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Meteorological Parameters and Seasonal Variability of Mosquito Population in Pune Urban Zone, India: A Year-round Study, 2017

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Abstract The re-emergence of vector-borne viral diseases in an emerging climate change scenario has raised considerable public health concern in rapidly expanding cities of India where altered land-use and environmental factors have resulted in increased mosquito breeding and occurrences of dengue and chikungunya in the recent years. The present year-round study was undertaken to investigate the seasonal variability and demographic diversity of mosquito population in the Pune urban-zone, western India, (18.52°N / 73.85°E), which is located on the eastern slope of the Western Ghats mountain range with considerable green cover. Mosquitoes were trapped from different localities (fixed trapping sites) representative of the urban-zone throughout the year covering all the four seasons. Specimens were identified, determined the demographic diversity, mosquito abundance and the association of the latter to meteorological parameters. Meteorological parameters were recorded daily and analyzed mathematically to obtain the derived parameters and fortnightly averages. Thirteen species of mosquitoes were found across the Pune urban-zone covering 4 genera*, i.e., Aedes*, *Anopheles*, *Armigeres* and *Culex. Culex spp.* was abundant throughout the year, while spurt in *Aedes* population was seen only during South West Monsoon (SW). Overall, mosquito abundance increased during the SW Monsoon due to low diurnal temperature range (DTR) along with increased rainfall and humidity, but decreased during winter followed by a slight increase during the Pre-Monsoon season. Seasonal variability of mosquito abundance and demographic diversity was observed in the study area which may form a basis for prospective systematic surveys and control measures.
Keywords Mosquito; Seasonal variability; Meteorology; Dengue; Pune; India; Chikungunya

Background

The dramatic increase in the emergence and re-emergence of vector-borne viral diseases (VBD) in the recent years across the globe has become a matter of concern (Sudeep and Parashar 2008; Angel et al., 2008; Manimunda et al., 2010; Gubler, 2010; Sudeep et al., 2011; Dash et al., 2013; Kumawat et al., 2014; Bueno-Mari and Jiménez-Peydró, 2015). Though the spread of VBDs depends on various factors like abundance and distribution of vectors and vector-pathogen-host interactions, recent research has revealed the possible role of climatic parameters in altering VBD transmission patterns in different regions of the world (Shope, 1991; Epstein, 1998; Rieter, 2001; Gould and Higgs, 2009; Lafferty, 2009; Roiz et al., 2014). In a recent study, Monaghan et al. (2016) explained that climate change is enhancing the propagation of vectors, especially mosquitoes which in turn is driving the spread of VBDs.

At any location the abundance, distribution and survival of mosquitoes depend on the climatic / environmental factors *viz*., maximum and minimum temperature, relative humidity and rainfall. It is getting established that diurnal temperature range (DTR) played important role in mosquito survival and abundance in equatorial and tropical settings (Murdocketal, 2017; Shil et al., 2017). Hence, knowledge of the effect of meteorological parameters on mosquito abundance (and seasonal variability) is essential for designing control measures and interventions.

In the recent years, India and Southeast Asian countries are witnessing a surge in the occurrence of mosquito borne viral diseases *viz*., dengue, chikungunya, Japanese encephalitis, etc (Dash et al., 2013; Dhiman et al., 2010). Most recently, the global spread of Zika virus has caused considerable damage in terms of human health and economy (Mourya et al., 2016). Since 2004, chikungunya virus (CHIKV) has re-emerged and caused massive outbreaks in the Indian Ocean islands, India and Southeast Asian countries (Schuffenecker et al., 2006; Tsetsarkin et al., 2007). CHIKV has become endemic in various parts of India causing sporadic outbreaks (Nandi et al., 2008).

Though India is diverse in terms of geographical regions and climate zones with a huge disease burden of vector-borne viral diseases, reports of organized surveys dealing with mosquito distribution and dynamics are sparse (Murty et al., 2010). Also, due to growing population and economic growth, many cities in India are facing rapid urbanization which is changing land-use patterns, inducing construction work, industrialization and growth of unorganized settlements (slums) (Nandi et al., 2008). This necessitates organized study of seasonal variability in mosquito population in rapidly growing cities which are affected by VBDs.

Earlier, Shil and co-workers (2017) have reported the mosquito diversity during South West Monsoon season in a particular locality of Pune city, India during 2016. In the present paper, we report the results of a year round survey (all four seasons) of mosquitoes covering the Pune urban zone, India.

1 Materials and Methods

1.1 Location and climate

The study was conducted in the Pune urban zone (centred on 18° 32" N and 73° 51" E), Maharashtra state, India with fixed trapping sites at five locations (Figure 1). Pune urban zone is located at an altitude of 560 m (1,840 ft) above sea level on the leeward side of the Western Ghats mountain range. It is a hilly city with undulating landscape and a few scattered hillocks located in and around the urban zone.

