Material and methods
Fig. 3. View of Gobindsagar Reservoir.
4. MATERIAL AND METHODS

The investigations on the various aspects of biology and fishery of *H. molitrix* from Gobindsagar reservoir were carried out during August 1998 - May 2000, except close seasons (June and July).

Silver carp (size range 235 - 1000mm) were collected monthly from commercial catches of this reservoir. The collections were conducted once or twice a month. Total length (TL), standard length (SL) and fork length (FL) of specimens were measured to the nearest mm on special measuring board. The fishes were weighed to the nearest 5g with an ATCO digital balance. Sex was recorded for each specimen.

Morphometric and meristic characters have been described after Jayaram (1999) (Fig. 5). These characters were compared with those recorded by Jhingran (1991) and Tandon *et al.* (1993).

**Food and feeding habits**

For gut analysis, the intestinal tract of individual fish was removed. Contents from anterior end of the intestine to its first loop (in some cases, slightly beyond the curvature) were collected as fore - gut contents. Contents
Fig. 4. Hypophthalmichthys molitrix (Val., 1844) from Gobindsagar Reservoir.
Fig. 5. *Hypophthalmichthys molitrix* (Val., 1844) indicating different morphometric measurements.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>DAF</td>
<td>Depth of anal fin</td>
</tr>
<tr>
<td>DBPV</td>
<td>Distance between pectoral fin and pelvic fin</td>
</tr>
<tr>
<td>DBVA</td>
<td>Distance between pelvic fin and anal fin</td>
</tr>
<tr>
<td>DDF</td>
<td>Depth of dorsal fin</td>
</tr>
<tr>
<td>ED</td>
<td>Eye diameter</td>
</tr>
<tr>
<td>FL</td>
<td>Fork length</td>
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<tr>
<td>HD</td>
<td>Head depth</td>
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<tr>
<td>HL</td>
<td>Head length</td>
</tr>
<tr>
<td>LAF</td>
<td>Length of anal fin</td>
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<tr>
<td>LCF</td>
<td>Length of caudal fin</td>
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<tr>
<td>LCP</td>
<td>Length of caudal peduncle</td>
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<tr>
<td>LDF</td>
<td>Length of dorsal fin</td>
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<tr>
<td>LPF</td>
<td>Length of pectoral fin</td>
</tr>
<tr>
<td>LVF</td>
<td>Length of pelvic fin</td>
</tr>
<tr>
<td>MABD</td>
<td>Maximum body depth</td>
</tr>
<tr>
<td>MIBD</td>
<td>Minimum body depth</td>
</tr>
<tr>
<td>PosDD</td>
<td>Postdorsal distance</td>
</tr>
<tr>
<td>PosOD</td>
<td>Postorbital distance</td>
</tr>
<tr>
<td>PreAD</td>
<td>Preanal distance</td>
</tr>
<tr>
<td>PreDD</td>
<td>Predorsal distance</td>
</tr>
<tr>
<td>PreOD</td>
<td>Preorbital distance</td>
</tr>
<tr>
<td>SL</td>
<td>Standard length</td>
</tr>
<tr>
<td>TL</td>
<td>Total length</td>
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in the intestine within 5cm from the anus were collected as hindgut contents according to Shei & Liu (1999).

Fore-gut and hindgut contents were immediately preserved in 5% formaldehyde solution in separate plastic vials. The gut contents were analysed qualitatively and quantitatively by drop method as described by Murphy & Willis (1996). Identification of food items was facilitated by the analysis of water samples taken from Gobindsagar reservoir. Prey was recognized at the genus level, when possible, but for the frequency-abundance analysis, they were grouped in to families or higher order taxa (Pirro et al., 1999).

Percent number, percent biovolume, and frequency of occurrence were used to estimate the dietary importance of each food category. Percent number is the number of individuals of a prey type divided by the total number of individuals as expressed as a percentage, after pooling the gut contents of all fishes. Percent biovolume is the equivalent measure for biovolume data (Garcia-Berthou, 1999). Frequency of occurrence is the percentage of guts where a food item was present. In mathematical terms, the percentage abundance (% Ai) and the percentage occurrence (% Fi) of prey i can be described by the equation:

\[
\% \text{ Ai} = \left( \frac{\sum Si}{\sum St} \right) \times 100
\]

\[
\% \text{ Fi} = \left( \frac{Ni}{N} \right) \times 100
\]

Where Si is the gut content (volume, weight or number) composed by prey i, St the total gut content of all intestines in the entire sample, Ni is the
number of predators with prey i in their intestine, and N is the total number of predators with intestine contents (Amundsen et al., 1996).

