3. Materials and methods

1.0 Vallabhsagar reservoir

The present study deals with the scale-based growth performance of Indian major carps namely catla (*Catla catla* Ham. 1822), rohu (*Labeo rohita* Ham. 1822) and mrigal (*Cirrhinus mrigala* Ham. 1822) in Vallabhsagar reservoir (Gujarat). Which is located on Tapi River and this river drain Madhya Pradesh, Maharashtra and Gujarat before emptying into the Gulf of Cambay at Surat district of Gujarat state. The basin of Tapi river is spread over (9,804 km²) in Madhya Pradesh, (51,504 km²) in Maharashtra and (3,837 km²) in Gujarat. Three main reservoirs namely Hathnur in Maharashtra, Kakrapar Weir and Vallabhsagar in Gujarat were built across this river. Among these reservoir Vallabhsagar is largest one and known as Ukai dam or Ukai reservoir which is situated on 21° 15' North longitude and 73° 35' East latitude geographical location in Tapi district of Gujarat. This multipurpose reservoir was completed in 1971 with catchment area 6225500 ha, water spread area of 520 km^2 at full reservoir level along with mean depth 11.8 m (Table 1).

1.1 Sampling stations

Allocation of the sampling station is the first and significant step before beginning of any research. The selection of sampling stations is generally linked with the study objectives, types of data, quality of data, equipment applied and sampling methods. After collection of detail information on physical parameter, biological parameters, geographical location and availability of sufficient catch fish six sampling stations were selected for proposed study. In these selected sampling stations three (Serula, Ukai, Chacharbunda) are situated on left side and other three (Thuthi, Jamli, Parchuli) are situated on right side of the dam (Fig.1).

2.0 Sampling duration

For the proposed study, water samples and fish specimens were collected during first week of every month for two consecutive year (June 2013 to May 2015). Sampling duration was further classified as breeding season (June to September), post breeding (October to January) and pre-breeding (February to May) for water and fish growth parameter analysis (Silva and Steward, 2006).

3.0 Sample collection and preservation

3.1 Water sample

Water samples were collected in pre-rinsed plastic bottle of 3-liter capacity for the analysis of physicochemical parameter. The water sample tends to modify itself to the new environment hence its necessary to

preserve the water samples and prevent its integrity till analyzed. So, the water samples were preserved as per APHA (2005) and transported to the Department of Aquatic Biology, Veer Narmad South Gujarat University, Surat for further analysis. For Total Kjeldahl nitrogen analysis water samples were taken to Pollucon Laboratory Pvt. Ltd, Surat.

3.2 Plankton sample

A microscopic community of plants (phytoplankton) and animals (zooplankton), found usually free floating, swimming with little or no resistance to water currents, suspended in water, nonmotile or insufficiently motile to overcome transport by currents are called plankton. Phytoplankton are photosynthetic and usually occurs as unicellular, colonial or filamentous forms while zooplankton comprise of microscopic protozoans, rotifers, cladocerans and copepods.

The plankton net is a field-equipment to collect plankton. It has a polyethylene filter of defined mesh size and graduated measuring jar attached to the other end. About 25 litre water was filtered through plankton net with 60µ mesh size to collect phytoplankton in glass tube tied to the lower narrow end of net. The water samples collected into the 100 ml polyethylene vials and were preserved by adding 1 ml chloroform to act as the narcotizing agent and 2 ml of 4% formalin for preservation.

3.3 Chlorophyll-a sample

Chlorophyll is the green pigment in plant cells that carries out the bulk of energy fixation in the process of photosynthesis. Besides its importance in photosynthesis chlorophyll-a is probably the most-often use to estimate the algal biomass in aquatic system (Barber, 2014). Many types of chlorophyll can be found in plant such as chlorophyll *a, b, c,* and *d*. To collect the chlorophyll 250 ml water sample was filtered through Whatman's filter paper No. 42 and 0.2 ml $MgCO_3$ was added in filtrate. Sample was placed in a tissue grinder and crushed the residue by slowly adding the 90% of acetone. Sample was then transferred to a screw cap centrifuge tube and the volume was brought up to 10 ml with acetone solution (90%). Sample was covered with black paper and kept it in a refrigerator at 4 °C for overnight.

