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MATERIALS AND METHODS
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4.1. Study period

These general limnological studies were carried out for a period of two consecutive years (February 1995 to January 1997) in the two man made lakes viz. Rabindra Sarovar and Subhas Sarovar and for one year (February 1997 -- January 1998) in the ponds General's Tank and for ponds of Brace bridge Nature Park. All sampling were done at fortnightly intervals from three stations of each lake and two stations of Generals tank and ponds of BBNP in early in the morning (between 8.00 -9.00 hours). The studies were carried out for two annual cycles, the first (February,1995-January 1996) and the second (February 1996-January 1997). For detailed studies of the population structure, dynamics and biology of some important species of zooplankton, observations were carried out for a further a period of one year from February 1997 to March 1998 in Rabindra Sarovar only.

For seasonal analysis, the annual cycles were divided into 3 main seasons. These were Premonsoon (PRM), from February to March, Monsoon (MON) from June to September and Postmonsoon (POM) from October to November. Each season was divided in to an early (I) and late (II) parts.

4.2 Sample Collection

Water samples for physico-chemical analyses were collected from all the stations of lakes and ponds from a depth of 15 - 20 cm below the surface. For estimation of dissolved oxygen, water samples were collected in a 300 ml BOD bottle separately. The water was filled in the bottles taking extreme care, so as to avoid any air bubbles entering the bottle. Samples were further collected in a clean glass beaker to measure temperature, pH, alkalinity, hardness and conductivity of water in the filed itself. For the analyses of other parameters, water samples were collected in a clean glass stoppered bottle of 500 ml capacity and brought to the laboratory and analysed as quickly as possible.

Zooplankton samples were collected from littoral zone with the help of a plankton net made of bolting nylon cloth (# 21). Qualitative sampling for taxonomic and relative abundance studies were carried out by sweeping or towing the net several times. For quantitative analyses, 50 litters of water was filtered through the net with the help of a 5 lit bucket. The samples collected in the bottle of the net were transferred in a small enamel tray with little water. The inside of the net near the mouth of the bottle was also washed in the tray so as to collect any attached plankter. Few drops of formalin were added to the tray water for narcotization. Afterwards all the animals died as become narcotized/narcotized and settled, the supernatant water was separated slowly and
concentrated zooplankton samples were taken in sample tubes and were preserved in 4% formalin.

Phytoplankton samples were collected by filtering 1-2 l of water through Whatman filter paper no. 4. Then filter paper was washed in a tray, and preserved in Lugol's solution.

Qualitative benthic samples were collected with the help of a tray type sampler (Khan and Alfred, 1995) from littoral zones only. Samples were washed through a 0.5 mm mesh sieve and all fauna retained on the sieve were hand picked with the help of sable hair brush, blunt head soft forceps and a magnifying glass. All samples were preserved in 4% formalin.

4.3. Sample Analysis

4.3.1 Physico-chemical parameters

Twelve limnological parameters, viz., temperature, pH, turbidity, conductivity, total alkalinity, total hardness, dissolved oxygen, chloride, phosphate, nitrate, nitrite and ammonium were estimated from the water samples collected from the field.

Measurements of temperature, pH and conductivity were done with the help of electronic instrument. Temperature of water was measured by a digital centigrade thermometer and pH was measured by a pH meter (Model 320, Merck Germany), conductivity of water was determined by a conductivity meter (Model LF 320, Merck, Germany). Turbidity values were determined by Spectrophotometrically by SQ 118 (Merck, Germany).

Determination of chemical parameters were chiefly based on Standard Methods (APHA, 1989). Total alkalinity was determined by titrating 20 ml of water sample with 0.02 N sulfuric acid using phenolphthalein and methyl orange indicator. Total hardness was measured by EDTA titrimetric method where water sample was titrated with 0.01M EDTA titrant using EDTA Buffer and Eriochrome Black T dye -sodium chloride dry powder mixture as indicator.

For determination of dissolved oxygen content, azide modification of Winkler's Iodometric method was adopted.

Chloride was measured by Argentometric method where 0.0141 N silver nitrate was used to titrate the water sample with potassium chromate indicator solution. Phosphate (ortho) was measured by ascorbic acid method. Phosphate reacts with ammonium molybdate to form molybdophosphoric acid. This is
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transformed by reductants to form a blue complex which recorded spectrophotometrically.

Nitrate was determined colorometrically following phenol-disulphonic acid method. Nitrite was also estimated colorimetrically by developing a color with EDTA, sulphanilic acid and naphthylamine hydrochloride, sodium acetate. Nesslerization method was followed for estimation of ammonium. Zinc sulfate and sodium hydroxide were added to the sample and one drop of EDTA was added along with Nessler's reagent to develop the colour which was measured by spectroquant SQ 118.

