Cx. perexiguus	-0.003	0.05	0.31	0.38	0.35	-0.07
Cx. pipiens	-0.27	0.02	0.06	0.07	-0.21	0.07
Cx. pusillus	-0.08	0.22	-0.34*	-0.34*	0.03	0.23
Cs. longiareolata	-0.54	0.63	0.28	0.28	-0.15	0.87
Oc. caspius	-0.02	0.16	0.2	0.19	0.42**	-0.27
Ur. unguiculata		0.2	0.4	-0.6	0.4	-1.00**

*P<0.05, **P<0.01 (Spearman's Correlation)

Table 4: The association number and percentage of mosquito larvae in Damietta region, Egypt, March-December 2019.

Species association	n	Abundance (%)
An. pharoensis		
Alone	128	23.66
An. tenebrosus, Cx. antennatus	1	0.18
Cx. antennatus	276	51.02
Cx. antennatus, Cx. perexiguus	53	9.8
Cx. antennatus, Cx. perexiguus, Cx. pipiens, Oc. caspius	1	0.18
Cx. antennatus, Cx. pipiens	71	13.12
Cx. antennatus, Cx. pipiens, Oc. caspius	5	0.93
Cx. antennatus, Oc. caspius	4	0.74
Cx. perexiguus, Cx. pusillus, Oc. caspius	2	0.37
Total	541	100
An. tenebrosus		
An. pharoensis, Cx. antennatus	35	30.43
Cx. antennatus	28	24.35
Cx. antennatus, Cx. perexiguus	17	14.78
Cx. antennatus, Cx. perexiguus, Cx. pipiens	7	6.09
Cx. antennatus, Cx. perexiguus, Cx. pipiens, Ur. unguiculata	6	5.22
Cx. antennatus, Cx. pipiens, Ur. unguiculata	22	19.13
Total	115	100

Cx. antennatus		
Alone	1546	17.9
An. pharoensis	1682	19.47
An. pharoensis, An. tenebrosus	30	0.35
An. pharoensis, Cx. perexiguus	101	1.17
An. pharoensis, Cx. perexiguus, Cx. pipiens, Oc. caspius	26	0.3
An. pharoensis, Cx. pipiens	774	8.96
An. pharoensis, Cx. pipiens, Oc. caspius	4	0.05
An. pharoensis, Oc. caspius	25	0.29
An. tenebrosus	121	1.4
An. tenebrosus, Cx. perexiguus	289	3.35
An. tenebrosus, Cx. perexiguus, Cx. pipiens	131	1.52
An. tenebrosus, Cx. perexiguus, Cx. pipiens, Ur. unguiculata	88	1.02
An. tenebrosus, Cx. pipiens, Ur. unguiculata	72	0.83
Cx. perexiguus	38	0.44
Cx. perexiguus, Cx. pipiens	55	0.64
Cx. perexiguus, Cx. pipiens, Cx. pusillus	75	0.87
Cx. perexiguus, Cx. pipiens, Ur. unguiculata	5	0.06
Cx. perexiguus, Cx. pusillus	250	2.89
Cx. perexiguus, Cs. longiareolata	1586	18.36
Cx. perexiguus, Oc. caspius	4	0.05
Cx. pipiens	1225	14.18
Cx. pipiens, Cs. longiareolata	254	2.94
Cx. pipiens, Oc. caspius	202	2.34
Cx. pusillus	54	0.62
Total	8637	100
Cx. perexiguus		
An. pharoensis, Cx. antennatus	2	0.34
An. pharoensis, Cx. antennatus, Cx. pipiens, Oc. caspius	150	25.21
An. pharoensis, Cx. pusillus, Oc. caspius	78	13.11
An. tenebrosus, Cx. antennatus	68	11.43
An. tenebrosus, Cx. antennatus, Cx. pipiens	16	2.69
An. tenebrosus, Cx. antennatus, Cx. pipiens, Ur. unguiculata	16	2.69
Cx. antennatus	24	4.03
Cx. antennatus, Cx. pipiens	23	3.87
Cx. antennatus, Cx. pipiens, Cx. pusillus	44	7.39
Cx. antennatus, Cx. pipiens, Ur. unguiculata	5	0.84
Cx. antennatus, Cx. pusillus	130	21.85
Cx. antennatus, Cs. longiareolata	30	5.04
Cx. antennatus, Oc. caspius	3	0.5