Costello's (1990) graphical method was used to describe prey importance and feeding strategy of this fish.

Overlap in the diets of males and females silver carp was assessed using the Schoener's (1970) index (Krebs, 1989), which is widely used (Wallace, 1981; Pedersen, 1999; Pirro et al., 1999; Chiaramnote & Pettovello, 2000).

**Schoener's index :**

\[ D = 1 - 0.5 \sum_{i=1}^{n} |P_{x,i} - P_{y,i}|, \quad \text{Schoener (1970).} \]

where: D is overlap index, P_{x,i} is the proportion of food category i in the diet of male, P_{y,i} is the proportion of food category i in the diet of female and n is the number of category. This index gives values from 0 (no overlap) to 1 (complete overlap). According to Zaret & Rand (1971), Wallace (1981) and Vamosi et al. (2000), a, D value > 0.60 is thought to be biologically significant. However Pederson (1999) defined D above 0.74 as arbitrarily to mean high overlap, 0.25 - 0.74 as moderate and D less than 0.25 as low overlap.

**Length - weight relationship**

The length - weight relationship was calculated by the method of least squares using parabolic equation suggested by Le Cren (1951).

\[ W = a L^b. \]

The relationship was fitted to the straight line through the logarithmic
transformation.

$$\log W = \log a + b \log L$$

Where $a$ is the y-axis intercept and $b$ is the slope of the equation.

Fulton condition factor ($K$) was computed as:

$$K = \frac{W}{L^3} \times 100,000$$

Where $W$ is weight (g) and $L$ is length (mm) (Treasurer, 1994).

**Age and growth studies**

Scales were removed from a key sample area from the left side of the body between lateral line and dorsal fin (preferably from 16th - 18th rows). The scales were first washed in tap water, kept in 1% KOH solution for a few minutes to remove extraneous material and again washed. 5 - 6 cleaned and dried scales were arranged between two glass slides fastened with adhesive tape and coded. These scales were examined under Carl Zeiss DL 5.3 VEB Documator at a magnification of 21.2 X. The annual increments of scales were measured from the focus to the lateral margin and recorded as the lateral radii (Fig. 6). Regenerated and abnormal scales were excluded.

![Fig. 6. Mode of measurement of scale. O, focus; A₁ - A₃, Annuli; A, margin.](image)

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For the present study cleithra, postcleithra, opercula and urohyal bones were removed from fresh specimens and muscles were separated by dipping them in water at 60° - 70°C for a few minutes. Care was taken to avoid boiling because on boiling, these bones become chalky or milky. The bones were then cleaned with a soft tooth brush and washed. The cleaned and dried bones were stored in ordinary envelopes with relevant data e.g. total length, standard length, weight, sex and date of collection. To prevent any mistake during the examination of bones, the fish number was written on each bone.

In case of cleithra, the distance from the origin to the annuli and from origin to the anterior edges was measured as the annual increment (Fig. 7) (Casselman, 1979; Horppila & Nyberg, 1999).

\[ \text{Fig. 7. Schematic view of cleithrum showing various cleithral measurements. The anterior cleithral radius for each annulus is the linear measurement from the origin (O) to each annulus (A_1, A_2, A_3). The total cleithral radius (OA) is the linear measurement from the origin (O) to the margin (A).} \]

In case of urohyal bones, annual increments were measured from the origin to the annuli and to the posterior edge (in lateral side of urohyal bones) along the anterior - posterior axis (Fig. 8).
The cross sections of first pectoral fin, postcleithrum and urohyal bone were obtained by the following method:

These bones were removed from fresh specimens, cleaned and air dried. Transverse sequential sections were then cut from the anterior tip of urohyal bone, the middle of postcleithrum and the base of pectoral fin using a fine jeweller’s saw. Each section was grinded and polished using carborandum stone and fine ground glass. The grinded and polished sections were either mounted on the glass slides in DPX or directly observed under transmission light using Carl Zeiss DL 5.3 VEB Documator or Getner Stereo-binocular Microscope. For photography, the sections mounted on the microslides have been used as negatives.

Sagittae and Lapilli otoliths of silver carp were removed by cutting the skull of the fish between eyes. Otoliths were cleaned, dried, and then stored in labelled plastic vials. The otolith weights were recorded to the nearest 0.001 g.
on an electronic digital balance. The length and width of the otoliths were measured under binocular microscope.

