CHAPTER - VII

SUMMARY AND CONCLUSION

In the present study, the effects of certain environmental conditions on some culicine and anopheline mosquitoes (Diptera : Culicidae) of public health importance and their seasonality have been carried out in laboratory as well as under field conditions at various places in Assam, India.

The laboratory colonies of the 3 mosquito species, namely, Aedes (Stegomyia) aegypti Linnaeus, Aedes (Stegomyia) albopictus Skuse and Culex (Culex) quinquefasciatus Say were raised from field collected gravid females. All the 3 colonies were maintained in separate air-conditioned rooms at 27 ± 1°C and 70 ± 5% RH. Light was provided by two 40 watt fluorescent tubes with a photoperiodicity of 12 hours light and 12 hours of darkness.

The biology of the 3 mosquito species was investigated at 27 ± 1°C and 70 ± 5% RH with a photoregime of 12L : 12D. For conditioning of the eggs of Ae. aegypti and Ae. albopictus, a period of 72 hours was found to be quite suitable as it yielded more than 95% hatchability. Out of 3 different media i.e. (i) filtered water, (ii) rearing medium and (iii) reduced pressure tried for hatching of the mosquito eggs, filtered
water was selected for routine use because of its easy availability and simplicity. It was noticed that eggs of *Ae. aegypti* and *Ae. albopictus* could be stored up to a period of 5 weeks under the normal laboratory conditions (27°C, 70% RH) as this storage period yielded reasonably good degree of hatchability (75.31 and 60.76% in *Ae. aegypti* and *Ae. albopictus* respectively).

Under laboratory conditions, the total larval duration of *Ae. aegypti* was approximately 7.3 days. Pupal duration was of 2.05 days. The ratio of male to female was 1:0.95. In *Ae. albopictus*, the average larval duration was of 7.85 days. Pupal period was of 1.75 days. Percentage of pupation was 85.68. Male to female ratio was 1:0.94. It may be concluded that in all the 3 mosquito species studied during the present investigations, the ratio of male to female was found to be 1:1.

The longevity of male *Ae. aegypti* ranged from 1-14 days, while the corresponding period for females was of 2-25 days. Male *Ae. albopictus* lived for 2-21 days, while its female's life was of 2-29 days. Likewise, longevity of male *C. quinquefasciatus* was found to be between 1-16 days, while the females showed much higher figures (2-32 days). It may be concluded that the adult longevity of the 3 mosquito species studied was quite variable under laboratory conditions.

The physico-chemical characteristics of breeding water of mosquitoes of various species were collected from
different places in Guwahati and its surrounding areas. These water samples were analysed for the following 8 parameters: temperature, pH, colour, turbidity, total alkalinity, nitrate, phosphate and chloride contents. The water temperature of different sites ranged from 17-32°C. In cut-bamboos, where the water temperature varied from 17-22.7°C, breeding of *Ae. albonictus*, *Ae. albolateralis*, *Ae. annandeili*, *Ar. kuchigenis* and *Ar. subalbatus* was generally found. pH of water of different habitats, having larvae of *C. quinquefasciatus*, ranged from 5-8. Colour (based on Platinum Cobalt scale units) of breeding water was found to be highly variable. Mosquito larvae were usually found in turbid water (range 0.1-220 units). As far as alkalinity in breeding water is concerned, *C. quinquefasciatus* seemed to prefer water with high alkalinity (upto 370 mg/l), while water samples of other mosquito species had alkalinity ranging from 80-100 mg/l. Likewise, nitrate, chloride and phosphate contents were also found to be quite variable in different water samples supporting breeding of various mosquito species. It was noticed that larvae of *C. quinquefasciatus* and other mosquito species had a wide range of adaptability to various physico-chemical parameters.

The biting rhythms of anophelines were studied in foot-hill areas of Kimin, Lakhimpur district, Assam, from July, 1981 to June, 1982. A total of 2931 anophelines
representing 11 species were collected while biting on human volunteers. *Anopheles maculatus* was found to be the most dominant species with very high man-biting rate (5.74 MBR). *Anopheles philippinensis* was the second dominant species with MBR of 1.19. Besides nocturnal biting rhythm, man-biting density based on hourly collections i.e. 1800 to 2400 hours were also studied. Most of the mosquito species were found to be biting between 1900 to 2300 hours only.

Nocturnal indoor resting collections were also made from 2400 to 0600 hours and per man-hour density was calculated accordingly.