Cx. pipiens, Oc. caspius	6	1.01
Total	595	100
Cx. pipiens		
Alone	2383	15.07
An. pharoensis, Cx. antennatus	166	1.05
An. pharoensis, Cx. antennatus, Cx. perexiguus, Oc. caspius	177	1.12
An. pharoensis, Cx. antennatus, Oc. caspius	1	0.01
An. tenebrosus, Cx. antennatus, Cx. perexiguus	16	0.1
An. tenebrosus, Cx. antennatus, Cx. perexiguus, Ur. unguiculata	67	0.43
An. tenebrosus, Cx. antennatus, Ur. unguiculata	18	0.12
Cx. antennatus	3166	20.02
Cx. antennatus, Cx. perexiguus	21	0.13
Cx. antennatus, Cx. perexiguus, Cx. pusillus	1140	7.21
Cx. antennatus, Cx. perexiguus, Ur. unguiculata	121	0.77
Cx. antennatus, Cs. longiareolata	523	3.31
Cx. antennatus, Oc. caspius	620	3.92
Cx. perexiguus, Oc. caspius	116	0.73
Cx. pusillus	81	0.51
Cx. pusillus, Cs. longiareolata	16	0.1
Cx. pusillus, Oc. caspius	453	2.86
Cs. longiareolata	6394	40.44
Oc. caspius	89	0.56
Oc. caspius, Ur. unguiculata	244	1.54
Total	15812	100
Cx. pusillus		
Alone	1830	33
An. pharoensis, Cx. perexiguus, Oc. caspius	5	0.09
Cx. antennatus	82	1.48
Cx. antennatus, Cx. perexiguus	95	1.71
Cx. antennatus, Cx. perexiguus, Cx. pipiens	71	1.28
Cx. pipiens	150	2.71
Cx. pipiens, Cs. longiareolata	160	2.89
Cx. pipiens, Oc. caspius	365	6.58
Oc. caspius	2787	50.26
Total	5545	100
Cs. longiareolata		
Cx. antennatus, Cx. perexiguus	4	0.33
Cx. antennatus, Cx. pipiens	4	0.33
Cx. pipiens	1213	99.1
Cx. pipiens, Cx. pusillus	3	0.24

Total	1224	100
Oc. caspius		
Alone	451	13.75
An. pharoensis, Cx. antennatus	1	0.03
An. pharoensis, Cx. antennatus, Cx. perexiguus, Cx. pipiens	10	0.3
An. pharoensis, Cx. antennatus, Cx. pipiens	1	0.03
An. pharoensis, Cx. perexiguus, Cx. pusillus	200	6.1
Cx. antennatus, Cx. perexiguus	3	0.09
Cx. antennatus, Cx. pipiens	78	2.38
Cx. perexiguus, Cx. pipiens	27	0.82
Cx. pipiens	316	9.64
Cx. pipiens, Cx. pusillus	220	6.71
Cx. pipiens, Ur. unguiculata	7	0.22
Cx. pusillus	1965	59.93
Total	3279	100
Ur. unguiculata		
An. tenebrosus, Cx. antennatus, Cx. perexiguus, Cx. pipiens	17	27.87
An. tenebrosus, Cx. antennatus, Cx. pipiens	8	13.12
Cx. antennatus, Cx. perexiguus, Cx. pipiens	25	40.98
Cx. pipiens, Oc. caspius	11	18.03
Total	61	100

Table 5: Matrix of co-occurrence (above diagonal) and association index (below diagonal) between pair of mosquito species in Damietta region, March-December 2019.

Index of Association	**Total occasions**	***An. pharoensis***	***An. tenebrosus***	***Cx. antennatus***	***Cx. perexiguus***	***Cx. pipiens***	***Cx. pusillus***	***Cs. longiareolata***	***Oc. caspius***	***Ur. unguiculata***
An. pharoensis	31		1	27	4	5	1	-	4	-
An. tenebrosus	9	-0.89		9	4	3	-	-	-	2
Cx. antennatus	67	-0.335	-0.807		17	25	6	2	5	3
Cx. perexiguus	19	-0.496	-0.634	-0.316		10	5	1	4	2
Cx. pipiens	54	-0.949	-0.983	-0.268	-0.766		9	13	10	4
Cx. pusillus	47	-0.998	-	-0.912	-0.862	-0.772		1	27	-
Cs. longiareolata	14	-	-	-0.625	-0.963	-0.043	-0.952		-	-
Oc. caspius	46	-0.883	-	-0.941	-0.754	-0.753	0.256	-		1
Ur. unguiculata	4	-	-0.398	-0.951	-0.808	-0.936	-	-	-0.989	