**Back-calculation of lengths**

In order to ascertain the differences or similarity between different back-calculation methods, the back-calculated lengths were estimated with the following equations:

1. \( L_i = \left( \frac{S_i}{S_c} \right) L_c \) or direct proportional method where \( L_i \) = total length of the fish at age \( i \), \( L_c \) = total length of the fish at the time of capture, \( S_i \) = scale radius (or cleithrum length or urohyal length) at age \( i \) and \( S_c \) = Scale radius (or cleithrum length or urohyal length) at the time of capture.

2. \( L_i = -\left( \frac{a}{b} \right) + \left( \frac{L_c + a}{b} \right) \left( \frac{S_i}{S_c} \right) \) or SPH, which is based on the regression of scale radius (or other hard parts) on fish length, where \( a \) = intercept and \( b \) = slope of this regression line.

3. \( L_i = \left[ \frac{(c + d S_i)}{(c + d S_c)} \right] L_c \) or body proportional hypothesis (BPH) equation based on the regression of body length on the hard part, where \( c \) = \( L \)- intercept of the regression line and \( d \) = slope of the line.

4. \( L_i = \left( \frac{S_i}{S_c} \right) (L_c - c) + c \) or Fraser - Lee equation - where \( c \) is as in (3) (Francis, 1990; Horppila & Nyberg, 1999). The lengths obtained with different methods were compared using paired " \( t \) " test. The percent difference of back-calculated lengths from observed lengths was calculated using the following equation:
Percent difference = \[ \frac{U - L_0}{L_0} \times 100 \], where \( U \) = back-calculated length and \( L_0 \) = observed length (Klumb et al., 1999a,b). The data were analysed with SPSS Windows, version 10.5 (SPSS Social Science, 1999).

**Scanning Electron Microscopy of scales**

To study, microstructures of scales, scales were gently removed with fine forceps from the left side of the body, between dorsal fin and lateral line. The scales were cleaned mechanically and then were inserted in a solution of sodium hypochlorite (one part of commercial solution in 12 parts of distilled water) for 30 to 60 seconds (Lippitsch, 1990, 1998) and rinsed with distilled water. For further cleaning, they were subjected to sonication for 5 - 10 minutes and again rinsed with distilled water. Cleaned scales were then dehydrated in 30, 50 and 70\% of ethanol (100\% ethanol caused the scale margins to curl), dried on filter paper. Cleaned and dried scales were then mounted on the metallic stubs by double adhesive tape (with dorsal surface upwards and ventral surface sticking to the tape). As scales are non-conductive specimens, they were coated with a thin layer (100 A\(^\circ\)) of gold in gold coating unit so as to overcome the problems of “charging” and “beam damage”. An additional advantage of coating is an improvement in the strength of secondary electron signal from the specimen surface. Since high molecular weight materials yield stronger secondary electron signals, coating with gold increased the yield of secondary electrons. The scales were viewed under vacuum in the JEOL JSM - 6100 scanning electron microscope, at an accelerating voltage of 15/20 kv at low probe current. Various images of the scales were then recorded on the photographic film in the form of negatives. When not being viewed, the specimens were stored in a dessicator. To
characterize the morphology of osteoclasts and resorption sites in silver carp scales, the scales were removed from fresh specimens and frozen in liquid nitrogen. In the laboratory, they were jet washed in Hank’s solution using a pasteur pipette, to remove mucus from them (Persson et al., 1999). After which they were fixed in a mixture of 0.5% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4, for 3h. The scales were then treated with 2% tannic acid in 0.1 M cacodylate buffer containing 5% sucrose, pH 7.4, 4°C for 3h. After buffer washing, the samples were dehydrated in a graded series of ethanol (30%, 50%, 70%, 90%, 100%) and then were placed into amyl acetate and dried in a critical point dryer with liquid CO₂. The dried scales were coated with gold in a sputtering unit and then examined with JEOL JSM - 6100 scanning electron microscope.

**Elemental composition of scales and otoliths**

For the quantitative analysis of elements in the scales and otoliths of silver carp, Energy Dispersive X-ray Microanalysis (EDX) and Energy Probe Microanalysis (EPMA) were undertaken. For EDX study, the cleaned and dried scales and otoliths were mounted on the stubs. The specimen stubs were handled only with the forceps so as to prevent skin oils from contaminating the stubs and the microscope column. The samples were then coated with a thin layer of 100 Å of Gold Palladium Alloy in the sputter coating unit. The elemental composition of different region of the samples were determined by Leica EDX Analyser attached to Leica Stereoscan 360 Scanning Electron Microscope. The data was analysed by Hewlett Packard Pentium 120 MHz Computer attached to the system (The gold peak was deleted).
For EPMA study carbon coated specimens were analysed using JSM - 840 A/WDS/EDS system attached to JEOL JXA - 8600 M Electron Microscope.

