CHAPTER 3

Methods of study

Mosquito bionomics

Although this study is intended to deal primarily with the entomological component in vector borne diseases programmes and the techniques to be utilized in studying the bionomics of the vectors, a general outline of the bionomics of mosquito vectors is necessary.

Vector bionomics and related aspects

Bionomics is that part of biology which deals with the relationships of a given species and its environment. Basic studies on the bionomics of mosquitoes include the development of immature stages, i.e., eggs, larvae, and pupae as well as the life of the adults under the influences exercised by the environmental conditions. Genetic factors, which govern the basic lines of behaviour and ecological factors, which may produce different type of reaction in a population having the same genetic characteristics.

The larval and adult stages of mosquitoes exist in two different environments, each stage being under the influences of its immediate surroundings. The adaptations of eggs, larvae, pupae and adults to certain environmental patterns constitute the influence of factors controlling the seasonal and geographical distribution of the species.

Among the ecological factors, the phenological one is of considerable importance. For example, the ability of mosquitoes to breed in fresh or salt water, or both, is controlled by genetic factors. Host preference seems also to be governed by genetic factors, but intensity of feeding on a certain host may vary from palace to place and even from day to day, not only with the availability of hosts, but also with the changes in meteorological conditions. The duration of development of mosquitos is dependent mainly on climatic factors. The rate of metabolic processes of insects is largely controlled
by temperature, hence certain biological events such as the duration of aquatic stages, the duration of blood digestion and maturation of ovaries and consequently the frequency of feeding varies according to temperature. These features underline the importance of careful recording of the environmental factors during such studies.

Different patterns of distribution and density of mosquito species are dependent upon several important environmental and man-made factors. The environmental factors like atmospheric temperature and rain fall, also availability of breeding sites and resting places. Knowledge of breeding, resting and biting habits and longevity of a vector species is therefore essential for organizing anti-vector measures and the evaluation of the impact of such measures. Each species is adapted to live in a particular “niche” in the community. In short, the environmental factors which govern the distribution, abundance and density of mosquito are climate, physical and chemical condition of habitat, hosts, and enemies and in some cases competition. This study is an effort to assess the impact of above mentioned factors on the mosquitoes.

3.1 Identification of mosquitoes (larvae and adult)

Larval Identification: The collected larvae were morphologically identified up to genus as per following references,

*Aedes* (Roy and Brown, 1971; Patel, 2002; Sumit, 2008).

*Anopheles* (Puri, 1957; Roy and Brown, 1971; Das *et al.*, 1990; Patel, 2002; Sumit, 2008).

*Culex* (Roy and Brown, 1971; Patel, 2002; Sumit, 2008).

*Armigers* (Roy and Brown, 1971; Patel, 2002; Sumit, 2008).

Adult Identification: Collected and emerged adults from larvae were identified as genus and species using following literature,

*Aedes* (Roy and Brown, 1971; Patel, 2002; Sumit, 2008).
Anopheles  (Puri, 1957; Roy and Brown, 1971; Rao, 1981; Das et al., 1990; Patel, 2002; Sumit, 2008).

Culex  (Roy and Brown, 1971; Patel, 2002; Sumit, 2008).

Armigers  (Roy and Brown, 1971; Reuben et al., 1994; Patel, 2002).

3.2. Classification by morphological characters of adult mosquitoes

1. Order – Diptera: Hind wing is modified in to club-shaped organs called halters, metamorphosis is complete.

1a. Suborder – Nematocera: Antenna long and filamentous and compressed of a large number of segments which are more or less similar in appearance, maxillary palps are elongated and flexible and are commonly composed of 4 to 5 segments.

2. Family – Culicidae: Second longitudinal vein forked only once, body and wings may or may not be covered with scales.

2a. Sub-family – Culicinae: Long and slender proboscis, mouth parts of many species adopted for piercing and for sucking.

