3

19

MATERIALS AND METHODS

(a) Field Laboratory :

A comparative study of primary productivity of higher aquatic plants, periphyton and phytoplankton in the Ajwa Reservoir was undertaken from March 1969 to October 1970. This investigation was carried out every month for three days at the reservoir site. A field laboratory very close to the intake tower was also established in one of the retiring rooms attached to the reservoir, where the author spent 3 days every month during the period of this investigation.

(b) Location of Sampling Station in the Reservoir :

As shown in Fig. 2. two stations hereinafter referred to as Station A and Station B in the reservoir were established for initiating the programme of primary productivity measurements. In

selecting the two stations, the main object was to compared primary productivity in two biologically different situations in the reservoir. Station A, located near the intake tower, represented a place where there was practically no macrophytic vegetation and was the deepest spot in the reservoir; while Station B represented an equally deep place where there was abundant macrophytic vegetation at all times of the year. The station B was located right in the midst of macrophytes and the two stations were indicated by two long galvanized iron pipes driven deep into the bottom and projecting about a meter above the water surface when the reservoir level was at its maximum. At Station B, a two square meter open space was cleared among the hydrophytes for obtaining samples from different depths. The boat was slowly rowed to the spot half an hour before taking the sample each time, so that conditions at the Station might become normal before the actual sample collection. Estimations of productivity made at Station A represented the primary production essentially due to phytoplankton and those made at

21 B were considered as being due to macrophytes (and periphyton and Phytoplankton together to some extent).

(c) Methods adopted for estimating primary productivity by (i) phytoplankton; (ii) macrophytes and (iii) periphyton.

"Primarily the investigator should try to select methods which, within the limits of the available resources, will give the most meaningful results when applied to the particular community to be studied. If possible, however, he should also try to apply some other methods to facilitate comparisons" (IBP Handbook No.12, 1969, p.114). The author has adopted simple, unsophisticated methods which are easily employed according to Ganapati and Pathak (1970). The author regrets that he could not obtained equipments such as those used by Wetzel (1964) in his comparative studies of primary production by higher aquatics, periphyton and phytoplankton in Borax lake in U.S.A. Secondly it was not

22 possible to use electrical probes as there was no electric power supply in the field and the battery operated probes were not available in the laboratory.

(i) Phytoplankton :

Pomeroy (1961) has discussed the advantages and disadvantages of the four methods usually employed in determining primary productivity in natural waters. Two of them are the most commonly used methods, viz. (i) the classic method of estimating oxygen changes in clear and dark bottles and (ii) measurements made directly in the aquatic environment shortly after dawn and before sunset i.e. at the beginning and at the end of the period of illumination taking advantage of community metabolism. In principle, the measurement of photosynthesis is the same in both the methods, and Talling (1957) has discussed the several sources of error inherent in them, no method being without them. He advocates the carrying out of experiments relating the diurnal changes over a period of several days for two reasons: (a) for checking the

reliability of the methods and (b) for correcting sources of loss due to respiration and gas exchange across the air-water interface using the mean rate of oxygen depletion during the night.

Again, Talling (1957) has stated that in shallow water bodies of low transparency and with simple morphometry with uniform wind action, the most favourable data are likely from observations made over several days. The observed oxygen changes during the day should be corrected for (a) respiration losses generally assumed constant throughout day and night; (b) for turbulent exchange with the atmosphere, and (c) for irregularities from advection of other water mass which is generally neglected. Gross and net primary production, respiratory losses, rate of photosynthesis, seasonal changes in production, annual production and photosynthetic efficiencies, have been calculated with the data obtained by second method, that is, by the diurnal changes in the community metabolism. Surveys of primary production were made at intervals

of about 30 days as suggested by Sorokin (1969 p.124).

(ii) Macrophytes :

Westlake (1969) has advocated the determination of diurnal changes in the concentration of oxygen, or CO_2 and pH over long hours on non-isolated natural community as a better measure of the primary productivity of plants, provided the rate of input and output of the substances measured to and from other sources other than the plant is known or known to be negligible. Following his suggestion, estimations of the total combined primary productivity of macrophytes periphyton and phytoplankton were made during 1969-1970 for Station B, as the macrophytes were always covered with a brownish coating of marl-like deposits, which on treatment and examination were found to consist of hundreds of several species of minute diatoms. The primary productivity of periphyton (which mainly consists of diatom species) was determined separately also.

25 From the biomass of the phytoplankton component, it was assumed that its contribution to primary productivity was negligible. The Winkler method was used in estimation of dissolved oxygen in both the cases.