**Growth parameters**

In order to understand the complex phenomenon of growth, parameters such as growth characteristic ($C_m$), growth constant ($C_z$), specific rate of linear growth ($C_i$), specific rate of weight increase ($C_w$), index of species average size ($\phi h$) and index of population weight growth intensity ($\phi C_w$) have been calculated using the following formulae.

1. $\phi h = \frac{\sum_{j=1}^{n_{i+a}} h}{\sum_{j=1}^{n_{i+a}} n} \quad$ (Balon, 1971)

2. $C_i = \frac{1_{n} - 1_{n-1}}{1_{n-1}} \cdot 100 \quad$ (Chugunova, 1963)

3. $C_{tb} = \frac{\log 1_{n} - \log 1_{n-1}}{0.4343} \cdot 1_{n-1} \quad$ (Chugunova, 1963)

4. $C_w = \frac{W_n - W_{n-1}}{W_{n-1}} \cdot 100 \quad$ (Chugunova, 1963)

5. $\phi C_w = \frac{\sum_{j=1}^{n_{i+a}} C_w}{\sum_{j=1}^{n_{i+a}} n} \quad$ (Balon, 1971)

6. $C_z = \frac{\log I_n - \log I_{n-1}}{0.4343} \cdot \frac{t_2 + t_1}{2} \quad$ (Chugunova, 1963)

where,

$I_n, I_{n-1} =$ back calculated length of fish at ultimate and penultimate ages

$W_n, W_{n-1} =$ back calculated weight of fish at ultimate and penultimate ages
using the equation $W = aL^0$

$j$ = juvenile

$a$ = adult

$n$ = number of juveniles and adults

$h$ = absolute increase in the length

$t_2, t_1$ = time intervals between ultimate and penultimate classes

$W$ = weight (calculated from the length - weight relationship)

$L$ = length

Growth parameters derived from von Bertalanffy growth function (VBGF) also have been used to compare growth rate of male and female silver carp.

$L_t = L_\infty \left[ 1 - e^{-k(t-t_0)} \right]$ von Bertalanffy 1938

where $L_\infty$ (L-infinity) is "the mean length of very old (strictly: infinitely old) fish". It is also called the "asymptotic length" (Sparre & Venema, 1992) or $L_\infty$ is, the mean length of the fish of a given stock would reach if they were to grow indefinitely (Craig, 1999). $K$ is a "curvature parameter" which determines how fast the fish approaches its $L_\infty$. $t_0$ determines the point in time when the fish has zero length. The growth performance index was computed according the formula given by Munro & Pauly (1983).

$\phi' = 2 + \log L_\infty + \log K$

**Harvestable size**

The theoretical harvestable size was determined from the crossing points of the following curves: (a) length increment in percentage of length of the
first growth season and (b) length (at ages) in percentage of length of the final
growth season. The percentages were plotted on the y-axis and age classes
on the x-axis (Tandon & Johal, 1996; Johal et al., 1999).

**Mortality - survival rates**

These rates were calculated by using Jackson method (1939) and
Robson & Chapman method (1961) which are as follows:

\[
S = \frac{N_2 + N_3 + N_4 + \ldots + N_t}{N_1 + N_2 + N_3 + \ldots + N_r-1} \quad \text{Jackson (1939)}
\]

\[
S = \frac{T}{n + T - 1} \quad \text{Robson & Chapman (1961)}
\]

\[
M = 1 - S
\]

where \(N_1, N_2, N_3, N_4 = \text{Number of specimens in each year class}\)

\(S = \text{Survival rate}\) \quad \(T = \text{a static}\)

\(M = \text{Mortality rate}. \quad n = \text{size of sample}\)

**Reproduction**

To study reproductive biology of silver carp, the fish were dissected
and sex was ascertained. Colour, and texture of the gonads were recorded
and a maturity stage assigned following a modified Nikolsky scheme
(Nikolsky, 1963).