3. Tribe – Anophelini: The palpi are as long as the proboscis in both male and female, abdomen never uniformly invested by broad overlapping scales.

3a. Genus – Anopheles Meigen. : Wing dark scaled or readily with more than two pale spots on costa.

4. Tribe – Culicini: Free edge of scutellum distinctly trilobed, mesonotum without bristles, palpi short in the female and long in male, more or less uniform.

4a. Genus – Culex Linn. : Spiracular and post spiracular setae absent.

4b. Genus – Aedes Meig. : Proboscis fairly slender straight, body including thorax conspicuously ornamented with white scales, tarsi usually have white bands.

4c. Genus – Armigeres Theob. (Subgenus – Armigeres Theob.): Proboscis rather stout and distinctly flattened laterally and curved downwards at the tip, silvery – white ornamentation of thorax not very conspicuous, pale scales round the margin of the mesonotum.
**Figure 3.1** Morphological features for the adult *Anopheles* female mosquito identification.

A - Antenna, Fe - Femur, FL – Fore Limb, HL – Hind Limb, ML – Mid Limb, MP – Maxillary Palp, P - Proboscis, Ta – Tarsomeres (1-5), Ti - Tibia, W – Wing; Wing: C - Costa, F – Fringe, Sc - Subcosta, V – Vein (1-6)
Figure 3.2 Morphological features for the adult *Culex* and *Aedes* female mosquito identification.

Morphological features for the adult *Anopheles, Culex* and *Aedes* female mosquito identification.

**Figure 3.3**


**Figure 3.4**


**Figure 3.5**

A – Alula, C – Costa, F – Fringe, NCV – Numerical Cross Vein (1 to 6), Re – Remigium (Stem vein), Sc – Sub costa, Sq – Squarna
Figure 3.6 Morphological features for the adult *Anopheles stephensi* mosquito identification.


*Anopheles stephensi*

- four dark patches on costa and sub costa of wing
- Maxillary palps speckled and apical and sub apical white bands are equal
- Hind limb speckled

*Anopheles annularis*

- Bifurcation of vein 5 the area is dark
- Palpi with three pale bands
- Second tarsal segment is pale at the apical end
Figure 3.7 Morphological features for the adult *Anopheles subpictus* mosquito identification.


- Wing with costa spotted black and white
- Palpi with apical pale band as wide as pre-apical dark band

*Anopheles vagus*

- Fringe spot at all the veins
- Four or More than four pale areas involving costa, subcosta and vein I
Figure 3.8 Morphological features for the adult *An. culicifacies* mosquito identification.


- Wing with pale fringe spots only at apex of veins 4.2 or 5.1 or both
- On the wing nearly the whole of the third vein is dark, basal dark area on costa interrupted by a white spot
- Three pale bands on maxillary palp
Figure 3.9 Morphological features for the adult *Culex quinquefasciatus* mosquito identification.

Head: MP – Maxillary Palp, P – Proboscis, A – Antenna

*Culex quinquefasciatus*

- Proboscis without pale band
- Postspiracular area without pale scale patch
- Tarsal segments are without basal pale bands
**Figure 3.10** Morphological features for the adult *Culex tritaeniorhynchus* mosquito identification.

Head: MP – Maxillary Palp, P – Proboscis, A – Antenna; HF – Hind Femur

*Culex tritaeniorhynchus* (A, B)

- Proboscis with the presence of pale band (median pale ring) and accessory pale patches or stripe
- Legs are dark brown but hind femur has dark ring near knee joint

*Culex vishnui* (C)

- Hind limb femur pale with distinct narrow dark ring near knee joint
- Two pale band with Tip of proboscis brown and rest entire proboscis pale

*Culex univittatus* (C, A)

- Proboscis is dark brown
- Legs are dark brown and fore leg has knee spot
- Abdominal pale bands on tergites are narrow
- White patches on postspiracular area
Figure 3.11 Morphological features for the adult *Aedes aegypti* mosquito identification.