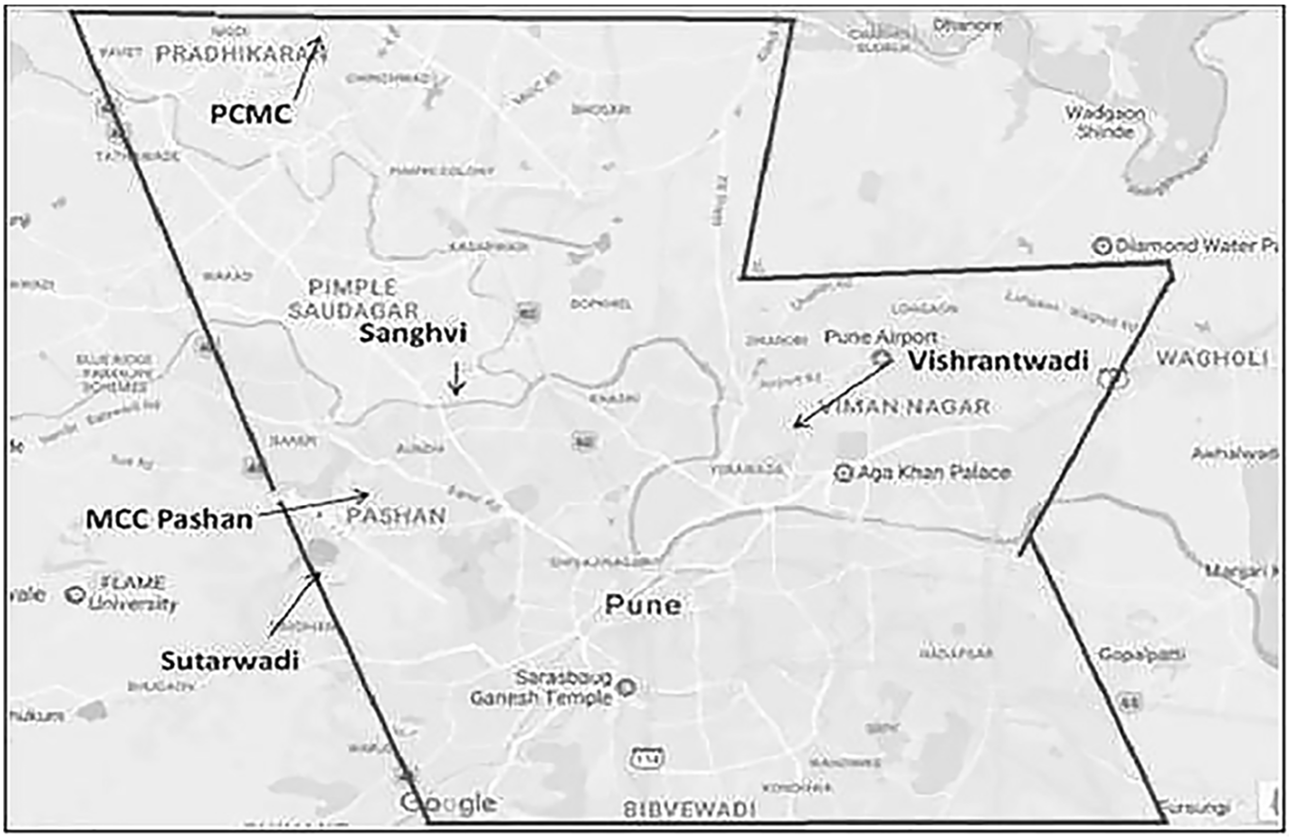

Figure 1 Map of the study area with trapping locations indicated by arrows

Pune Metropolitan Area occupies 7,000 km² comprised of i) urban zone (Pune old city, Pimpri-Chinchwad municipal areas) and ii) semi-rural and rural areas notified for future urbanization. Only the urban zone of Pune metropolitan area (UPMA) was covered in the present study. Locations were selected as representative of the different urban characteristics as described below: a) MCC Pashan (18.32°N / 73.51°E): Pashan area is a populated urban set-up with wide roads and lot of vegetation, apartment blocks and bungalows (with gardens) growing around a small hillock covered with grass and deciduous trees. b) Sanghvi (18.57°N / 73.81°E): Old

Sanghvi is a densely populated middle-class neighborhood with apartment blocks, fewer trees, and narrow roads. c) Vishrantwadi (18.53°N / 73.8°E): Vishrantwadi is an upcoming township with some apartments, bungalows and clusters of low cost housing. The area is little low in cleanliness and water management system. d) Sutarwadi (18.5°N / 73.7°E): Sutarwadi area is densely populated with mostly low cost housing with deficient drainage and waste management system. This area is bound by a small lake to the Southwest. e) Chinchwad (18.6°N / 73.7°E): Chinchwad, PCMC is a populated neighborhood consisting of apartments, bungalows with wide roads and lots of vegetation. Thus, we tried to cover the different areas of the city to get a cross section of the UPMA. At each location, premises of housing apartments or residential bungalows were used for trapping with consent from the owner/residents.

