CHAPTER TWO

MATERIAL AND METHODS

2.1 The habitat selected
2.2 Morphometry of the habitat
2.3 Hydrobiological parameters
2.4 Primary production
2.5 Zooplankton analyses
2.1 THE HABITAT SELECTED

The Nelligudda reservoir is located near Bidadi, along the Bangalore-Mysore Road. It is 26 km from the Bangalore University, Jnana Bharathi Campus. The reservoir is rain-fed but perennial and has a maximum depth of 10 m and an extent of 80 ha (ha = Hectare; 1 ha = 2.451 acres) at Full Reservoir Level (FRL).

2.2 MORPHOMETRY OF THE HABITAT

The morphometric data was obtained from the Irrigation Department. Mean water-spread area and shoreline development (DL) were calculated by standard methods (APHA 1989). The details of the habitat are given in Chapter 3.

2.3 HYDROBIOLOGICAL PARAMETERS

Water samples were collected from different depths of the water column at a fixed station near the centre of the reservoir, using a modified Mac Vuit sampler (Littrick and Mavuti 1985) (Fig. 2.1).

The details of the parameters studied, periodicity of collections and depth of sampling are summarised in Table 2.1. The location of sampling stations are shown in Figure 3.1.

2.3.1 WATER LEVEL

The water level was recorded from the 'Level Gauge' installed at the sluice. The gauge is graduated from 0' at the Dead Storage Level to 24' at the FRL. At each sampling station the water column depths were recorded using a graduated sounding line.

2.3.2 TEMPERATURE

Temperature of the water (°C) was recorded from the surface to the bottom, at intervals of every meter, using a mercury thermometer, graduated at 0.1°C interval.
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameter</th>
<th>Sampling depth</th>
<th>Frequency</th>
<th>Period</th>
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<tr>
<td>2.</td>
<td>Water temperature</td>
<td>Intervals of every meter from the surface to the bottom</td>
<td>Same as for 1</td>
<td>Same as for 1</td>
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<td>3.</td>
<td>Secchi disc transparency</td>
<td>-</td>
<td>Same as for 1</td>
<td>Same as for 1</td>
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<tr>
<td>4.</td>
<td>Dissolved oxygen</td>
<td>Same as for ?</td>
<td>Same as for 1</td>
<td>Same as for 1</td>
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<tr>
<td>5.</td>
<td>Alkalinity Conductivity Total phosphorus</td>
<td>Intervals of every two meters from the surface to the bottom</td>
<td>Fortnightly</td>
<td>Aug 1993 - Aug 1995 (BMI)</td>
</tr>
<tr>
<td>6.</td>
<td>POC Chlorophyll 'a'</td>
<td>Uppermost two meters - integrated</td>
<td>Same as for 5</td>
<td>Jan 1994 - Apr 1995 (BMI)</td>
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<td>7.</td>
<td>CPUE</td>
<td>-</td>
<td>Same as for 1</td>
<td>Same as for 6</td>
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<td>8.</td>
<td>Primary productivity</td>
<td>Every one meter depth</td>
<td>Monthly</td>
<td>Nov 1993 - Aug 1994 (BMI)</td>
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<td></td>
<td></td>
<td></td>
<td>Fortnightly</td>
<td>Sep 1994 - Aug 1995 (BMI)</td>
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<tr>
<td>9.</td>
<td>Zooplankton</td>
<td>Every two meters from the surface to the bottom - integrated</td>
<td>Weekly</td>
<td>Aug 1993 - Nov 1994 (BMI)</td>
</tr>
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</table>
2.3.3 TRANSPARENCY

Water transparency (cm) was measured using a Secchi disc of 20 cm diameter painted white.

2.3.4 DISSOLVED OXYGEN

The dissolved oxygen content (mg/l) of the water samples collected at every 1 m depth was estimated titrimetrically by the Azide modification of the Winkler's method (APHA 1989).

2.3.5 pH

The pH of the water samples was measured in the laboratory within three hours of collection, using a pH meter fitted with a glass electrode (model Toshniwal, Type CL 24).

2.3.6 TOTAL ALKALINITY

Total alkalinity (mg CaCO₃/l) was determined in the water samples using bromocresol green as the indicator (pH 4.5). This is known to produce a sharp end point colour change (APHA 1989).

2.3.7 SPECIFIC CONDUCTIVITY

The specific conductivity of the water samples (µS at 25°C) was measured in the laboratory, using a conductivity bridge (ELICO model). Prior to the measurement, the temperature of the sample was recorded.