(iii) Periphyton :

Few attempts have been made to assay the growth rate of natural populations of periphyton in situ (Wetzel, 1964, 1965, 1969). The data of this parameter are often useful in view of the periphytic heterogeneity on natural substrates inspite of the numerous sources of errors in estimating production of periphyton. A modification of the method followed by Vollenweider and Samaan (1958) was adopted in this investigation for these estimates. They vertically suspended glass rods of known surface area at various depths among emergent macrophytes for simulated colonization of periphyton. The rods colonized with algae were carefully placed into bottles of prefiltered lake water taken at depths at which they (rods) were

26

originally suspended. Periphyton was removed from the rods, homogenized in a small aliquot of water and a portion filtered on to a membrane filter for radio assay. In the present study, glass slides of blue seal quality were thoroughly cleaned, sterilized and put in slide boxes open on both the sides. These boxes were hung by wire from a horizontal pole at Station B (Figure 1). The pole was attached to two vertical galvanized iron pipes driven into depths at Station B. The slide boxes with slides were hung below the water surface at depths of 10 cm., 2.5 m. and 5 m depth. These boxes were adjusted every month to keep them at the respective depths. Each box contained 100 glass slides. This incubation experiment was started on October 23, 1969.

Once every month (keeping the interval constant as far as possible)several slides were removed from the boxes and taken to the field laboratory in wide-mouthed glass bottles containing filtered water into which they were carefully placed in a slanting position. The growths attached to the slides were removed later by means of a

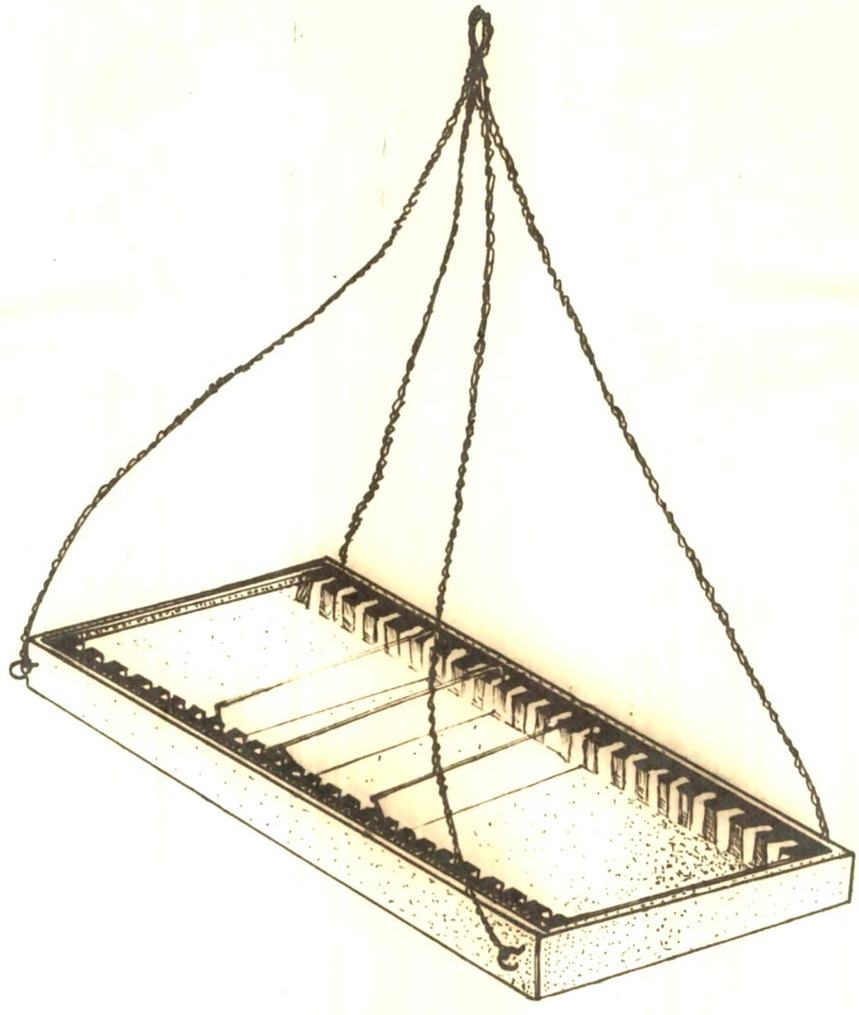

Figure 1 Reck with glass slides suspended in the reservoir

27 blade, then transferred to a pre-weighed filter paper and dried at 55°C for 1 to 3 hours until two consecutive weights remained the same. The area of the slides was measured and the average growth per unit area was calculated. The periphyton growths consisted essentially of hundreds of minute diatoms. From another aliquot, the diatoms were counted for seasonal variations in all the monthly collections. From a third aliquot dry weight, ash weight and organic matter were estimated.