3.4 Fish sample

Fish specimens of three commercially important Indian major carps *viz.* catla (*Catla catla* Ham. 1822), rohu (*Labeo rohita* Ham. 1822) and mrigal (*Cirrhinus mrigala* Ham. 1822) were collected because these are the fastest growing species among carps (Jhingran, 1952) and remarkable contribution in the fish production of Vallabhsagar reservoir. The collected fish species were randomly identified with the help of standard reference of Day (1875), Shaw and Shebbeare (1937) and Misra (1959).

The detailed description of Indian major carps is described as:

3.4.1 Catla (*Catla catla*, Ham. 1822)

Catla's body is short somewhat laterally compressed, body depth is more than head length covered with large cycloid scales. Its large head is devoid of scales, blunt round snout and large eyes are visible from underside of the head. Mouth is upturned and wide with prominent protruding lower jaw thick lower lip and no barbels. Lower jaw with a movable articulation at symphysis, without a prominent process, gill rakers long and fine, pharyngeal teeth in three row, 5.3.2/2.3.5 pattern, dorsal fin inserted slightly in advance of pelvic fins, with 14 to 16 branched rays, the simple rays non-osseous, anal fin short, pectoral fins long extending to pelvic fins, caudal fin forked, lateral line with 40 to 43 scales. Greyish on back and flanks, silvery-white below, fins dusky (Fig. 2 A).

3.4.2 Rohu (*Labeo rohita*, Ham. 1822)

Its body is bilaterally symmetrical, moderately elongate, dorsal profile more arched than the ventral profile. Body is covered with cycloid scales while head without scale, snout depressed, projecting beyond mouth, without lateral lobe. Eyes are dorsolateral in position, not visible from outside of head, mouth small and inferior, lips thick and fringed with a distinct inner fold to each lip and a pair of small maxillary barbells concealed in lateral groove of fish. No teeth on jaws but pharyngeal teeth are present in three rows. Three or four dorsal fin rays are Simple and unbranched while 12 to 14 are branched, dorsal fin inserted midway

between snout tip and base of caudal fin, pectoral and pelvic fins laterally inserted, pectoral fin devoid of an osseous spine, caudal fin deeply forked, lower lip usually joined to isthmus by a narrow or broad bridge, pre-dorsal scale 12-16, lateral line distinct running along median line of the caudal peduncle, lateral line scales 40 to 44, lateral transverse scale-rows six or six and a half between lateral line and pelvic fin base, snout not truncate, without any lateral lobe, color bluish on back, silvery on flanks and belly (Fig. 2 B).

3.4.3 Mrigal (*Cirrhinus mrigala*, Ham. 1822)

Its body is bilaterally symmetrical and streamlined, body depth about equal to length of head, body with cycloid scales, head without scales, snout blunt, often with pores, mouth broad, transverse, upper lip entire and not continuous with lower lip, lower lip most indistinct, single pair of short rostral barbells, pharyngeal teeth in three rows, 5.4.2/2.4.5 pattern, lower jaw with a small post-symphysial knob or tubercle, origin of dorsal fin nearer to end of snout than base of caudal, dorsal fin as high as body with 12 or 13 branched rays, last unbranched ray of dorsal fin non-osseous and non-serrated, pectoral fins shorter than head, caudal fin deeply forked, anal fin not extending to caudal fin, lateral line with 40-45 scales, lateral transverse scale rows 6-7/5½-6 between lateral line and pelvic fin base, usually dark grey above, silvery beneath, dorsal fin greyish, pectoral,

pelvic and anal fins orange-tipped especially during breeding season (Fig. 2 C).

These fish specimens were used for the collection of morphometric data like total length, standard length and weight for length weight relationship, scale for age and growth study and gut contents for gut content analysis as well as gastrosomatic determination.

3.5 Morphometric data

Morphometric measurement such as total length (TL), standard length (SL) and weight (WT) were collected from 1585 specimens of catla, 1490 specimens of rohu and 1408 specimens of mrigal for the analysis of length weight relationship. Total length (TL) of fishes were measured from tip of snout to the end of caudal fin, standard length from tip of the snout to caudal peduncle corrected up to 0.5 cm with the help of measuring tape and body weight (WT) was taken with the help of digital weighing machine top loading single pan balance (Sartorius 'PT' 600) corrected up to 1.0 gm.