The effects of photoperiod and temperature were studied on egg-hatchability, duration of larval period, pattern of pupation and adult emergence of the 3 laboratory reared mosquito species, viz., *Ae. aegypti*, *Ae. albopictus* and *Culex quinquefasciatus*.

To determine the effects of photoperiod and temperature on the eggs of the mosquitoes during the present work, we tried 5 photoperiods i.e. 4L : 20D, 8L : 16D, 12L : 12D, 16L : 8D and 20L : 4D at 20°C and 80 ± 5% RH as well as in outdoor conditions. It was noticed that eggs of *Ae. aegypti* yielded more than 70% hatching between 5–10 days of exposures
except in the photophase of 4L:20D. In the eggs of *Aedes albopictus* conditioning for 5 and 7 days in 4L:20D periods yielded more than 70% hatching. In *C. quinquefasciatus*, high hatchability (more than 70%) always occurred during 3 days exposure in all the photoregimes, except in 4L:20D.

It was concluded that photoperiod has a role to play in the regulation of hatching process in aedine eggs, but the effect of photoperiod on *C. quinquefasciatus* eggs was found to be less effective. On the other hand, low temperature inhibits the egg-hatching process of all the 3 mosquito species studied during the present investigations.

To investigate the effect of photoperiod and temperature on mosquito larvae of the 3 species, namely, *Ae. aegypti*, *Ae. albopictus* and *C. quinquefasciatus*, the 1st instar larvae of each were reared in short-day (8L:16D) and long-day (16L:8D) photoperiods at 20°C and 80 ± 5% RH in the laboratory. Simultaneously, the rearings were maintained in outdoor conditions.

It was noticed that larval period of *Ae. aegypti* took a minimum of 7.75 days and maximum of 17.05 days to pupate in short-day (8L:16D). The mean duration of larval period was calculated to be 12.40 days. In long-day (16L:8D) regime, the duration of larval period was shorter (10.05 days). In *Ae. albopictus* an average of 12.6 days were needed for the
The results of the present investigations clearly indicate that photoperiod is an important factor in the regulation of duration of larval life of 3 mosquito species; however, temperature also plays a significant role in this respect. In long-day regime, the duration of larval period is shortened as compared to that of short-days.

The effect of photoperiod and temperature was also studied on the pattern of male and female pupation in the 3 mosquito species (Ae. aegypti, Ae. albopictus and C. quinquefasciatus).

In Ae. aegypti, the first pupation of both male and female, in long-day (16L : 8D) started from day 11 and rose gradually and finally terminated in both the sexes on 23rd day. In case of male, the maximum pupation (66.32%) occurred...
between 15th and 18th day, while in females, the maximum pupation (88.99%) took place between 16th and 20th day. In short-day (8L : 16D), the male and female pupation started on 13th day after hatching. The rate of pupation increased and then there was a gradual decline and finally pupation terminated on 27th day in both, male and female. Under control conditions, the first pupation of male and female insects started on 6th and 7th day respectively. The highest pupation rate was noted on 7th and 8th day in male and female respectively.

The pupation pattern in *Ae. albopictus*, under the same photoperiodic conditions, temperature and humidity revealed that both male and female pupation started on day 10 in long-day (16L : 8D). The highest rate of pupation of male was found the next day (i.e. 11th day) and then it gradually fell to end the process on 21st day. In contrast, the female pupation in the same LD was apparently a multimodal type as the pupation continued till 22nd day. As far as pupation in short-day (8L : 16D) is concerned, the male pupation started on day 12 and reached its peak on 13th day. The process gradually declined and terminated on 24th day. Female pupation also began on 12th day, however, the pattern continued to be multimodal till the end (24th day). In control, the pupation followed a definite pattern in both males and females.
In C. quinquefasciatus, the appearance of male pupae in long-day began on day 10, while that of female it was on 11th day after hatching. The pupation terminated on 16th and 17th day in male and female respectively. In short-day regime, the days of commencement of pupation and its cessation in both male and female pupae was similar, and the process was completed within 7 days. Under ambient conditions, the male and female pupation started on 9th day. The male pupation ascended gradually, reached its peak on day 11, then descended and finally terminated on day 17. Likewise, the female pupation reached its peak on day 12 and thereafter dropped gradually for termination on 17th day.

It may be concluded that photoperiodic action in the determination of pupation pattern has been found in all the 3 mosquito species. Moreover no diurnal rhythm in pupation pattern was observed in any of the 3 species studied. Once pupation started, it continued uninterrupted.