FF – Fore Femur, MF – Mid Femur, HF – Hind Femur, FT – Fore Tibiae, MT – Mid Tibiae, HT – Hind Tibiae, Ta – Tarsus

*Aedes aegypti*

- Lyre-shaped white markings present on scutum of thorax
- White knee spot present on all femora of fore, mid and hind limb
- Mid femur with a narrow white longitudinal stripe on ventral half of anterior surface
- Tibiae of fore, mid and hind limb anteriorly dark, without any white band
Figure 3.12 Morphological features for the adult *Aedes vittatus* mosquito identification.

FT – Fore Tibiae, MT – Mid Tibiae, HT – Hind Tibiae, Ta – Tarsus

*Aedes vittatus*

- Scutum with 3 pairs of distinct, small and white spots of narrow scales present
- Tibiae of fore, mid and hind limb dark, each with a sub basal white spot and a white band present
- Tarsomeres (1-4) of hind limb with a presence of white band
Figure 3.13 Morphological features for the adult *Armigeres obturbans* mosquito identification.

Head: MP – Maxillary Palp, P – Proboscis, A – Antenna; Ab – Abdomen

*Armigeres obturbans*

- Proboscis is stout and curved downwards and flattened laterally
- Silvery white patches or spots on the lateral side of abdominal tergites
3.3. Morphological characters for identification of the mosquito species found in the study area

* Aeles aegypti *
- Proboscis without white band
- Thorax, Scutum black or brown with a pair of submedian – longitudinal white strips, with white lyre – shaped markings
- Femora with white knee spot
- Midfemur with three large white patches on anterior surface
- Hindtarsomere entirely white

* Aeles vittatus *
- Mesonotum with 4 to 6 small round white spots
- Femora with complete pre-apical white rings
- All tibiae with white rings

* Anopheles stephensi *
- Palpi with speckling
- apical and subapical band are equal separated by a dark band
- speckled appendages
- four dark bands on costa and sub costa
- sixth vein has two dark spots

* Anopheles annularis *
- Apical pale and pre apical pale dark bands nearly equal in length
- Second tarsal segment is pale at the apical end
- Bifurcation of vein 5

* Anopheles culicifacies *
- Four or More than four pale areas involving costa and sub costa
• Third vein is entirely dark
• Hind tarsomeres 3 and 4 entirely dark
• Tip of the leg is dark
• Pale bands on tarsal segments are absent

*Anopheles subpictus*
• Palpi with apical pale band as wide as pre-apical dark band
• Wing with costa at base spotted black and white
• Dark area on first vein
• Fore leg tarsal segments have broad pale bands

*Anopheles vagus*
• Pre-apical dark band is 1/4\(^{th}\) or 1/5\(^{th}\) of apical pale band.
• Wing with four or more than four pale areas involving costa, sub costa and vein I
• Fringe spot at all the veins
• Fringe spot between vein five and six
• Tip of the leg is dark
• Fore leg tarsal segments have broad pale bands.

*Armigeres obturbans*
• Proboscis is stout and curved downwards at the tip and distinctly flattened laterally
• Silvery white patches or spots on the mesonotum are absent

*Culex quinquefasciatus*
• Proboscis without pale band
• Hind femur with pale stripe on anterior surface
• Abdominal tergite segments with narrow pale band
• Tarsal segments are without basal pale bands
Legs are dark brown, pale posteriorely

**Culex tritaeniorhynchus**

- Proboscis with pale band
- Abdominal tergite with narrow pale band
- Legs are dark brown but hind femur has dark ring near knee joint
- Tarsal segments are with basal pale bands

**Culex univittatus**

- Abdominal pale bands on tergites are narrow
- Fore leg has knee spot
- Proboscis is dark brown
- White patches on postspiracular area
- Legs are dark brown

**Culex vishnui**

- Tip of the proboscis is brown and rest entire proboscis is pale
- Mesonotum covered with brown and some lighter scales
- Mid and hind tibiae with a more or less distinct pale stripe on the out side
- Proboscis with pale band