3.6 Scale sample

For the age and growth study fish scale were collected from above of lateral line to below of dorsal fin. About 5-10 scales from each specimen of catla (1585), rohu (1490) and mrigal (1408) were collected and preserved in paper envelop with key note information of fish like, total length, standard length, total weight, name of fish species, landing centre, date of sampling etc.

3.7 Gut sample

For gut contain analysis and gastrosomatic index determination gut samples were randomly collected from 105 specimens of catla, 110 specimens of rohu and 106 specimens of mrigal during the study period. Each fish specimen was weighed to the nearest 1.0 gm with the help of top loading single pan balance and gut were removed after dissecting the fish. For the gastrosomatic index determination the weight of gut was taken, and its contents were preserve in 4% formalin solution for gut contents analysis.

3.8 Data collection

The fish production data of Gujarat and Vallabhsagar reservoir of last 8 years were collected from Deputy Director Fisheries (Anon, 2015) and rain fall data of the study area during the study period were also collected from Irrigation Department (Anon, 2015).

4.0 Analysis of sample

4.1 Water parameters analysis

The water quality parameters *viz*. Temperature, Dissolve Oxygen (DO), Total Solid (TS), Total dissolve Solid (TDS), Total suspended solid (TSS), Hardness, Ammonical-Nitrogen, Nitrate-Nitrogen, Nitrite-Nitrogen, Silicate, Phosphate, Chlorophyll (a) were analyzed following the standard methods of Trivedy and Goel (1986) and APHA (2005).

4.1.1 Temperature (^{0}C)

Temperature plays a very important role in reservoir dynamism affecting the various parameters such as alkalinity, salinity, dissolved oxygen, electrical conductivity etc. hence water temperature affects fish life.

Method: Thermometric

Apparatus: Thermometer

Procedure: Temperature measurement in ^{0}C was made by immersing the thermometer in surface water of reservoir for sufficient time to stabilize the reading and the reading were noted down.

4.1.2 Turbidity (NTU)

Turbidity is an expression of optical property and scattering of the light is proportional to the turbidity. Turbidity in water is caused by suspended and colloidal matter such as clay, silt, fine organic and inorganic matter, plankton and other microscopic organisms. Turbidity affects light penetration, scattering, absorption and aesthetic appearance of water.

Method: Nephlometric

Apparatus: Digital turbidity meter

Reagents: 40 or 400 NTU standard solution

Procedure: Digital turbidity meter was calibrated by distilled water for zero NTU and standard turbidity suspension of 40 NTU or 400 NTU. The thoroughly shaken water sample was taken in cuvette and placed in digital turbidity meter for turbidity reading. Reading were noted down from display monitor.

4.1.3 Dissolve oxygen (mg/L)

It is a very important parameter as it serves as an indicator of the physical, chemical and biological activities of the water body. The main sources of dissolved oxygen are diffusion of oxygen from the air and photosynthetic activity.

Method: Titrimetric (Winkler's Iodometric method)

Apparatus: BOD bottles (300 ml), measuring cylinder, conical flasks, etc.

Reagents: Manganous sulphate, Alkaline iodide-azide reagent, sulphuric acid (concentrated), starch indicator and sodium thiosulphate (0.25N).

Procedure: The water samples (300 ml) were collected in BOD bottles, to which 2 ml of manganous sulphate and 2 ml of potassium iodide were added and sealed for the oxygen fixation. Oxygen fixation was visible in the form of precipitation and at that stage 2 ml of concentrated sulphuric acid was added to dissolve the precipitate. Fixed water sample (50 ml) was transferred into the conical flask and titrated against 0.025N sodium thiosulphate using starch as an indicator. The endpoint was the change of color from blue to colorless.

Calculations:

$$\text{Dissolve Oxygen (mg/L)} = \frac{V_1 \times N \times 8 \times 1000}{V_2}$$

Where:

V_1 = Volume of titrant
N = Normality of titrant
V_2 = Volume of sample titrated

4.1.4 Total solids (mg/L)

Total solids are the term applied to the material residue left in the vessel after evaporation of the water sample and its subsequent drying in an oven at a temperature of 103-105 ^{o}C.

Method: Gravimetric

Apparatus: Evaporating dishes or porcelain dish 100 ml, steam bath, drying oven, desiccator, digital balance and measuring jars.