4.3.2 Primary productivity

Primary productivity and respiration were measured by classical light and dark bottle technique (Gaarder and Gran, 1927). Modified Winklers method was used for estimation of oxygen.

Water samples were collected from several depth with the help of a depth sampler during morning hours. After determining the initial dissolved oxygen content, samples of different depth were filled in separate sets of light and dark bottles of 300 ml capacity BOD bottles and hanged at respective depth with the help of a bamboo pole or rope with float and heavy anchor. After an in situ incubation period of 6 hours, the bottles were taken out and their oxygen contents were fixed and determined.

Net and gross productivity and respiration were determined by the following formulae:

Let oxygen in light bottle (mg/l) = X
in dark bottle (mg/l) = Y
in initial bottle (mg/l) = Z

Gross productivity = \( \frac{(X - Y) \times 0.536}{PQ \times N} \) mgC / l / hr

Net productivity = \( \frac{(X - Z) \times 0.536}{PQ \times N} \) mgC / l / hr

where PQ is photosynthetic quotient = 1.2. N is incubation period and 0.536 is the factor to convert mg of oxygen to mgC.
4.3.3 Phytoplankton

Preserved phytoplankton samples were identified under a high powered binocular microscope. The generic identification were done following Smith (1950), Edmondson (1959) and Needham and Needham (1962).

4.3.4 Macrophyte

Macrophyte strands were collected qualitatively from different ponds of the each wetlands and identified up to generic level.

4.3.6 Benthos

Benthic samples were identified following Edmondson (1959) and Subba Rao (1989).

4.3.6. Zooplankton

Preserved zooplankton samples were examined under a binocular microscope with different magnification. Detailed taxonomic identification were carried out following Edmondson (1959), Pennak (1978), Michael and Sharma (1988), Sehgal (1983), Battish (1992) and Arora (1966). Key characteristic taken into consideration for the identification of Rotifera, Copepoda and cladocera were as follow.

a) Rotifera :- Body wall, foot, trophi, Antennae, Corona and reproductive system.

b) Cladocera :- Carapece, Ocellus, Antennae, and Post abdomen.

c) Copepoda :- Body shape, fifth leg, first antennae.

d) Ostracoda :- Size and shape of the shell, 2nd antennae, spines of maxillary process. Third Thoracic leg, caudal furca.

For quantitative analysis of relative abundance and density, identification and enumeration were done simultaneously in a Sedgwick Rafter counter by taking 1 ml subsample and then raising to total volume of water filtered. The sedgwick Rafter counting chamber has 1000 squares with 1ml capacity. The no of plankton counted in 100 squares was converted into units of plankton present per litre by the following formula:

\[ \text{Plankton per litre} = \frac{25 \times \text{No of plankton counted in 100 squares}}{100} \]
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\[
\text{No. of zooplankton / litre} = \frac{N \times 10 \times D}{C} / \text{litre.}
\]

where \( N \) = no of zooplankton in 100 square.

\( D \) = Dilution volume (in ml).

\( C \) = Collected samples (in litre).

Relative distribution of different species was analysed by examining 100 animals randomly and making records species-wise. The size was measured with the help of ocular micrometer.

4.3.5.i. Population Dynamics

Detailed population dynamics studies were carried out on a cladoceran *Simocephalus expinosus* Sars and a copepoda *Mesocyclops leuckarti* (Claus) both under laboratory condition and in field (Rabindra Sarovar).

4.3.5.i.A. Population Dynamics of *Simocephalus expinosus*

4.3.5.i.A.1. Laboratory Culture Studies

Laboratory culture was maintained by keeping mixed zooplankton collected from field in a large enamel bowl containing filtered pond water under normal laboratory condition (29 ± 3°C). Freshly collected pond water was filtered through a relatively fine meshed bolting cloth, which allowed the retention of all insects and crustacean zooplankton but allowed protozoan, rotifers, algae, bacteria and micro detritus. This was treated as normal culture medium. Routine change of such filtered water in the culture supplemented nutrients, oxygen and food supply.

Before commencement of the study, a single adult parthenogenetic female was separated and kept in a small petridish containing 10 ml filtered pond water and allowed to breed. Newly born young ones were again separated and cultured individually in separate petridishes. Further, all the studies were carried out taking these offspring only. This was done so as to have all animals of single conhot. Screening of the petridishes was done daily and detailed observation were made on moulting and change of instars by observing the empty shell. The moulting and duration of each instars, size, growth and number of young ones produced by each adult instar were measured daily. Medium was also changed at the same time.
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Thirty neonates were reared individually for the study of embryonic developments. The methods adopted for rearing the animals were as per Murugan and Sivaramakrishnan (1973).