According to the Koppen classification system (Critchfield, 1983; Rubel et al., 2011), Pune has a 'tropical wet and dry' climate type with rainfall occurring in the South West Monsoon (June / July to September) and mostly dry for rest of the year. Summer day temperature may rise as high as 41°C (April-May) and the average winter minimum temperature is around 10°C (January). The four seasons for the Indian subcontinent are: South West Monsoon (SW Monsoon from June / July to September), Post-Monsoon (October-November, a period of transition between end of monsoon and winter), winter (December-February) and Pre-monsoon season (March-early June), consists of spring and summer till the arrival of the SW monsoon).

1.2 Mosquito collection

Mosquitoes were collected using CDC sentinel traps in dusk-to-dawn operations as per protocols described elsewhere (Shil et al., 2017). Briefly, traps were operated from one hour before sunset till one hour after sunrise, thus covering the dawn and dusk activity for *Aedes* mosquitoes as well. Traps were setup in 5 fixed locations in the Pune Urban Zone as described earlier.

At each location, outdoor trapping took place once every week. Trapped mosquitoes were transported to the laboratory on ice, identified, pooled and stored at -80°C. Pooling of mosquitoes was based on gender and location. The standard taxonomic keys were used for species identification (Barraud, 1934; Knight and Stone, 1977). *Aedes* mosquitoes were considered for virus detection and isolation as per established protocol.

Period of the study: 1st December 2016 till 30th November 2017 covering all the seasons (winter: 1st December 2016 - 28th February 2017; *Pre-Monsoon*: 1st March - 2nd June 2017; *SW Monsoon*: June - September 2017 and *Post-Monsoon*: October -November 2017).

1.3 Virus detection

The method described by Sudeep et al. (2011), was followed for virus detection and isolation. Only *Aedes* mosquitoes were considered for virus detection studies. Briefly, individual pools of mosquitoes were triturated in 1ml MEM (Minimum Essential Medium, Gibco, USA), clarified by centrifugation at 5000 rpm for 10 min at 4ºC. The supernatant was filtered with 0.22 µm filter (Millipore, India) and inoculated over Vero E6 cell line in 24 well plates (Nunc, Denmark). The cultures were observed daily for the signs of cytopathic effects.

1.4 Recording and calculations for meteorological data

Every day, meteorological data was recorded from the website of Indian Meteorological department (IMD) or the weather bulletins issued by IMD. Maximum and minimum temperature (MXT and MNT respectively), maximum and minimum Relative Humidity, daily rainfall (RF) and sunrise and sunset times were recorded. From these we calculated the average day temperature (AVT) and diurnal temperature range (DTR) (which is the difference between MXT and MNT for a particular day). For all parameters, fortnightly averages were obtained.

1.5 Statistical analyses

Graph plotting and imagery executed in MS Excel. Statistical analyses were performed using the R software package.

1.6 Study design

The present study was conducted to ascertain the abundance, composition and seasonal variations in mosquito population in Pune urban zone covering all seasons in 2017. Variations in mosquito abundance with meteorological parameters, if any, were determined. Mosquitoes were trapped at five fixed locations in Pune urban zone as representative of the various urban settings. Collected mosquitoes were identified and mosquito abundance determined in terms of fortnightly averages of counts per trap. To understand the effects of environmental factors, for each season mosquito abundance was compared with fortnightly averages of meteorological parameters like rainfall (RF), maximum and minimum temperature (MXT and MNT), diurnal temperature range (DTR) and relative humidity (RH).Relevant statistical analyses were performed in R software package.

2 Results

2.1 Mosquito composition

A total of 3163 mosquitoes were collected during the study period and 13 species were identified belonging to four genera *viz*., *Aedes*, *Anopheles*, *Armigeres* and *Culex*. Among the genera, *Culex* was the most diverse (5 species) followed by *Aedes* (4) and *Anopheles* (3). The top four most abundant species was found to be: *Culex quinquefasciatus* (48.5%), *Culex tritaeniorhynchus* (17.2%), *Culex gelidus* (12.8%) and *Aedes vittatus* (10.7%). In addition we found *Aedes albopictus* (4.3%), *Ae aegypti* (2.8%) and *Anopheles subpictus* (1.8%). A few members each of the following were also found: *Ae vexans vexans* (20), *An vegas/subpictus* (15 male), *An vegas* (5), *Ar subalbatus* (11) and *Cx pseudovishnui* (7). A single specimen of *Cx bitaeniorhynchus* was captured in the month of May from Sanghvi.

Figure 2 presents the seasonal variations in the top four most abundant species. While Culex *quinquefasciatus* remained the most abundant species throughout the year (all four seasons), *Ae vittatus* were trapped only during the SW Monsoon and Post-Monsoon seasons. *Cx gelidus* were most abundant in Pre-Monsoon season compared to SW Monsoon and other seasons.