The use of nets for collecting phytoplankton for studies on primary production is not recommended as all the nanno and ultra plankton is lost from the sample (IBP Handbook No.12). However, Lund and Talling (1957) have pointed out the usefulness of plankton nets when quantitative estimations by other methods would be impossible. Rawson (1953) also has used a plankton net for quantitative sampling for establishing fundamental relationship between lake morphometry and production. The author

28 considered it useful for the purpose of this study, since filtering a few litres of the reservoir water was not helpful in collecting sufficient phytoplankton. So, a plankton net of silk bolting cloth, 15 cm in diameter, containing 77 threads to a centimetre and measuring nearly 60 cm. in length was used in all these investigations, unless otherwise mentioned. The net was attached to one end of a 6 m long rope and the other end was tied to a post in the boat. The boat was rowed by four men, slowly and steadily, at a uniform rate between two fixed points i.e. the intake tower and the second landing place at a distance of about 200 m. from the tower. The time taken for rowing this distance was about 30 minutes on each occasion. The net was towed very gently to reduce displacement of water from the mouth of the net. The net plankton was carefully washed and the material was transferred to a specimen glass tube and the volume made to measure 15 ml and later taken to the field laboratory. The collection was made between 8 and 10 a.m. on every occasion.

29 At the field laboratory near the intake tower, the sediments were measured after centrifuging for 15 minutes in a hand centrifuge and the sediments were made upto a known volume again. An aliquot part of the sediments was preserved with enough formalin to make a 4% solution for identifying the plankton organisms later.

Immediate examination of the catches always showed that they consisted largely of organic debris and more Zooplankton organisms and very little of phytoplankton of dimensions greater than 60μ. The organic debris being heavier easily and quickly settled down, and the zooplankton organisms could be seen as white specks evern with the naked eye in the clear water column above when the tube was viewed against light. Only a very few phytoplankton organisms could be seen with a hand lens.

(iv) Extraction and estimation of Chlorophyll a per gram dry weight of seston in the net plankton.

Since Harvey's (1934) first introduction

of a method for measuring chlorophyll in marine phytoplankton using visual standards, many research workers (Kozmlanski, 1938; Manning and Juday, 1941; Richards and Thompson, 1952; Parson and Strickland, 1963 etc.) have used colorimetric or spectrophotometric and fluorimetric techniques. For the simultaneous determination of chlorophyll a,b and c as well as astacin and non-astacin types of carotenoids the polychromatic spectro-photometric methods of Richards and Thompson as modified later by Parsons and Strickland are widely used. But Vollenweider (1969) suggested that only chlorophyll a is important in the process of photosynthesis and limnologists should confine themselves only to chlorophyll a, the most important and abundant pigment in the living material. Following this suggestion only chlorophyll a estimations have been made in this study.

Extraction and estimation of Chlorophyll a :

The plankton mass from the seston was treated in a known volume of 90% aqueous mixture

31 of acetone (the method of Richards and Thompson, (1953) and the clear supernatant coloured liquid was used for the estimation of chlorophyll a using the wave length 665 m/u in a Carl Zeiss SPEKOL Spectrophotometer using the formula of Talling and Driver (1963).

Westlake (1964) has reported that in tropical aquatic communities with an almost constant biomass, it will not be profitable to search for the annual maxima. The seasonal changes of biomass in tropical lakes are very small or almost negligible.There is no well marked periodicity.So without the rates of turn over of the crop of community it would be very difficult to generalize the validity of the production rates from the standing crops.

(v) Biomass of Vegetation :

The distribution of biomass of rooted, submerged and floating vegetation is related to environmental conditions. In general, the vertical

distribution of biomass is determined by water depth, and the physical characteristics of the bottom sediments.To investigate first the distribution of the standing crop of rooted aquatic plants, the littoral region was divided into 200 sectors at various depths and at each sector sampling of rooted aquatic plants was made several times by means of a locally improvised bottom dredge, mostly when level of water in the reservoir was lowest in May.For estimating the standing crop the whole plants in a constant area of 1 m by 1 m were removed and weighed. In this way, the quantitative relationships obtained by both the methods were taken into consideration. About 100 stations were picked up at random at various depths and slopes in the littoral region. The average dry weight of the aquatic plants was calculated as 10% of the fresh weight as most of the rooted plants had about 90% water content.For the entire reservoir, the total weight of the standing crop in a unit area was multiplied with the total area, (Jayangaudar,1964).