4.3.5.i.A.1. Field Study

a) Population size

Zooplankton samples were collected from Rabindra Sarovar at alternate day interval by filtering of 50 litres of water. Zooplankton samples were preserved in 4% formalin. Individuals of Simocephalus expinosus were separated from the 1 ml sub-samples of mixed zooplankton samples and counted and classified into three categories, viz. Neonates, Juveniles and Adults. As neonates and Juveniles resembled adult except the size. It was easy to identify and enumerate, relative composition of various size / age groups viz. neonates, Juveniles and adults were then calculated. Mean density of the species was expressed as population size.

b) Population growth rate

The determination of population growth-rate was done following Hall (1964). The number of new born occurring during an interval of time divided by the number of animals already existing is defined as finite birth rate (B). Average brood size and rate of development of egg, expressed as the fraction of development of eggs accomplished per day (1/D) were calculated from the laboratory culture. Finite birth rate was calculated by following equation (Edmondson et al 1972):

\[
B = \frac{N_A \cdot \bar{E} (1/D)}{N_0}
\]

where, \( N_0 \) = Population size, \( N_A \) = no of adults.

\( \bar{E} \) = Average brood size, 1/ D = rate of Development per day.

Instantaneous birth rate (b) and population growth-rate(r) were calculated by using the following formula (Edmondson et al 1962)

i) \( b = \ln (1 + B) \)

ii) \( r = \frac{\ln N_t - \ln N_0}{t} \)
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where $N_t$ and $N_0$ are the population densities at time $t$ and $o$ respectively and $t$ is the time interval between sampling.

c) Reproduction

The species reproduced parthenogenetically. No male of the species were even recorded. Average number of eggs / sac or average brood size was determined from the laboratory culture which served as a population reproductive index. The criterion of reproduce maturity is somewhat arbitrary, since no morphological distinction can be made between mature and immature Simocephalus unless the adults are reproduction. Carapace length at the onset of reproduction was determined. The egg ratio was calculated by multiplying the mean broods size to reproductive ratio of the population (total number of females with broods / total number of adult females).

B) 4.3.5.i.B. Population Dynamics of *Mesocyclops leuckarti*

Like *Simocephalus expinosus*, the population of this copepod was followed throughout the year based on samples collected from field. Life cycle was also studied also under laboratory conditions.

4.3.5.i.B.1. FIELD STUDIES

Ghers and Robertson (1975) method was followed for the study of the population of *Mesocyclops leuckarti* in the field. Zooplankton samples containing *Mesocyclops leuckarti* were collected from field at alternate days interval. Individuals of *Mesocyclops leuckarti* were identified from the mixed samples and total density were enumerated. The entire population was divided into five major classes, viz. (i) egg, (ii) Nauplii, (iii) copepodite I - III, (iv) copepodite IV - V, and (v) copepodite VI (adult). Stages were recognised by appearance and state of development. The sizes of each stage were measured. Since no other cyclopoid was present in noticeable numbers, it was easier to enumerate and measure *M. leuckarti*.

4.3.5.i.B.2. LABORATORY STUDIES

The laboratory culture of *Mesocyclops leuckarti*, was maintained by the method similar to that of *Simocephalus expinosus*. From the stock culture ten
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Pairs of male and female were taken and each couple was placed in separate petridishes containing 10 ml filtered pond water.

As soon as first nauplii appeared, they were separated and kept in separate petridishes. Petridishes were examined daily and records of developmental duration of eggs and various instars, total life span and fecundity were made.

The size of breeding population was recorded by establishing the ratio of ovigerous female to the total female population, clutch size was established by counting the number of eggs per sac for at least 20 animals, egg stock of the population was calculated by multiplying the mean clutch size to mean number of ovigerous females and number of eggs in each clutch was recorded in laboratory and individual fecundity was worked out.

Number of clutches produced by females in the lake was recorded indirectly by dividing the number of ovigerous females to egg development time (Chapman 1969).

Population birthrate, death-rate and population growth-rate of *M. leuckarti* were calculated by the same formula as described in case of *Simocephalus expinosus*.

4.4. INTERRELATIONSHIP BETWEEN ZOOPLANKTON DENSITY AND ABIOTIC FACTORS

Several statistical methods have been used to analyse the relationship of zooplankton with physiochemical factors of environment. An attempt was made to assess the relative effectiveness of various techniques for the variables on the zooplankton density. Techniques used for the present study included transformation, correlation, multiple regression, one way analysis of variance, principal component analysis and redundancy analysis following Jongman et al. (1995).

4.4.a) Data Processing

Fortnightly station-wise data on the zooplankton density and physico-chemical factors are pooled together month-wise for each waterbody separately. All the statistical analyses were carried out using SPSS (Statistical Programme for Social Science) package.