The effect of photoperiod and temperature on adult emergence (in block of 4 hours) of the 3 species (Ae. aegypti, Ae. albopictus and C. quinquefasciatus) was studied in long and short-day regimes at 20 ± 1°C and 80 ± 5% RH. In Aedes aegypti, the maximum male emergence (31.5%) occurred between 72-76 hours and minimum (1.5%) in the course of 88-92 hours in long-day. In short-day, the highest male emergence (37.11%)
took place between 76-80 hrs and lowest (0.63%) between 104-104 hrs, whereas in the control, maximum emergence of males (36.98%) was observed during the period of 60-64 hours and minimum (1.57%) during 48-52 hours. Likewise, in LD, maximum (50.16%) and minimum (0.53%) emergence of adult females took place at 80-84 and 60-64 hours respectively, while the period of emergence of females in SD was between 80-84 (23.41%) hours and 64-68 (0.54%) hours respectively. In outdoor conditions, the maximum female emergence (32.85%) took place in 64-68 hrs block and minimum (5.4%) between 72-76 hrs.

In Ae. albopictus, the maximum (34.14%) male emergence in LD regime occurred between 72-76 hours, and the minimum (1.8%) between 88-92 hrs. In short-day length, the corresponding periods were obtained between 76-80 hrs (32.65%) and 88-92 hrs (1.04%), whereas in ambient condition this happened between 60-64 (27.68%) and 48-52 (3.13%) hours respectively. On the other hand, the maximum female emergence (40.53%) was noted during the period between 76-80 hrs and minimum (1.31%) between 60-64 hours in LD regime. In SD photophase (8L : 16D), the highest female emergence (35.65%) was noted between 80-84 hrs and the lowest (1.49%) in 64-68 hrs after pupation. In outdoor conditions, maximum female emergence (33.8%) was recorded during 64-68 hrs, while the minimum (0.47%) in 48-52 hour blocks.
In *C. quinquefasciatus*, very high male emergence (64.11%) was recorded in 68-72 hrs in LD, while the lowest (0.32%) was noted in 80-84 hrs. The maximum male emergence (50.78%) was seen at 72-76 hrs in SD regime, while in control, the highest emergence (46.76%) was observed at 60-64 hr. Contrary to this, the minimum male emergence in SD photoperiod was noted at 80-84 hrs, while in control the minimum male emergence (5.03%) took place at 48-52 hrs. The maximum female adult emergence (43.29%) in LD photoperiod closely followed its male counterparts with a lag of 4 hr, while in SD, the maximum emergence (both male and female) was recorded during the same period of time (72-76 hours). On the other hand, the minimum female emergence was observed during 64-68 hr in SD. In the control, the highest female emergence (52.83%) took place at 64-68 hrs followed by emergence at 60-64 (25.47%), 56-60 (11.32%) and 68-72 hrs (10.83%).

It may, therefore, be concluded that a single pupa of *Ae. aegypti* (without considering its sex) took a period of mean 97.04 hours, while *Ae. albopictus* and *C. quinquefasciatus* needed a mean period of 80.51 hours respectively for emergence at 20 ± 1°C, 80 ± 5% RH in a photoregime of 8L : 16D.

A detailed mosquito survey was carried out at 4 places in Cachar, 5 in Lakhimpur and 4 in Kamrup district of Assam.
during the present investigations. Aquatic stages (larvae and pupae) were collected from different breeding habitats.

During the present survey in Assam, mosquitoes of 46 species belonging to 9 genera i.e. Anopheles (14 species), Culex (13 species), Aedes (9 species), Mansonia (5 species), Armigeraes, Ficalbia, Malaya, Toxorhynchites and Tripteroides (one species each) were encountered. The maximum larval collection (26.24%) of a single species was that of Aedes albopictus, followed by Anopheles vagus (25.65%) Culex quinquefasciatus (12.95%) and Armigeraes subalbatus (10.24%). Among the adult mosquitoes trapped, Culex tritaeniorhynchus (23.25%) was the most dominant one. The second position was secured by Culex quinquefasciatus (11.31%).

Two mosquito species, namely, Culex mimeticus and Aedes vittatus are being reported for the first time from Assam.