The weight of the gonad relative to the body weight, the gonado-
somatic index (GSI), was calculated using the modified formula described by
Nikolsky (1963):

\[
GSI = \frac{\text{weight of ovary}}{\text{weight of fish} - \text{weight of ovary}} \times 100
\]
This formula is widely used (Ntiba & Jaccarini, 1990; Kaunda - Arara & Ntiba, 1997; Robards et al., 1999). To determine ova diameter, the ovaries were preserved in 10% formalin solution. After they had attained hardness, they were kept between the folds of filter paper to remove excess water. Sub samples were taken from anterior, posterior and middle regions of ovary for homogeneity. The diameter of 100 ova from the sub samples was measured using stereoscopic microscope fitted with an ocular micrometer. The monthly frequency of ova diameter, various gonad stages and mean monthly GSI were plotted by month to determine the reproductive cycle and breeding season of the fish.

Absolute individual fecundity (Fa) was determined gravimetrically in 27 females. The weights of the preserved ovaries were recorded. Three samples from the anterior, middle and posterior part of ovary were taken, mixed for homogeneity, and weighed. The ova were separated by teasing and then counted. For the purpose of fecundity, only ripe specimens (stage V) were considered.

Absolute individual fecundity (Fa) were calculated using the following formula:

\[
Fa = \frac{\text{Weight of ovary} \times \text{No. of ova in the sub sample}}{\text{Weight of the sub sample}}
\]

Fa was then correlated with total length, (TL) total weight (W), eviscerated weight (Wo) and ovary weight using the least squares method. The correlation co-efficient (r) between fecundity and total length; total weight, eviscerated weight and ovary weight were calculated by the method of Karl
Pearson (Snedecor & Cochran, 1967). Relative individual fecundity (Fr) was calculated as the quotient of the Fa on 1 g of total weight (The number of the eggs per gram total body weight). Chi square test was used to assess deviation from 50 : 50 : sex ratio (Robards et al., 1999).

**SEM of eggs**

To study surface structure of ripe eggs, the fixed samples were first dehydrated in a graded series of ethanol. The samples then were placed in amyl acetate and critical point dried with liquid CO₂. The mounted samples were coated with gold palladium in a gold sputtering unit. The rest of the process is similar to the methodology, adopted in case of scale studies.

**Histological studies of ovary**

Sub samples of ovaries were dissected out from the fish and washed in 0.9% NaCl to make them blood free. Following histological procedure was followed:

1. Tissue was fixed in Bouin’s fixative* for 24 ours.
2. It was kept overnight in running water and then in 70% alcohol to remove extra Bouin’s fluid.
3. Dehydration was done in 90% alcohol followed by absolute, for about 1h in each.
4. The dried tissue was passed sequentially through a mixture of absolute alcohol and benzene in following ratios 3 : 1, 1 : 1, 1 : 3 and finally in pure benzene, for 30min in each mixture.

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* Bouin’s fixative : 75ml saturated solution of picric acid + 25 ml formalin + 5ml glacial acetic acid.
5. The cleared tissue was given a change twice in a mixture of benzene and wax (1:1) for 30min.

6. It was then immersed in molten paraffin wax (m.p. 58°C) and three changes were given at an interval of 30min.

7. Suitable sized cavity block was selected for block making. A layer of glycerine was smeared on it and was filled with molten wax. Finally tissues were placed in it with proper orientation. The wax was allowed to freeze at room temperature; blocks were taken out and trimmed carefully for sectioning.

8. Sections of 5 - 7 μ thickness were cut using a rotary microtome.

9. Sections were fixed on glass slides having a very thin layer of albumin and were stretched in warm water (50°C).

10. The slides were left in oven (37°C) for overnight.

11. The sections were deparaffinized in xylene and were brought to water after downgrading through a series of alcohol (100 - 30%).

12. The sections were stained with hematoxylin for 5 min. and were washed in running water for about 10min. For differentiation, the slides were shifted to mild acid and base water, respectively.

13. They were dehydrated in alcohol grades upto 70% stained in eosin for 30sec. and further dehydrated through 90% and 100% alcohol.

14. Sections were cleared in xylene and mounted in DPX.

**Physico-chemical analysis**

Parameters like water temperature (°C), pH, dissolved oxygen (mg/l) and conductivity (μS/cm) were measured on the spot using "Multi line F/SET-3 P4 Water Analysis Kit." Air temperature (°C) was measured with streamline
thermometer. Total dissolved solids (TDS) were estimated using a Merck TDS Scan Meter. Penetration of light was measured with a Secchi disk. The rest of parameters, like free carbon dioxide (mg/l), total alkalinity (mg/l), total hardness (mg/l), calcium and magnesium hardness (mg/l), chloride (mg/l), inorganic phosphates (mg/l) and nitrate (mg/l) were analysed according to the standard methods given in APHA (1998).