2.3.8 PARTICULATE ORGANIC CARBON (POC)

The POC (mg C/l) was determined by the 'wet oxidation' procedure as detailed in Wetzel and Likens (1991). Integrated water sample of the uppermost 2 m of the water column, collected with the help of Lund's tubing (Lund 1954) was used for the analysis. Unfiltered ('Whole') and 35 µm-filtered aliquots of the sample were filtered on to pre-ashed (500°C) GF/F (Whatman) filters. The filter papers with the residue were stored at 4°C prior to analysis, which was carried out within 24 hours of collection.
2.3.9 TOTAL PHOSPHORUS AND AMMONIA

Total phosphorus (mg/l) was determined after persulphate digestion by ascorbic acid method and ammonia (NH$_4$-N µg/l) by phenol-hypochlorite method, as described in Wetzel and Likens (1991).

2.3.10 CHLOROPHYLL 'a'

Chlorophyll 'a' content (µg/l) of the integrated sample of the uppermost 2 m of the water column was determined by filtration through GF/C filter. The filters were stored over a desiccant and deep frozen until analysis, which was undertaken within 24 hours. The pigment was extracted in alkaline acetone by effectively grinding in a glass/glass tissue grinder, under subdued light. After centrifugation, the concentration was determined by spectrophotometric analysis at 665 and 750 nm. No correction was made for the degradation products. Lorenzen's (1967) equation (cf. Wetzel and Likens 1991) was used to estimate the pigment concentration.

2.4 PRIMARY PRODUCTION

Primary production in aquatic environments is measured most accurately by techniques that monitor the flux of metabolically labile molecules, especially oxygen and carbon dioxide. The most common of these techniques currently in practice involves the measurement of differential metabolism in transparent and dark bottles or C-14 labelled carbon dioxide. The 'oxygen difference method' was used during this study. The details and limitations of this technique are outlined in IBP Handbook No.12 (Vollenweider 1969). The oxygen-difference incubations were carried out in 250 ml Borosil bottles. These bottles were rinsed in weak thiosulphate solution to clear them of iodine, then washed thoroughly in distilled water each time before use. Dark bottles were designed by covering them with a double layer of black plastic scotch tape. To prevent the entry of light around the ground glass joint, before suspension the neck
portion of the dark bottle was covered with aluminium foil. The light and dark bottles (in duplicates) were filled as rapidly as possible, flushed and then stored in dark until all the samples had been collected. Simultaneously, duplicate oxygen bottles were also filled at each depth and fixed chemically for the measurement of initial oxygen concentration. Using a graduated suspension line (marked at each metre intervals) and starting with the samples from the greatest depth, the bottles were suspended with light bottles at the ends and dark bottles in the centre. Incubations were done at 0.1, 1, 2, 3 and 4 m below the surface. From September 1994 onwards, incubation was also made at 0.5 m below the surface. The incubation was always started around 10°Ch and terminated after 3-4 hrs. Rates of photosynthesis often have been observed to be greater during the early part of daylight and thereafter to decrease markedly, in the afternoon periods of intense light accompanied by increased concentrations of dissolved oxygen and higher pH values. Hence, incubations from the mid-morning to the mid-afternoon (from 10°00 to 14°00 hours) are recommended, to compensate for these variations and to yield an average value (Wetzel and Likens 1991). The oxygen values were converted to carbon, using a factor of 0.375. Both the photosynthetic and respiration cc-efficients were taken to be 1.0. Using these values, net and gross production rates as well as community respiration rates were calculated.

To estimate the rate of photosynthetic productivity through the water column of the euphotic zone (below one square meter of water surface), the productivity values were plotted against the depth and the area of the curve was integrated planimetrically.

The productivity values obtained for the incubation period of 4 hours (from 10°00 to 14°00 hours) were converted to daily values, by using a diurnal expansion factor of 2 (see section 5.4 for details).
Annual primary productivity was estimated by integrating the area under the daily productivity using planimetry.

2.4.1 SPATIAL HETEROGENEITY OF PRIMARY PRODUCTION

Primary production may vary over the surface of a lake as the phytoplankton respond to local variations in sunlight, nutrients, cropping and other factors. Spatial heterogeneity of primary production was assessed by estimating the production at four different stations, in addition to the central station, covering the entire expanse of the reservoir. The quantum of production at a fixed depth near the point of maximum fixation (which was 0.1 m below the surface during the present study) was assumed to be representative of each of the five stations (see also Lewis 1974). The samples collected from all stations at 0.1 m depth were incubated at the central station, together with the samples of the central station. Since all the samples were incubated at the central station, it is believed that all variables are either due to the phytoplankton or due to the chemistry of the water from which they were collected and not due to variations in sunlight.