33 (d) Solar energy and its relation to production :

The visible portion of sunlight with wave lengths of 400 m/u to 700 m/u is the prime factor for photosynthetic oxygenation, and therefore, the knowledge of the geographical distribution of the photosynthetically active light is of basic importance.This value was not measured directly by means of a pyrheliometer but was obtained from Smithosonian Meteorological Tables (1951) for the corresponding latitude of Baroda near which the Ajwa reservoir is situated (Ganapati,1970). The values for visible radiation for the particular latitude corrected for sky clearance factor, elevation etc., have been taken as a quantitative measure of the energy available for photosynthesis for the twelve months of the year (Ganapati and Sreenivasan 1970). The quantity of oxygen produced is given by the relation $W.O_2$ (mg. per litre per day) = F x S/3.68, where $W.O_2$ is the weight of oxygen, S the amount of visible solar energy penetrating a smooth water surface in calories per sq.cm. per day, F the efficiency of conversion of

34 light energy conversion to chemical energy expressed as a decimal, 3.68 is the energy in gram-calories required to produce 1 mg of oxygen through photosynthesis.

The utilization of the solar energy by algae developing in the presence of nutrients in the reservoir under study results in the proliferation of new algal cells and the concomitant production of molecular oxygen. A relationship has been established by Oswald and Gotaas (1957) between the algal cell material synthesised and the oxygen released on account of photosynthesis.

(e) Oxygen-algal weight ratio :

The amount of energy associated with oxygen production is well established. It has been found in the case of C hlorella, Scenedesmus and other planktonic organisms that the energy required is 3.68 calories per miligram of oxygen production; that the production of planktonic algae per gram is associated with 1.25 to 1.75 gm of oxygen; and that

the unit heat of combustion of algae is about 6000 calories per gram or 6 calories per miligram. Hence the ratio of the weight of oxygen released to the weight of algae synthetised is termed 'p' which corresponds to a value of 1.63 (Arceiwala 1964) in the range of 1.25 to 1.75 gm for India.

(f) Calculation of Efficiency of light energy conversion :

The photosynthetic efficiency 'F' has been taken as nearly equal to the ratio of energy 'H' (in calories) in the algal cells produced per unit area per day to the amount of visible solar energy 'S' (in Langley) received at the water surface per unit area each day, i.e. F = H/S (Oswald et al 1957). 'H' can be determined from the gross photosynthetic oxygen data by multiplying the values by 3.68 or from the algal weight which is calculated by dividing the oxygen values by the 'p' value of 1.63 (Ganapati and Sreenivasan 1970).

56 (g) Sample collection from the two Stations A and B :

Samples of surface water were taken ten centimeters below the surface. Vertical series of samples (at 6.00 a.m., 10.00 a.m., 2.00 a.m. 6.00 p.m., 10.00 p.m., 2.00 a.m. and 6.00 a.m. next day) were obtained from different depths by a modified Winkler's sampler. The temperature was recorded by means of a mercury bulb thermometer calibrated to a tenth of a degree immediately after the bottles were hauled up to the surface. The samples for dissolved oxygen were taken separately in a similar way and analysis were carried out by Winkler's method immediately in the field. The water samples for other tests were stored in airtight polythene bottles for further analysis in the laboratory.

(h) Estimation of respiratory uptake of oxygen and gross and net primary production.

The rate of respiratory uptake of oxygen taking place in the water by the aquatic communities was estimated from the nocturnal rate of oxygen decline

37 from 6.00 p.m. to 10.00 p.m., from 10.00 p.m. to 2.00 a.m. and from 2.00 a.m. to 6.00 a.m. series of samples.The average rate of oxygen decline per hour at night has been taken as the average rate of community respiration in water column (Talling, 1957).

Samples of water were obtained from 0, 1, 2, 3, 4, 5 metre depths once a month at intervals of 4 hours in 1969 and at 6 hours in 1970, and included those taken near sunset and sunrise and the period of sample collection extended over more than one diurnal cycle of 24 hours. The day series of samples taken for dissolved oxygen represented the values of net photosynthetic oxygen production and the night samples, the values for respiration of aquatic communities; and the sum of the two values the gross production. The exchange of O_2 between air and water was calculated separately.

(1) Other tests :

pH, dissolved oxygen, ortho-phosphate,

carbonate, bicarbonate, silicate, nitrate, nitrite, ammoniacal nitrogen (direct nesslerisation) and hardness were determined at the field laboratory. All the tests were done according to the Standard Methods (APHA, 1965).

Numerical estimation of plankton was made by the 'drop-sedimentation technique' detailed in the Standard Methods (APHA, 1965).