Procedure: A known volume of the well-mixed water sample (50 ml) was measured into a pre-weighed dish and evaporated to dry at 103 ^{0}C on a steam bath. The evaporated water sample was dried in an oven for about an hour at 103-105 ^{0}C, cooled in a desiccator and recorded for constant weight.

Calculation:

$$\text{Total solids (mg/L)} = \frac{(A-B) * 1000 * 1000}{V}$$

Where:

A = Final weight of the dish in gm
B =Initial weight of the dish in gm
V = Volume of water sample taken in ml

4.1.5 Total dissolve solids (mg/L)

Total dissolved solids are the measure of all kinds of dissolved solids present in water. Water with high dissolved solids generally of inferior palatability may induce an unfavorable physiological reaction for aquatic animals.

Method: Gravimetric

Apparatus: membrane filter funnel, drying oven and Grooch crucible etc.

Procedure: Total dissolved solids were determined as the residue left after evaporation of the filtered water sample. A known volume of the filtered water sample (50 ml) were measured into a pre-weighed dish and evaporated to dryness at 103 ^{0}C on a steam bath. The evaporated water sample was dried in an oven for about an hour at 103-105 ^{0}C, cooled in a desiccator and recorded for constant weight.

Calculation:

$$\text{Total dissolved solids (mg/L)} = \frac{(A-B) * 1000 * 1000}{V}$$

Where:

A = Final weight of the dish in gm
B = Initial weight of the dish in gm
V = volume of the water sample taken in ml

4.1.6 Total suspended solid (mg/L)

Total suspended solids are the measure of all kinds of suspended solids present in water. Total suspended solids were determined as the difference between total solids and total dissolved solids.

Total suspended solids (mg/L) = Total solids – Total dissolved solids

4.1.7 Total hardness (mg/L)

Hardness is predominantly caused by divalent cations such as calcium, magnesium, alkaline earth metal such as iron, manganese, strontium, etc. The total hardness is defined as the sum of calcium and magnesium

concentrations both are express in mg/L. Carbonates and bicarbonates of calcium and magnesium cause temporary hardness whereas sulphates and chlorides cause permanent hardness.

Method: Titrimetric

Apparatus: Burette, pipette, conical flask, beakers etc.

Reagents: Ammonium buffer solution (10.0±0.1), Eriochrome black-T indicator and EDTA titrant (0.01 N).

Procedure: Exactly 50 ml of the well-mixed water sample was pipetted into a conical flask, to which 1 ml of ammonium buffer and 2-3 drops of Eriochrome black -T indicator was added. The mixture was titrated against 0.01 N EDTA until the wine-red color of the solution turns pale blue at the endpoint.

Calculation:

$$\text{Total hardness (mg/L)} = \frac{\text{Vol. of EDTA} * 1000}{\text{Vol. of Sample}}$$

Nitrate – Nitrogen (mg/L)

Nitrates are the most oxidized forms of nitrogen and associated with reservoir productivity. It is not poisonous but high concentration in reservoir water causes algal bloom.

Method: Spectrophotometric

Apparatus: Pipette, conical flask, beakers etc.

Instrument: UV-Spectrophotometer (Shimadzu UV- 1800)

Reagents: N-(1-naphthyl)-ethylenediamine dihydrochloride, sulphanilamide and amalgamated cadmium filing.

Procedure:

Standard nitrate solution:

Dissolved 0.722 g of KNO_3 in distilled water to prepare solution contains 100 mg/L nitrate-nitrogen. Dilute it to 100 times to prepare a stock solution of 1 mg/L concentration. From this stock solution 10 ml solution was diluted to 100 ml to prepare solution of 0.1 mg/L nitrate–nitrogen concentration.

Standard graph:

Based on the range of nitrate-nitrogen to be determine, the series of standard nitrate-nitrogen solution with the concentration in the range of 0.0 to 1.0 mg/L prepared by pipetting appropriate volume of stock solution and dilute with nitrate free water.

Sample analysis:

A filtered 10 ml water sample was reduced with amalgamated cadmium granules by stirring the water sample for 5 minutes. The nitrate was determined by adding 0.3 ml sulfanilamide along with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye. Its absorbance was measured, spectrophotometrically at wavelength 540 nm. Distilled water with same amount of reagent was used as blank.