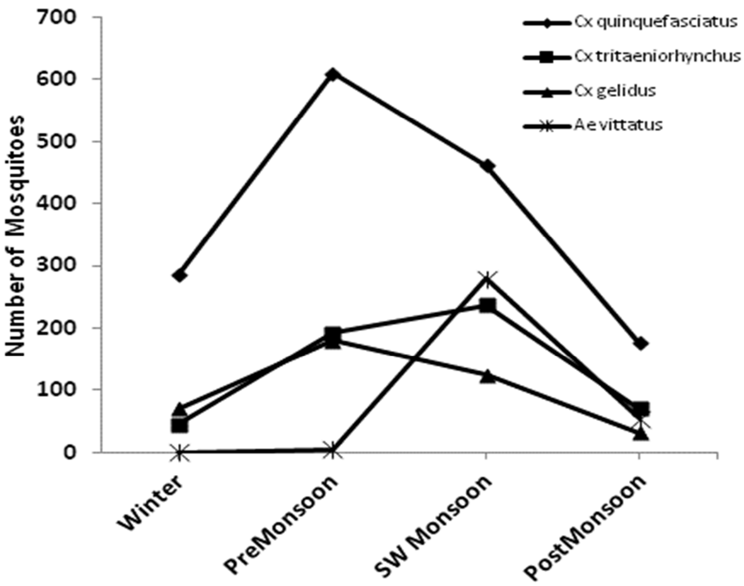

Figure 2 Top four most abundant species covering various seasons in urban Pune in 2017

2.2 Seasonal variation of mosquito abundance

a) Winter season (Dec 2016 to Feb 2017)

Mosquito abundance in terms of fortnightly averages of mosquito count per trap (M) was correlated with the averaged maximum and minimum temperature (MXT and MNT) as well as with maximum and minimum Relative Humidity (maxRH and minRH). Figure 3A shows the variation of mosquito counts with temperature for the winter season. The Pearson's correlation coefficient for M vs MXT was found to be: r= -0.38210 (95%

confidence, p-value= 0.457) and that for M vs MNT was found to be r= -0.4884 (95% confidence, p-value= 0.3256). Figure 3B shows the mosquito abundance variation correlated with maximum and minimum relative humidity (maxRH and minRH) respectively. The Pearson's correlation coefficient for M vs maxRH was found to be r = 0.3106 (95% confidence, p-value=0.5491) while that for M vs minRH was found to be r= 0.6055 (95% confidence, p-value=0.2077).

There was no rainfall recorded during the winter season.

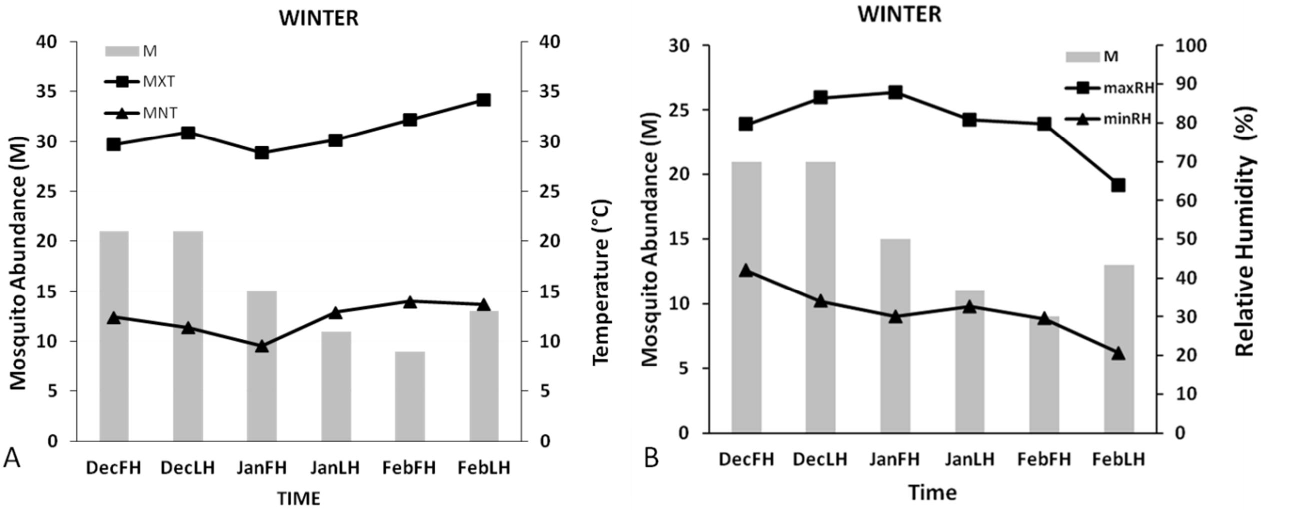

Figure 3 Average Mosquito abundance (M) and meteorological parameters for the Winter season (1st December 2016 to 28th February 2017): A) M, averaged maximum temperature (MXT), averaged minimum temperature (MNT) vs time; B) M, averaged maximum Relative Humidity (maxRH), averaged minimum Relative Humidity (minRH) vs time

b) Pre-Monsoon season (March to May 2017)

Figure 4A shows the variation of mosquito counts with temperature for the Pre-Monsoon season. The Pearson's correlation coefficient for M vs MXT was found to be: r= 0.5658 (95% confidence, p-value= 0.2418) and that for M vs MNT was found to be r= 0.1879 (95% confidence, p-value= 0.7214). Figure 4B shows the mosquito abundance variation correlated with maximum and minimum relative humidity (maxRH and minRH) respectively. The Pearson's correlation coefficient for M vs maxRH was found to be r= -0.4873 (95% confidence, *p*-value=0.3268) while that for M vs minRH was found to be r= -0.1835 (95% confidence, p-value= 0.7277). No significant rainfall was recorded in this season.