### 3.4. Site selection

Villages around Rajkot city were selected to carryout necessary studies on vector habits, resting; biting habits, distribution in domestic and outskirt shelters was possible. Preliminary survey has been done in the said areas. The sites were selected on the bases of WHO manual; methods and techniques were also used as per WHO (WHO, 1975). On the bases of preliminary survey seven villages were selected around Rajkot and fixed sampling sites for domestic and outskirt larval and adult mosquito collection.
Larvae and adults of the mosquitoes found at the study sites were collected monthly from 1\textsuperscript{st} January 2006 to 31\textsuperscript{st} December 2007. All types of domestic and outskirt sites were village wise surveyed for the presence or absence of larvae and adult mosquitoes. Larval habitats are non-perinnial; sites where the larvae were found (positive sites) were further examined for determining larval density for a given time. Number of samples was fixed on the bases of the houses in the village. Minimum six and maximum ten collection sites were fixed and larvae were collected from places ranging up to 500 m from the domestic site.

At outskirt, six larval collection sites and at domestic, ten larval collection sites were fixed for sampling in each village. In domestic nine sites were fixed for adult collection in each village; sampling was done in each village once every month. Minimum 15 minutes of time was spent in each house for adult collection. Adult collection has been done during morning hours. Standard methods and techniques were adopted shown by WHO (WHO, 1975).

3.5. Classification of mosquito breeding sites

a. Large or medium size habitats

Permanent or semi-pemanent standing water
- Marshes or marshy shores of waterbodies
- Swamps, ponds, pools, cheddam
- Seepages, reservoirs, wells

Running water
- Springs, streams, rivers, canals etc.

Temporary breeding places
- Rain pools
- Pools on river banks
• Small irrigation water channels
• Surface ditches for the evacuation of waste water or for drainage of temporary waters

b. Domestic habitats
• Cattle shed tank
• Iron, plastic barrels
• Water cisterns, cement tanks, over head tanks, under ground tanks
• Pots for storage of water
• Empty tins

3.6. Classification of domestic resting habitats mosquitoes

(1) Human dwelling:
This is a habitat with human residing areas including constructed cemented houses and huts and working areas.

(2) Mixed dwelling:
This is a habitat of human residence either cemented houses or a hut, with presence of domestic cattle.

3.7. Larval and adult collection

During field work, larvae were collected by dipping method, using a beaker of 250 ml capacity as a dipper. Collected larvae were placed in collection plastic tubes; brought to the laboratory for identification and rearing. Larvae were reared in small plastic jars covered with cotton cloth. Larvae were fed upon yeast and biscuit powder mixture. (Das et al., 2003; Helge et al., 2002.; Joseph et al., 2004; Khamre and Kaliwal, 1988; Marrippan et al., 1992; Rajni, 1996; Yadav et al., 1989)

Resting mosquitos were collected by standard hand collection method, using aspirator and a test tube. Mosquitoes were collected and were brought to the laboratory
for morphological of female identification. (Das et al., 2003; Hans et al., 2003; Jagdish and Jagbir, 2003; Joshi, 2005; Pemola and Jauhari, 2004; Pemola and Jauhari, 2006; Yadav et al., 1989)

3.8 Study of larval density (quantitative study)

By using dipping method, collected data were analyzed to calculate larval density. Average monthly and village wise larval and adult densities were also calculated.

- Larval density (LD) was calculated by using following formula:
  
  \[ \text{LD} = \frac{\text{number of larvae collected}}{\text{number of dips made}} \]

3.9 Study of adult density

Collected data were utilized to calculate adult density of each mosquito species separately. Average monthly and village wise larval and adult densities were calculated.

- Adult density was calculated as Man per Hour Density (MHD) by formula given below:

  \[ \text{MHD} = \frac{\text{Total number of mosquito collected}}{\text{Total time of collection}} \]