Standard curve was prepared between concentration and absorbance of know concentrations for nitrate nitrogen solutions. The absorbance of water samples was measured to know the concentration of nitrate nitrogen with the help of standard graph.

Nitrite-Nitrogen (mg/L)

Nitrite can be formed by the oxidation of ammonia or the reduction of nitrate. Its concentration is less than the nitrate and ammonia but its high concentration is very poisonous to aquatic organism.

Method: Spectrophotometric

Apparatus: Pipette, conical flask, beakers etc.

Instrument: UV-Spectrophotometer (Shimadzu UV- 1800)

Reagents: N-(1-naphthyl)-ethylenediamine dihydrochloride and sulphanilamide.

Procedure:

Standard nitrite solution:

Dissolved 1.232 g $NaNO_3$ in distilled water and dilute to 1 litre, this solution containing 250 mg/L Nitrite nitrogen. Took 4 ml of this solution (250 mg/L NO_2 -N) to make it 1000 ml stock solution which containing 1 mg/L concentration. Now from that stock solution 10 ml solution was transferred for further dilution to make 100 ml solution with 0.1 mg/L concentration.

Standard graph:

Based on the range of nitrite-nitrogen to be determine, the series of standard nitrite-nitrogen solution with the concentration in the range of 0.0 to 1.0 mg/L prepared by pipetting appropriate volume of stock solution and dilute with nitrite free water.

Sample analysis:

The nitrite was determined by adding 0.3 ml sulfanilamide along with 0.3 ml, N-(1-naphthyl)-ethylenediamine dihydrochloride in 10 ml filtered water sample to form a highly colored azo dye. Its absorbance was measured spectrophotometrically at wavelength 540 nm. Distilled water with same amount of reagent was used as blank.

Standard curve was prepared between concentration and absorbance of know concentrations for nitrite nitrogen solutions. The absorbance of water samples was measured to know the concentration of nitrite nitrogen with the help of standard graph.

Ammonical- Nitrogen (mg/L)

Ammonia is a colorless pungent gaseous compound of hydrogen and nitrogen that is highly soluble in water. It's a biologically active compound found in most water as a normal biological degradation of nitrogenous organic matter.

Method: Spectrophotometric

Apparatus: Pipet, conical flask, etc.

Reagents: K-Na (Rochelle salt) and Nessler's reagent

Instrument: UV-Spectrophotometer (Shimadzu UV-1800)

Procedure:

Standard ammonia solution:

Dissolved 3.819 g of anhydrous NH_4Cl in distilled water to prepare 1 liter of stock solution. That stock solution contains 1000 mg/L of ammonical nitrogen. Dilute 10 ml of that stock solution to 100 times to prepare the solution containing 0.1 mg ammonical nitrogen.

Standard graph:

Based on the range of ammonical-nitrogen to be determine, the series of standard ammonical-nitrogen solution with the concentration in the range of 0.0 to 5.0 mg/L prepared by pipetting appropriate volume of stock solution and dilute with ammonia free water.

Sample analysis:

For ammonia estimation, 1 ml of K-Na solution and 1 ml of Nessler's reagent was added in 50 ml filtered water sample. After 5 minutes, yellow color was developed, and its absorbance was measured with the help of spectrophotometer at wavelength 425 nm. Distilled water with same amount of reagent was used as blank.

Standard curve was prepared between concentration and absorbance of know concentrations for ammoniacal nitrogen solutions. The absorbance of water samples was measured to know the concentration of ammoniacal nitrogen with the help of standard graph.

4.1.11 Total Kjeldahl Nitrogen (mg/L)

Total Kjeldahl Nitrogen (TKN) is the organic and ammoniacal nitrogen content in the water sample which is determined as NH_4^+ after mineralization of organic nitrogen with sulfuric acid to form ammonium sulfate and in the presence of selenium as a catalyst. These parameters were analyzed from Pollucon Laboratory Pvt. Ltd., Surat.

4.1.12 Organic Nitrogen (mg/L)

It is the by-product of living organisms which includes proteins, peptides, nucleic acids, urea and numerous synthetic organic materials. Typical organic nitrogen concentrations vary from a few hundred micrograms per litre in some lakes to more than 20 mg/L in raw sewage (APHA, 2005). Organic nitrogen can be determined by subtracting the concentration of ammonia from the Kjeldahl nitrogen values.