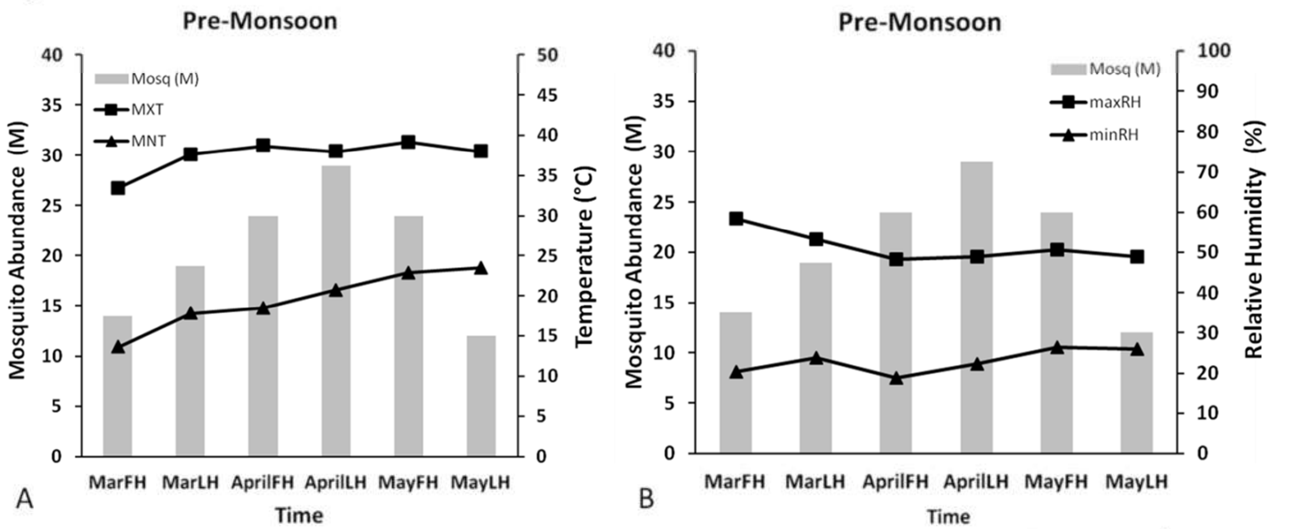

Figure 4 Average Mosquito abundance (M) and meteorological parameters for the PreMonsoon season (1st March to 30th May 2017): A) M, averaged maximum temperature (MXT), averaged minimum temperature (MNT) vs time; B) M, averaged maximum Relative Humidity (maxRH), averaged minimum Relative Humidity (minRH) vs time

c) SW Monsoon season (1st June - 30th September 2017)

In the first two weeks of June 2017, Pune experienced fluctuations in weather conditions as the South west monsoon winds arrived and by end of the month monsoon rainfall system was well established in the region. Figure 5A shows the variation of mosquito abundance with temperature for the SW Monsoon season. The Pearson's correlation coefficient for M vs MXT was found to be: r = -0.7238 (95% confidence, p-value = 0.0423) and that for M vs MNT was found to be r = - 0.1690 (95% confidence, p-value= 0.689). Figure 5B shows the mosquito abundance variation correlated with maximum and minimum Relative Humidity (max RH and min RH) respectively. The Pearson's correlation coefficient for M vs max RH was found to be r =0.3342 (95% confidence, *p*-value = 0.4184) while that for M vs min RH was found to be r = 0.5197 (95% confidence, p-value = 0.1868).

In this season, Mosquito abundance showed positive correlation with rainfall, with Pearson's correlation coefficient, r= 0.60957 (95% confidence, p-value = 0.1086).

