Chapter IV- MATERIALS AND METHODS
Physico-Chemical Properties of water

Physico-chemical parameters of water were studied in the Keibul Lamjao National Park, River Nambol, River Moirang River and Nambul River at monthly intervals from September 2009 to September 2011. Water samples were collected from the sampling site during morning hours. Three replicate samples were taken. The samples were analysed in the laboratory within 24-72 hours.

Temperature of air (AT), water (WT), depth (Dpt), Transparency (Trns) was noted during each collection of sample with the help of a mercury thermometer, Secchi-Disc; pH were measured with a pH meter (Scan 3 Double junction) and Conductivity (Con) by conductivity meter (EC Scan 0 to 1990 μ Siemens) respectively. Total alkalinity (TA) was measured titrimetrically against .01 NHCl (Trivedy & Goel, 1984., APHA, 2005). Dissolved oxygen (DO) was estimated by Winkler Method and Biological Oxygen Demand (BOD) was estimated by Winkler Method after five days incubation. Free Carbon dioxide (FCO₂) by titrating against 0.05 N sodium hydroxide with phenolphthalein indicator (Trivedy & Goel, 1984). Chloride (Cl⁻), Salinity (Sal), were analysed and estimated as per standard methods (APHA, 2005). Nitrate (NO₃⁻) was estimated by the phenol disulphonic acid digestion method and Phosphate (PO₄³⁻) by the stannous chloride–ammonium molybdate method using the Spectrophotometer UV and Visible Spectrophotometer, 118, (Systronic). Sodium (Na) and Potassium (K) were estimated by Flame Photometer (Digital Flame Photometer 125). Heavy metals like Lead (Pb), Mercury (Hg), Iron (Fe) etc. were analysed using Atomic Absorption Spectrophotometer AA 203 Model Chemito.

Statistical Analyses were done by statistical package SPSS 20.0.

Aquatic insects

Members of different aquatic insect groups were collected through an extensive survey around the Keibul Lamjao National Park, Nambol river, Moirang river and Nambul river. Larval collection were made at monthly intervals from September 2009 to September 2011 with a hand net having a mesh size of 60 μm.
fixed to a square wrought iron frame. For representative collection of a given population a ‘Kick’ method (Brittain, 1974) was used whereby the vegetation was disturbed and the net dragged around the vegetation for an unit of time (Macan and Maudsley, 1968; Subramanian and Sivaramakrishnan 2007). Three such drags constituted a sample. Three replicate samples were collected and the insects sorted and counted. Larvae were preserved in 6% formaldehyde solution. Insects were later identified using Dewinter Advance Stereozoom Microscope with the help of standard keys (Wright and Peterson, 1944; Kumar, 1973 a, 1973 b; Pennak, 1978., Westfall and Tennessen 1996, ZSI, 2004; Thirumalai, 1994; Thirumalai, 2007) and by the experts from the Zoological Survey of India, Calcutta.

Diversity indices like Shannon-Wiener Index ($H'$), Evenness Index ($J$), Berger Perker Index of Dominance, Cluster Analysis and Margaleff Index for the insect community collected in Keibul Lamjao National Park, Nambol river, Moirang river and Nambul river for the month of September 2009 to September 2011 were worked out using the package (Biodiversity Professional Version 2 for Windows 1997, The Natural History Museum and Scottish Association for Marine Science). The statistical analysis such as Analysis of Variance (ANOVA) and Coefficient of Correlation Analysis were done by SPSS 20.

Canonical Corespondence Analysis (CCA) were used to classify, investigate patterns in species distribution and correlate the patterns with environmental variables respectively by using CANOCO software V 4.5.

The SIGNAL (Stream Invertebrate Grade Number-Average Level index (Chessman, 2003) is a measure of water quality using the factors of indicator animals and abundance. It has been developed for Australian waters. Animals are identified up to family level classification with each family assigned a grade between 1 and 10 depending on the tolerance to common pollutants (higher value represent lower level of tolerance). Each species is then assigned for abundance on a 4 point scale. Scores for each type are calculated from the product of grade and abundance. This provides a comprehensive ecological indicator that takes into account the number and abundance of pollutant sensitive animals.
Signal indices are classified into 4 levels:
Less than 4 = probable severe pollution
4-5 = probable moderate pollution.
5-6 = doubtful quality, possible mild pollution, greater than 6 = clean water.
A low grade number means that the macroinvertebrate is tolerant to a range of environmental conditions, including common forms of water pollution. A high number means that the macroinvertebrate is sensitive to most forms of pollution. The higher the number, the greater the average sensitivity. The signal score is also in use for wetland studies (Chessman, 2003).

The formulae for calculation of the indices (Magurran, 1988) are as follows:

**Shannon-Wiener Index**

\[ H' = -\sum p_i \ln p_i, \]
where \( p_i = n_i / N \)
\( n_i = \) the abundance of the \( i \)th species
\( N = \) the total abundance.

**Evenness Index**

\[ J' = H' / H_{\text{max}} \]
where \( H_{\text{max}} = \log S \)
\( S = \) total number of species.

**Berger-Parker index**

\[ d = N_{\text{max}} / N \]
\( N = \) total number of individuals of all species collected.

**Margalef index**

\[ d = \frac{S-1}{\log_e N} \]
\( S = \) number of species.