Calculation:

Organic nitrogen (mg/L) = Kjeldahl nitrogen – Ammonical nitrogen

4.1.13 Phosphate (mg/L)

Phosphates occur in natural or wastewaters as orthophosphates, condensed phosphates and naturally found phosphates. Their presence in water is due to detergents, fertilizers and biological processes. They are essential for the growth of organisms and works as an essential nutrient while its deficiency limits the primary productivity of the water body. Phosphorus plays a dynamic role in aquatic ecosystems and can be estimated by the stannous chloride method.

Method: Spectrophotometric

Reagents: Ammonium molybdate and stannous chloride

Instruments: UV-Spectrophotometer (Shimadzu UV- 1800)

Procedure:

Standard solution:

Dissolved 4.388 g of dried anhydrous potassium hydrogen phosphate in distilled water to make solution containing 100mg/L phosphate concentration. Dilute this solution to 100 times to prepare stock solution containing 10 mg/L concentration. Now 1 ml of this stock solution diluted and brought to 100 ml to prepare 0.1 ml/l concentration.

Standard graph:

Based on the range of phosphate to be determine, the series of standard phosphate solution with the concentration in the range of 0.0 to 1.0 mg/L prepared by pipetting appropriate volume of stock solution and dilute with phosphate free water.

Sample analysis:

Filtered water sample (50 ml) was taken in a conical flask and 2 ml of ammonium molybdate was added followed by 5 drops of stannous chloride and mixed thoroughly which develop blue color. The absorbance of colored water sample was measured after five minutes but before 12 minutes of the addition of the last reagent at wavelength 690 nm using

spectrophotometer. A reagent blank was always run with same treatment with distilled water as sample.

Standard curve was prepared between concentration and absorbance of know concentrations for phosphate solutions. The absorbance of water samples was measured, and concentration of phosphate was calculated with the help of standard graph.

Silicate (mg/L)

Silica plays very important role in plants and animals life as occurring naturally in many forms like sand, quartz, opal, rock, sea floor and sediments. Specifically, biogenic silica is extracted from water by plants, microorganisms or invertebrates for building structural materials and growth. Primary producers such as Diatoms extract silica from water, which is required for growth, allowing blooms to occur and ultimately increasing the energy flow from lower to higher trophic levels in an ecosystem.

Method: Spectrophotometric

Apparatus: Pipet, conical flask

Reagents: Ammonium molybdate, oxalic acid solution and HCl + water (1+1)

Instruments: UV-Spectrophotometer (Shimadzu UV- 1800)

Procedure:

Standard silica solution:

Dissolved 671.4 mg of Sodium fluosilicate in 1-liter water by heating to prepare stock solution containing 100 mg/L concentration of silica. Dilute

this solution 100 times to prepare standard solution containing 1 mg/L of silicate.

Standard graph:

Based on the range of silicate to be determine, the series of standard silicate solution with the concentration in the range of 0.0 to 20.0 mg/L prepared by pipetting appropriate volume of stock solution and dilute with silicate free water.

Sample analysis:

Added 1 ml of HCl + water (1+1) in 50 ml of filtered water sample in a conical flask and immediately added 2.0 ml of ammonium molybdate solution. Oxalic acid 1.5 ml was added in the solution which imparted yellow color and absorbance was taken at wavelength 410 nm on the Spectrophotometer.

Standard curve was prepared between concentration and absorbance of know concentrations for silicate solutions. The absorbance of water samples was measured the concentration of silica was calculated with the help of standard graph.

Plankton analysis (No. /l)

About 25 litre waters filtered through plankton net of 60 µ mesh size and filtrate was collected in glass tube tied to the lower narrow end of net. Plankton containing filtrate were taken in glass tube and preserved by adding 4% formalin solution. Settled plankton were transferred to 10 m1

measuring cylinder and again kept for settlement. Lackey's drop method was used for the quantitative plankton analysis using trinocular microscope (Olympus CH 20i). Exactly 0.1 ml of preserved sample was used for plankton counting with the help of pipette and graduated slide. Counting of plankton were done by moving the microscope over mounted slide.