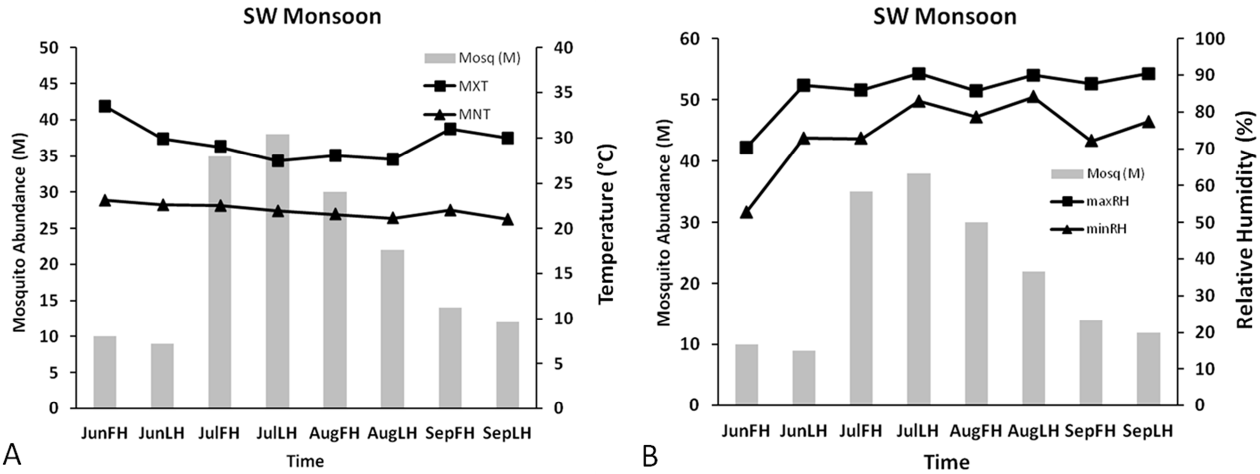

Figure 5 Average Mosquito abundance (M) and meteorological parameters for the SW Monsoon season (1st June to 30th September 2017): A) M, averaged maximum temperature (MXT), averaged minimum temperature (MNT) vs time; B) M, averaged maximum Relative Humidity (maxRH), averaged minimum Relative Humidity (minRH) vs time

d) Post-Monsoon Season (October - November 2017)

Figure 6A shows the variation of mosquito abundance with temperature for the Post Monsoon season. The Pearson's correlation coefficient for M vs MXT was found to be: *r* = 0.6630 (95% confidence, *p*-value = 0.3369) and that for M vs MNT was found to be *r* = 0.9511 (95% confidence, *p*-value = 0.0488). Figure 6B shows the mosquito abundance variation correlated with maximum and minimum Relative Humidity (maxRH and minRH) respectively. The Pearson's correlation coefficient for M vs max RH was found to be: *r* = 0.2161(95% confidence, *p*-value = 0.7839) while that for M vs min RH was found to be *r* = 0.7872 (95% confidence, *p*-value = 0.2127).

In 2017 there were a few cloudy days as withdrawal of SW Monsoon was somewhat delayed. Light rainfall occurred occasionally (few days). Mosquito abundance showed positive correlation with rainfall, with Pearson's correlation coefficient, *r* = 0.7320 (95% confidence, *p*-value = 0.2679).

2.3 Diurnal temperature variation and mosquito abundance

Figure 7 shows the variation of mosquito abundance with the variation in diurnal temperature range (DTR) for the various seasons. During winter, diurnal temperature range DTR remains high and the mosquito abundance is low. For winter season, DTR remains high (~20°C) and mosquito abundance remains low but there is a positive correlation with the variations (Pearson's correlation coefficient, *r* = 0.05332 with 95% confidence, *p*-value = 0.9201).

During Pre-monsoon season, hot and dry conditions prevailed and the DTR steadily decreased due to warmer nights (mid-April till end of May). There is an increase in mosquito abundance in April but decreases in second half of May due to intense heat and dry weather. There is a positive correlation between the DTR and mosquito abundance in this season Pearson's correlation coefficient, *r* = 0.1981 with 95% confidence, *p*-value= 0.7066).