Thus, the number of mosquito species of Assam (based on recent findings, including those of others also) clearly rises to 72 belonging to 10 genera, viz., Anopheles (30 species), Aedes (13 species), Armigeraes (3 species), Culex (15 species), Mansonia (4 species), Malaya (2 species), Coquillettidia (2 species), Ficalbia, Tripteroides and Toxorhynchites (1 species each).
High densities of *Anopheles annularis* and *Anopheles philippinensis* have been noted during the present survey. It may be pointed out that both these species have been incriminated as malaria vectors in Assam and in some neighbouring states of north eastern region. Furthermore, JE vectors i.e. *Culex bitaeniorhynchus*, *C. tritaeniorhynchus*, *C. vishnui*, *C. whitmorei*, *A. barbirostris* and *A. hyrcanus* group (which includes *A. nigerrimus* also) have been encountered in large numbers and formed 41.44% of the total catch. It is considered that the presence of JE vectors in large number all over Assam, may sometime result in an epidemic. However, prior to 1973, JE was not a major health problem in north eastern region, in recent time the JE cases are showing an upward trend in Assam. The complexity of JE problem is mainly increasing due to involvement of multiple vectors.

Seasonality of adult mosquitoes was studied by trapping them with CDC light traps. The collections were made fortnightly for a period of 2 years (June, 1982 to May, 1984) in residential area of Tezpur, Sonitpur district, Assam. A total of 23,964 adult mosquitoes were trapped during this period. Seventeen species belonging to 4 genera, viz., *Anopheles*, *Culex*, *Mansonia* and *Armigeres* were identified. The genus *Anopheles* comprised of 6 species (5051 specimens), *Culex* had 6 species (12,718 specimens), *Mansonia* included 4 species
(2853 examples), while Armigeres was represented by a single species only. Culex tritaeniorhynchus was found to be the most dominant species and formed 17.32% (in 1982-83) and 18.46% (during 1983-84) of the total mosquitoes trapped. Armigeres subalbatus was the next predominant species and constituted 15.66% and 12.19% of the total catch during 1982-83 and 1983-84 respectively. C. vishnui, C. quinquefasciatus and C. gelidus ranked 3rd, 4th and 5th dominant species respectively. C. tritaeniorhynchus and C. vishnui were found throughout the year in the study area with a peak during October each year. Both these JE vectors were prevalent in very high densities from July to November. It may be concluded that all the 3 species, (C. gelidus, C. tritaeniorhynchus and C. vishnui) showed the same trend of seasonal fluctuations of density and, therefore, seemed to have a close relationship with rainfall in the study area.

Among the 6 anopheline species encountered in Tezpur, Assam, 3 species, viz., A. annularis, A. barbirostris and A. vagus were found almost throughout the year. Other 3 species i.e. A. nigerrimus, A. kochi and A. philippinensis were found to be altogether absent during the winter months i.e. January to March. During these months, temperature varied from 15.9 to 23°C, while the average RH was 62.5 to 75% and the total rainfall was 123.15mm(i.e. from January to March 1983-84).
In Karbi-Anglong area of Assam, fortnightly collections were made by suction tubes from human dwellings and cattle-shed from June 1983 to May 1985. A total of 21,149 adult anophelines were collected during the present investigations. In all, 15 Anopheles species were aspirated. A. philippinensis was the most dominant species encountered followed by A. vagus and A. nigerrimus. These 3 species together formed nearly 85% of the total catch. Of the 15 species encountered, only 6 species namely, A. aconitus, A. annularis, A. nigerrimus, A. kochi, A. philippinensis and A. vagus could be collected during all the months, though they showed variations in their densities.

Seasonal studies of different mosquito species revealed that various species have different seasonal peaks of their prevalence; Kharif season (warm and wet) was found to be the most favourable one. It may be concluded that various blocks: (a) January to April dominated by C. quinquefasciatus and (b) May to December, when other mosquito species dominate.

It is therefore suggested that in view of persistent malaria transmission in Assam and due to increase in JE cases, conventional larval abatement and adulticiding should not be the only methods applicable for control of mosquitoes in Assam. Thorough knowledge of epidemiology should be acquired along with studies on bionomics and control of vector (s) mosquitoes. More emphasis should be laid on environmental
sanitation, hygiene and health education, proper utilisation of public health engineering systems and publicity through mass media. Integrated Pest Management techniques should be adopted by State Health authorities of Assam.

It is felt that the present study on effect of certain environmental conditions on some culicine and anopheline mosquitoes (Diptera : Culicidae) of public health importance and their seasonality in Assam, will help understand the prevalence of various mosquito species and their seasonal fluctuations. It will, in turn, protect the farmers and field workers and their livestock whose habitats are surrounded by prolific mosquito breeding and who are always exposed to this health hazard.