2.4.2 DIURNAL VARIATIONS IN PRIMARY PRODUCTION

It is suggested that for a number of reasons incubation be limited to 4 to 6 hours (cf. Vollenweider and Nauwerck 1961, Vollenweider 1965). This period is said to be not only long enough to allow measurement of carbon assimilated but is also sufficiently brief to minimise errors incurred from longer exposures as a result of bacterial growth on the inner surface of experimental bottles, respiratory recycling of CO₂, nutrient depletion and so on. To be meaningful, however, the results are expected to be extrapolated to a daily value by using correction factors.
Ideally, productivity measurements are believed to be taken from the euphotic zone, at about 4 hour increments from the dark to dark phase each day, for an entire year. Since this regimen is rather impractical, final estimates are suggested to be made from data that have been already obtained.

On two chosen dates, namely 08.03.1995 and 14.06.1995, sets of experimental bottles were incubated from 06° to 18° at every three hour intervals (06 to 09, 09 to 12, 12 to 15 and 15 to 18). March 8 was characterised by continuous bright sunshine with clear sky, whereas June 14 was completely overcast, with low sunshine. The calculated production, integrated over the depth, for each time-interval, was plotted at the mid point of its respective time-period. The area of the entire curve was integrated and compared with that of the typical 4-hour measurements (10° to 14°). The average of the area ratios of the fraction to the whole (for the two experimental dates), was used as the scaling factor, to extrapolate the 4-hour incubation to a daily value.

2.5 ZOOPLANKTON ANALYSES
2.5.1 THE SAMPLING DEVICE

As the population density of zooplankton represents the number of individuals in a given volume of water, choice of a right sampler that can make accurate quantitative collections is essential. For quantitative zooplankton collections description of various devices, with their merits and demerits are detailed in Downing and Rigler (1984). During the present studies, a self-closing Schindler-Patalas trap (Schindler 1969), made of transparent acrylic material was specially designed (Fig. 2.1). A large trap of 28.5 l capacity, equipped with a 35 µm mesh was used to minimise the escape of highly motile species and to trap sufficient number of organisms for counting even when their population was small. Plankton traps can be regarded as a special kind of
bottle, specifically designed for plankton collection. These quantitative samplers usually present certain advantages as compared to ordinary bottles (Van Dorn, Ruttner bottles). They have rapid closing systems and large mouths, both of which reduce the possibility of 'avoidance reactions', and allow for simultaneous collection and filtration of a large volume of water. Authors who have compared the efficiency of plankton traps to other sampling devices have opined that traps are highly efficient. This is specially true with regard to the trapping of species which display the strongest avoidance reactions viz. adult copepods and Daphnia sp. (Patalas 1954, Schindler 1969).

2.5.2 ZOOPLANKTON SAMPLING

a. Procedure

The routine weekly zooplankton sampling was made at the central station (see Fig. 3.1), during August 1993 and November 1994 (BMI). The samples were collected from the entire column of water at intervals of 2 m depth. The samples so collected were drained into one container, to represent the sample of entire water column of the station. On several occasions, additional samples were, also, collected from the entire water column at the south station to assess if the sampling at the central station was adequate as a reflection of the entire lake-plankton-profile.

b. Preservation

The plankton samples were preserved immediately on-board, using a solution of 40 g/l of sucrose and 4% buffered formaldehyde (Haney and Hall 1973). This method of preservation is known to reduce egg-loss and avoid ballooning of the carapace.

c. Sub-sampling

At the time of counting, the sample was brought to a certain fixed volume, depending on the density of the organisms, to yield sub-samples containing greater than 60
individuals (see Bottrell et al. 1976). With the help of wide-bore (4 mm) automatic pipette, well mixed sub samples of one ml were drawn. The flask containing the sample was swirled in a figure eight pattern, to ensure random distribution of organisms. While in motion, the pipette was inserted and the sub sample was drawn.

d. Counting

In the case of cyclopoids (except the ovigerous females) and rotifers, the sub samples representing 1 to 5 1 of lake water, were counted using a Sedgwick-Rafter counting cell, counting all the organisms in the sub sample. The counting was done at 100 X magnification using a binocular compound microscope. For calanoids and cladocerans, which were comparatively scarce, sub samples representing 25 to 50 ml of lake water were counted under a stereoscopic microscope at 52.5 X magnification. The ovigerous females of cyclopoids, which were low in abundance, were censused along with calanoids and cladocerans.