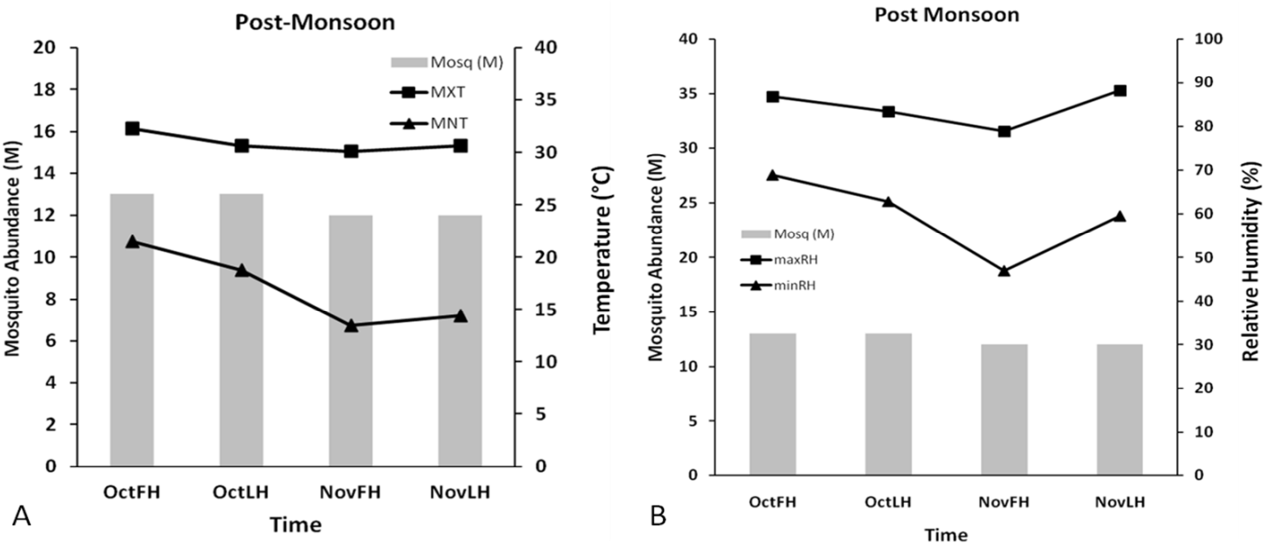

Figure 6 Average Mosquito abundance (M) and meteorological parameters for the Post-Monsoon season (1st October to 30th November 2017): A) M, averaged maximum temperature (MXT), averaged minimum temperature (MNT) vs time; B) M, averaged maximum Relative Humidity (maxRH), averaged minimum Relative Humidity (minRH) vs time

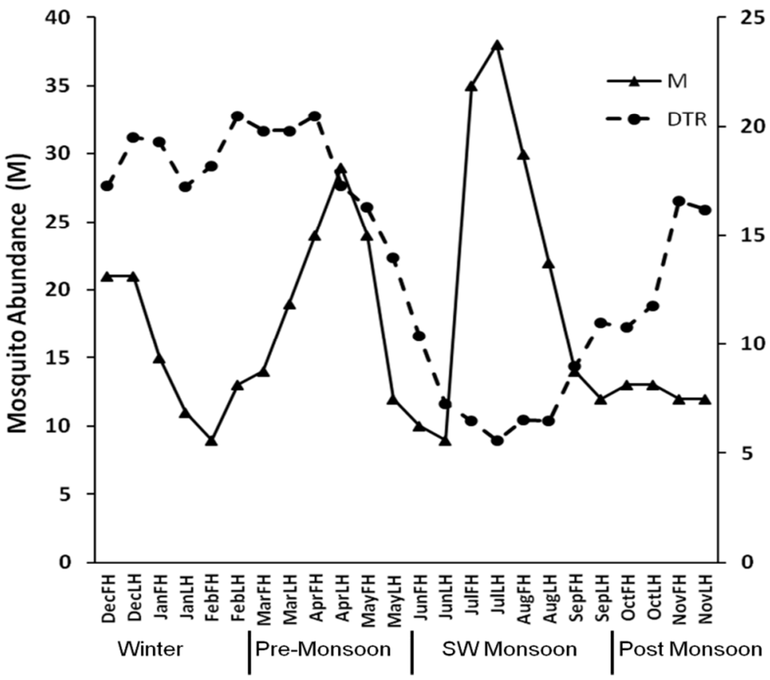

Figure 7 Variation of mosquito abundance with DTR covering all seasons in Pune urban zone in 2017

During the monsoon season, DTR remained low (-5°C) and the mosquito abundance was high. Statistical analyses revealed a negative correlation with Pearson's coefficient r = - 0.7751 with 95% confidence, *p*-value = 0.0238.

With the gradual withdrawal of monsoon in the Post-Monsoon season, DTR increased sharply and there was sharp decrease in the mosquito abundance. A negative correlation was observed between the mosquito abundance and the DTR (Pearson's correlation coefficient, *r* = - 0.9890 with 95% confidence (*p*-value = 0.1097).

3 Discussion

Due to their sensitivity to environmental parameters mosquitoes are considered as excellent indicator species. For any geographical location the demography and seasonal variation of mosquito population is influenced by various factors like climate, natural vegetation, host availability and human activities like land-use and urbanization or agricultural practices (Dhiman et al., 2010).

In the recent years, there has been an increase in dengue and chikungunya cases in Pune urban zone. The present study was undertaken to understand the diversity of mosquito population and seasonal variability in abundance in the Pune urban zone. Location and urban structure of the different trapping sites have been described in the Materials and Methods section. In a preliminary study, five different localities representative of Pune urban zone features were considered as trapping locations. Trapping was done weekly at each site from 1st December 2016 to 30th November 2017 (1 year, covering all four seasons).

Our study revealed the existence of 13 species of mosquitoes covering four genera (*Aedes*, *Anopheles*, *Armigeres* and *Culex*) in the Pune urban zone. Among these *Culex* was the most prevalent both in terms of species diversity (5) and overall abundance. Four most abundant species encountered during the study were *Cx quinquefasciatus, Cx tritaeniorhynchus*, *Cx gelidus* and *Ae vittatus.* A small population of *Ae vexans vexans*, and *Ae albopictus* were detected from Sutarwadi and Pashan areas only. A few specimens of *Cx pseudovishnui* were found in the Pashan region. One specimen of *Cx bitaeniorhynchus* was also found in the Sanghvi area.