Calculation:

$$\text{No. of planktons/ml} = \frac{A*B}{C*D*E}$$

Where:

A = No. of organisms counted in all fields
B = area of cover slip, mm^2
C = Area of one microscopic field mm^2
D = no. of field counted
E = volume of sample in cover slip

4.3 Chlorophyll-a analysis (mg/m^3)

Chlorophyll-a is a specific form of chlorophyll used in oxygenic photosynthesis. It absorbs most energy from wavelengths of violet-blue and orange-red light. This photosynthetic pigment is essential for photosynthesis in eukaryotes, cyanobacteria and prochlorophytes because of its role as primary electron donor in the electron transport chain.

Method: Spectrophotometric

Apparatus: Pipet, centrifuge tube, carbon paper etc.

Instruments: UV-Spectrophotometer ((Shimadzu UV- 1800)) and Centrifuge machine

Procedure: 250 ml of water sample was concentrated by filtering through Whatman's filter paper No. 42 and 0.2 ml magnesium carbonate was added. Filtrate sample was placed in a tissue grinder and crushed the residue by slowly adding of 90% acetone. Sample was transferred to a screw cap centrifuge tube and the volume was brought up to 10 ml with acetone 90%. These sample containing centrifuge tubes were covered with black paper and kept in refrigerator at 4° C for overnight. Extract was clarified by centrifuging it at 2000 rpm for 20 minutes and absorbance of clear extract was measured at wavelength 630, 645 and 663 nm by spectrophotometer.

Calculation:

$$\text{Chlorophyll (a) } (mg/m^3) = \frac{C * Ve}{Vs * L}$$

Where:

C = 11.64 E 663 - 2.16 E 645 + 0.10 E 630
Ve = Volume of acetone (10 ml)
L = Light path of cell (cm)
Vs = Volume of water centrifuged (10 ml)

Scale analysis

Information regarding fish age is necessary for stock assessments, develop management and conservation plans in fishery science. The most commonly used techniques involve counting natural growth rings on the scales, otoliths, vertebrate, fin spines, eye lenses, teeth or bones of the jaw, pectoral girdle, and opercular series (Helfman *et al.* 1997). Scales are the

most widely used aging structure in tropical area as effect of temperature variation is clearly visible on annulus formation (Al-Absy and Carlander, 1988). Counting the number of annuli (rings) on a scale provides the fish age and the spacing between rings is proportional to the growth of the fish (Silva and Stewart, 2006).

Method: Microscopic observations

Reagents: 1% KOH

Instrument: Scale reader 4P

Procedure: For the study, scales were dipped in 1 % KOH solution for 5-10 minute and rubbed with fingertip to remove extraneous matter and mucous. Clean and transparent scales were examined under the scale reader for scale radius (S) and radius of each annual rings (S_1, S_2, S_3 S_4…….S_n).

The existence of a relationship between fish length and scale radius is a key assumption underlining back-calculation. Linear regression analysis was performed to check the relationship between fish length and scale radius at the time of capture.

The length of the fish at the time of formation of annuli could be estimated through formula given by Bagenal and Tesch (1978).

$$L_n = a + \frac{S_n}{S} \times (L-a)$$

Where:

L_n = Lenght of fish when the annulus (n) was formed.
L = Lenght of fish when scale sample was obtained.
S_n = Radius of annulus (n)
S = Total scale radius
a = Correction factor (intersect point between TL ans Scale Radius)

Length weight relationship analysis

Length weight relationship is important in fisheries science as it can be used to predict weight from length measurements made in the yield assessment and to estimate fish biomass from natural water body.

Procedure: For analysis of length weight relationship the length and weight data of 1585 specimens of catla, 1490 specimens of rohu and 1408 specimens of mrigal were measured. The measured data were divided into different length group at 10 cm intervals and seasonal group like breeding, post breeding and pre-breeding. The pooled and grouped data were used to establish length weight relationship to evaluate the growth of fish.

The relationship between weight and length is not linear which can be expressed by non-linear equation given by LeCren (1951)

The length weight data were transformed in natural logarithm to establish linear relationship between length and weight parameters. The expression of the equation is represented by the following formula:

$\text{Log } W = \text{Log } a + b \text{ Log } L$ (LeCren, 1951)

Plot a graph between log weight and log length which shows the linear relationship in the variables and equation depict slope ‘b’, intercept ‘a’ and dependent variable. The constants ‘a’ represents the point at which the regression line intercepts the y- axis and ‘b’ the slope of the regression line.