e Identification of developmental stages

In the case of copepods (as only two species with large size difference were common viz Thermocyclops hyalinus and Heliodiaptomus viduus), it was possible to separate all the developmental stages namely nauplius, copepodite stages - 1 to 5, male and female. In temperate lake, advanced copepodite stages of cyclopoids are known to often undergo diapause in the sediment (Hutchinson 1967). As per the findings of many (Ravera 1954, Fryer 1957a, Burgis 1971, Lewis 1979), in warm water lakes the cyclopoids are known to remain planktonic throughout their life cycle. The counts are thus believed to represent all the developmental stages adequately. Adults of both the species were distinguished on the basis of urosomal segments and/or species-specific secondary sexual characteristics.
All adults of rotifers were distinguished species-wise but the eggs were counted together without separating them species-wise.

f. Fecundity

For copepods, at least ten ovigerous females were randomly picked every alternate week for fecundity studies. The organisms were measured from the tip of cephalothorax to the point of insertion of the caudal setae. The egg-sacs were separated from the mother, the eggs separated from one another and counted. In the case of rotifers, the eggs were censused simultaneously, while counting the organisms for their density.

g. Biomass

The linear measurements as determined were converted to biomass using the length-weight relationship data of each genus/species documented in earlier literature (Downing and Rigler 1984).

2.5.3 CALCULATION OF POPULATION PARAMETERS

The accuracy and reliability of population estimates of birth and death rates are known to depend on the frequency and precision of numerical sampling (Prepas and Rigler 1978, De Mott 1980, Keen and Nassar 1981). Keen and Nassar (1981) recommend sampling intervals roughly equal to the length of the embryonic duration. For entomostracans inhabiting waters above 15-20°C, this would require samplings twice or thrice in a week (Herzwig 1983). Investigators attempting to meet this ideal situation face heavy sampling and analytical loads. Statistical procedures now exist which yield confidence limits for sampling variability, for the instantaneous population parameters (Keen and Nassar 1981) and permit reasonably reliable assessment of population estimates. The calculation of birth rates (natality rate) from 'egg-ratio' technique introduced by Elster (1954) and Edmondson (1960, 1965, 1968) has invoked much discussion.
Objective guidelines which assist in the choice of an appropriate formulation for this purpose now exists (Taylor and Slatkin 1981). Based on these "Paloheimo's birth rate formula" (Paloheimo 1984) which has gained acceptance in recent accounts (Keen and Nassar 1981, Taylor and Slatkin 1981) was chosen for use during the present studies:

\[ b' = \ln \left( \frac{E}{N+1} \right) / D \]

Where \( b' \) is the instantaneous birth rate
- \( E \) is the egg density (number of egg-bearing females x mean clutch size + detached eggs or embryos)
- \( N \) is the density of post embryos and
- \( D \) is the embryonic duration at the prevailing temperature.

Prime signs are used to denote that the parameters are determined indirectly. Embryonic duration of *Thermocyclops hyalinus* was obtained from Burgis (1970). Successive population estimates \((N_t, N_o)\) were used to calculate the instantaneous rate of population change \((r')\) over sampling interval \((t)\), using the following familiar exponential equation:

\[ r' = \frac{(\ln N_t - \ln N_o)}{t} \]

The instantaneous death rate (mortality rate) \((d')\) was estimated as the difference between instantaneous birth rate and rate of population change:

\[ d' = b' - r' \]

2.5.4 PRODUCTION ESTIMATES

Production can be determined from turnover rate derived from birth or death rate values (e.g. Burgis 1971, George and Edwards 1974, Hart & Allanson 1975, Waters 1977, Hart 1987,
Mason & Abdul-Hussein (1991). The merits and demerits of the approach are discussed in Rigler and Downing (1984). In equilibrium populations with a stable age distribution, replacement time or turnover time of numbers can be equalled with replacement time of biomass. Thus estimates of per capita finite birth rate ($\beta$) given by

$$\beta = \frac{F}{ND}$$

(with $F, N$ and $D$ defined above) multiplied by the standing stock biomass ($B$) gives values of production ($P$)

$$P = \beta \times B$$

This approach has been applied to populations which violate the assumptions of steady state. Despite valid criticisms (Rigler and Downing 1984), this method offers an approximate estimate of production.