Seasonal variability as well as diversity was observed in the mosquito population in Pune urban zone. *Cx* species were most abundant in all regions of the city through-out the year, though their abundance peaked in the Pre-Monsoon season. *Anopheles* species were found throughout the year in low numbers with an increase in Post-monsoon season. *Aedes* species were captured only during Monsoon and Post-Monsoon seasons. An increase in *Culex* abundance during the Pre-Monsoon season may be attributed to the human activities *viz.* stagnant drains and civil construction works, which occur mostly during March-May.

During winter the overall mosquito abundance was low. With the increase in temperature, mosquito abundance increased in the Pre-Monsoon season especially in end of March and April. With very hot and dry climatic conditions prevailing in the last half of May, the mosquito count decreased drastically. This is consistent with findings elsewhere that maximum day temperature (MXT) greater than 36°C is detrimental for mosquito survival and activity (Carrington et al 2013b). The South West Monsoon season extend from June to September. June marks transition in weather patterns as SW Monsoon gets established over India. Initial days in June were very hot, which gave way to a few days of thunderstorms. Some days were hot and sunny while others were windy with light clouds and scanty rainfall. Second half of June experienced onset of monsoon with strong winds, cloudy sky and intermittent rainfall. Due to fluctuations in the weather and windy conditions fewer mosquitoes were trapped in outdoor collections. This is the probable reason for lower mosquito abundance, M (as determined in terms of counts per trap) for the month of June 2017. With the onset of SW Monsoon, the maximum and minimum temperature decreased and relative humidity increased. There was rapid increase in the mosquito abundance in July and August followed by a gradual decrease in September as rainfall decreased and more sunny days appeared. October - November marked the Post-Monsoon season; a period of transition as Monsoon slowly withdrew and wind-flow patters altered over the Pune region. Though generally marked by warmer days and cooler nights, in 2017 a few days with cloudy, humid conditions with light-shower occurred in Pune on the account of cyclonic circulations over peninsular India in the Post-Monsoon season (IMD Bulletin, 2017).

Diurnal temperature range (DTR) is known to play an important role in mosquito survival and activities (Carrington et al., 2013a). Figure 7 shows the plot of DTR with mosquito abundance throughout the period of study. During winter (1st December 2016 to 28th February 2017), DTR showed positive correlation with mosquito abundance. The trend continued in Pre-Monsoon season. A negative correlation during SW Monsoon indicated that mosquito abundance is high when the DTR is low. This is consistent with findings elsewhere (Beck-Johnson et al., 2013; Beck-Johnson et al., 2017). Earlier study on mosquito population in one particular recently urbanized locality in the fringe of Pune urban region, Shil et al. (2017) found similar trends for monsoon 2016. In a study conducted in Thailand, Carrington et al. (2013a) also found that large DTR adversely affected mosquito biology by extending the larval phase of development, adult survival and reproductive capacity in females. Our findings reflect trends found in a variety of studies dealing with DTR and mosquito lifecycles (Mudrock et al., 2017; Lambrechts et al*.,* 2011; Carrington et al., 2013b). In the Post monsoon season, dramatic increase in DTR resulted in a decrease in mosquito abundance.

Aedes mosquitoes were considered for virus detection in our laboratory. However, no dengue or chikungunya viruses were detected from the specimens collected. Abundance of *Culex* species is a cause of public health concern as these mosquitoes have potential for transmitting viral diseases like Japanese encephalitis (JEV) and West Nile viruses. However, the study area is not suited for JEV epidemiology. The increase in abundance of *Culex* species in the Pre-Monsoon season may be a result of various human activities like construction, scrap items, as well as rocky pools along riverbeds, old wells etc., which favour mosquito breeding (www.mymedicalmantra.com, 2017).

Considering the dynamic nature of mosquito distributions in space and time (Crans et al., 2004), and the disease potential, it is necessary to undertake systematic study of mosquito population in urban centres in India. Also, to understand the long term effects of changing climate, sustained systematic mosquito survey is required, which may be taken up as future research.

4 Conclusion

The present study is a first ever survey of mosquito population covering different representative regions of Pune urban zone in western India. Presence of 13 species detected across the various pockets of Pune urban zone covering four genera *i.e*., *Aedes*, *Culex*, *Anopheles* and *Armigeres*. Seasonal variance of total mosquito abundance was observed along with seasonal variability in demographic diversity. While low diurnal temperature range along with rainfall and high humidity favours high mosquito abundance during SW Monsoon, the abundance decreases during the Post-Monsoon and remains low during winter. We hope the findings of the study will be of help to the public health and vectors control authorities and researchers. However, comprehensive surveys with environmental and land-use data can be taken up for future research.

Authors' contributions

Planning and designed the study: PS and ABS. Field work: AAP and SNG. Laboratory work: GNS and ABS. Data analyses and interpretation: PS. Manuscript writing: PS, GNS and ABS. All authors have read and approved the final manuscript.

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