All the above statistical calculations were done using soft wear SPSS (version 16) and then the graphs were plotted using the log of observed values.

Growth parameters based on scale studies

The growth parameters such as growth characteristic (Cth), specific linear growth (Cl), growth constant (Clt), specific rate of weight increase (Cw), index of species average size (Øh), and index of population growth intensity (ØCw) were estimated from the following formulaewhich can be used in tropical aquatic condition (Johal & Tandon, 1992 and Ujjania *et al.* 2007) :

(i) Specific rate of linear growth

$$C_l = \frac{L_n - L_{n-1}}{L_{n-1}} \times 100$$ (Chugnova, 1963)

(ii) Growth characteristic

$$C_{th} = \frac{\text{Log } L_n - \text{Log } L_{n-1}}{0.4343} \times L_{n-1}$$ (Chugnova, 1963)

(iii) Growth constant

$$C_{lt} = \frac{\text{Log } L_n - \text{Log } L_{n-1}}{0.4343} \times \frac{t_2 + t_1}{2}$$ (Chugnova, 1963)

(iv) Specific rate of weight increase

$$C_w = \frac{W_n - W_{n-1}}{W_{n-1}} \times 100$$ (Chugnova, 1963)

(v) Index of species average size

$$Øh = \frac{\sum h = 1}{nj + a}, \quad h = nj + a \qquad \text{(Balon, 1971)}$$

(vi) Index of population weight growth intensity

$$ØC_w = \frac{\sum C_w = 1}{nj + a}, \quad Cw = nj + a \qquad \text{(Balon, 1971)}$$

Where:

L_n, L_{n-1} = total length of fish at ultimate and penultimate age
W_n, W_{n-1} = weight of fish at ultimate and penultimate age
j = juveniles
a = adult
h = absolute increase in length
t_1, t_2 = time intervals between ultimate and penultimate age

Condition factor and relative condition factor

Condition factor (K) and Relative condition factor (Kn) evaluate the general well being of fish in aquatic environment and can be determine by using length and weight data of fish samples. It was calculated annully for different lenght groups and seasonally (breeding, post breeding and pre-breeding) using the formula given by LeCren (1951).

$$K = \frac{(W \times 100)}{L^3}$$

Where:

W = Weight of fish (gm)
L = Total length of fish (cm)

The relative condition factor (Kn) is associated for changes in form or condition with increase in length and it was calculated using equation given by LeCren (1951) from the length weight data.

$$Kn = \frac{W}{w}$$

Where:

W = Weight of fish (gm)

w = Calculated weight of fish (gm)

Gut analysis

The fluctuation in quality and quantity of intake food materials will influence the growth rate of the fish. The available food materials in a water body and preferred food materials by fish could be determine by analysis of gut contents (Kumar *et al*. 2007).

Gut contain analysis

For gut contain analysis preserved gut sample were transferred in petri dish and allowed for settlement. With the help of 1 ml pipet one drop of sample was transferred on the graduated slide and observed under the Trinocular microscope (Olympus CH 20i). The gut contents were analysed to follow the frequency occurrence methods of Hynes (1950) and Pillay (1952) and observations were grouped into zooplankton, phytoplankton, plant material, insects and decay matter.

Gastrosomatic index (GaSI)

Gastrosomatic index determine the feeding intensity of fish. For this the weight of gut and weight of fish body was taken before removing the gut. The value of Gastrosomatic Index was calculated using the formulae given by Desai (1970).

$$\text{Gastrosomatic Index (GaSI) \%} = \frac{\text{Weight of gut (gm)}}{\text{Weight of fish (gm)}} \times 100$$

Table 1: Morphometric feature of Vallabhsagar reservoir

S.N.	Features	Measurements
1	Name of water body	Vallabhsagar reservoir or Ukai dam
2	Year of construction	1971
3	Geographic location	
	Latitude (N)	21° 15'
	Longitude (E)	73° 35'
4	Catchment area (ha)	6222500
5	Surface area (km^2)	612
6	Length of Dam (m)	4,927
7	Height of the Dam (m)	80.772
8	Gross capacity (MCM)	7414
9	Live storage capacity (MCM)	6730