For applying certain statistical techniques like multivariate regression and principal component analysis, variables were standardized. That is, if $Y_{ij}$ is the
value (or score) of the k-th variable at i-th site, then the standardized value $Y_{ki}^*$ is computed as

$$Y_{ki}^* = \frac{(Y_{ki} - \bar{Y}_k)}{S_K}$$

where $\bar{Y}_k$ is the mean and $S_K$ is the standard deviation of the K-th variable ($Y_k$).

**4.4 (b) Multiple Regression Analysis**

A linear multiple regression model was used for identifying the important explanatory (Independent) variables. In a multiple linear regression model, the dependent variable is the one whose variation is explained with the help of other variables/ factors called independent variables. A linear relationship (described below) between dependent variable and independent variables has been estimated throughout this study whatever multiple regression analysis has been resorted to. The analysis also shows the extent to which the variation of the dependent variable is explained by the independent variables. The proportion of variance of dependent variable explained by the model (i.e., by the independent variables included in the model) is measured by $R^2$ and adjusted $R^2$. Adjusted $R^2$ is nothing but $R^2$ adjusted for the degrees of freedom, and is usually denoted by $\bar{R}^2$. The acceptability of the model is tested for statistical significance with the help of the F value in case of each regression. The test of statistical significance of individual coefficient of each independent variable is carried out with respect to the respective t-values.

Attempt has been made in this study to explain the fluctuation in zooplankton density with various physico-chemical factors. Zooplankton density is the dependent variable and the physico-chemical factors are the explanatory variables or independent variables or predictors.

Stepwise regression technique was followed in order to find out which variables have statistically significant impact on zooplankton density and which do not. Stepwise regression is essentially a method of selecting independent variables for a regression equation. To begin with, regression is carried out with the independent variable which had the highest simple correlation (by magnitude, ignoring the sign) with the dependent variable. Next, partial correlation coefficients of each of the remaining independent variables with the dependent variable are computed controlling for the variable(s) already entered into regression equation. The partial correlation coefficient of an independent variable $X_i$ with the dependent variable $Y$ controlling for the independent
variables $X_2, X_3, ..., X_k$ measures the direct impact of $X_i$ on $Y$ after separating out the indirect effect of $X_1$ on $Y$ through $X_2, X_3, ..., X_k$. In practical situation, a variable like water temperature often has an indirect effect on turbidity, dissolved oxygen, etc. in addition to its direct impact on zooplankton density. Partial correlation coefficient between water temperature and zooplankton density measures the direct impact of the former on the latter after taking out (controlling for) the indirect impact of water temperature on zooplankton density through turbidity, dissolved oxygen, etc.

The independent variable with highest partial correlation coefficient (irrespective of sign) was entered into the equation in the second step, and regression was carried out with two independent variables. Then, the third variable to be entered into the regression equation was selected as the one with the highest partial correlation coefficient (ignoring sign) with the dependent variable controlling for the first two variables already entered into the equation.

Actual analyses were carried out with SPSS (Statistical Package for Social Sciences), one of the most widely accepted computer packages for statistical analyses. In SPSS, stepwise regression analyses are continued till the step at which the p-value crosses some pre-specified limit. Variables are entered into the equation on the basis of highest partial correlation coefficients of variables not in the equation with the dependent variable controlling for the variable(s) already in the equation.

At every step, the value of adjusted $R^2$ keeps on increasing, but up to a certain point. Results of regression analyses are reported here up to the step till which the adjusted $R^2$ keeps on increasing starting from the first step.

All equations were estimated in linear multiple form:

$$Y = b_0 X_1 + b_2 X_2 + \ldots + b_n X_k$$

where $Y$ = dependent variable (zooplankton density)

$X_k$ = independent variable (physicochemical factors)

A total of 12 independent variables ($k = 12$) is considered. For each independent variable, the t-test and its probability, the full regression coefficients of determination $R^2$ and the degrees of freedom are reported. $R^2$ shows the extent of variation in the dependent variable explained by the model, i.e., by the set of independent variables. The standard error of estimation of $Y$, $F$
test and its probability are also reported. Since the sample size is not large (24 observations for Rabindra Sarovar and Subhas Sarovar each, 12 observations for General’s Tank and Brace Bridge Nature Park each) as compared with the number of predictors (12), adjusted $R^2$ is reported as well. This is the squared multiple correlation $R^2$ adjusted for the degree of freedom, and is given by the formula,

$$
\bar{R}^2 = 1 - (1 - R^2) \frac{n - 1}{n - k}
$$

where

$R^2$ = the original squared multiple correlation

$n$ = the sample size

$k$ = the total number of predictors/ independent variables in the equation

For each model, $\bar{R}^2$ indicates the variations in density explained by the $k$ variables.