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The Nangal wetland was assessed for a period of two years (January, 2010-December, 2011) on monthly basis for various physico-chemical and biotic parameters. Fish sampling was done during the months of May, 2011, October, 2011 and March, 2012 and the permission for the same was obtained from Chief Wildlife Warden, Divisonal Forest Officer, Punjab.

4.1. Fish fauna

(i) Collection and identification

Random sampling was done at the wetland with the help of gill net with hired help of fishermen in the area (Fig. 4.1).

![Collection of fish with the help of gill net at Nangal wetland](image)

Fig. 4.1 Collection of fish with the help of gill net at Nangal wetland

The representative specimens of the different fish species were preserved in ten percent formaldehyde solution and brought to the laboratory for identification using standard references of Day (1875-1878), Tilak and
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Husain (1977), Johal and Tandon (1979, 1980), Talwar and Jhingran (1991) and Jayaram (2010). Various morphometric measurements were done according to the criteria laid down by Jayaram (2010).

4.1.1. Species composition
It is the variety of different types of organisms which inhabit an area.

4.1.2. Relative diversity and Abundance
It is the proportion of an individual species to the total number of individuals inhabiting an area.

4.2. Length-weight relationship
The length-weight assessment is of great importance in fishery assessments. The parameters of the length-weight relationships were calculated by the following equation:

\[ W = aL^b \]  
(Le Cren, 1951; Ricker, 1973; Pauly, 1983)

Where

- \( W \): Weight of the fish in grams (gm)
- \( L \): Total length of the fish (cm)
- \( a \): Constant (intercept)
- \( b \): the length exponent (slope)

The length-weight pairs were plotted initially in order to identify and delete the possible outliers. The “\( b \)” is an exponent with value ranging between 2.5-3.5 demonstrating normal growth dimensions or the interpretation of relative well-being (Bagenal, 1978; King, 1996 a, b). Linear transformation was made by using the natural logarithm at the observed lengths and weights proposed by Zar (1984). The expression of the equation is represented by the following formula:

\[ \log W = b \log L + \log a \]

A graph of the \( \log W \) against \( \log L \) forms a straight line with slope of “\( b \)” and a Y-axis (\( \log w \)) intercept of \( \log a \). Invariably, “\( b \)” is close to 3.0 for all species. In the previous versions and in much of the fishery literature, the regression constant is represented by “\( c \)” rather than “\( a \)” and the regression slope is represented by “\( n \)” rather than “\( b \)”. Equations in the form of natural logarithms (base e) and power functions are commonly used instead of log 10 (Schneider et al., 2000). All the above statistical calculations were done using the software SPSS (Version 16) and then the graphs were plotted using the observed values and log of observed values.
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4.3. Fish Morphometry

The morphometric measurements of all specimens were done in laboratory from the left side of the body of the fish. The total lengths of the fish were recorded with an accuracy of ± 0.5 cm. The morphometric readings were then converted into percentages and were expressed with respect to total length and head length of the fish. These percentages were then used to compute mean, standard deviation (S.D.) and range. The actual values were used to calculate correlation coefficients (r), regression lines. For computation of mean, standard deviation (S.D.), range differences, correlation coefficient, regression equation and plotting of the regression lines, the standard version of computer program SPSS (version 16) was used. Measurements were analyzed in two groups:

- in one group; the measurements were taken with respect to the total length;
- in the second group; the measurements were taken with respect to the head length.

All the measurements were taken in cms and the weight was recorded in gms. The following morphometric characters have been recorded:

**Total length of fish vs.**

1. Standard Length (SL)
2. Pre-dorsal Distance (PreD)
3. Post-dorsal Distance (PostD)
4. Length of Dorsal Fin (LDF)
5. Depth of Dorsal Fin (DDF)
6. Length of Anal Fin (LAF)
7. Depth of Anal Fin (DAF)
8. Pre-Anal distance (PreA)
9. Length of Pectoral Fin (LPF)
10. Length of Pelvic Fin (LP’F)
11. Minimum body width (MBW)
12. Maximum body width (MB’W)
13. Distance between Pectoral and Pelvic fins (DPP)
14. Distance between Pelvic and Anal fins (DPA)
15. Length of Caudal Fin (LCF)
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16. Length of Caudal Peduncle (LCP)
17. Length of Rostral Barbel (B)
18. Length of Maxillary Barbel (B’)

Head length (HL) vs.
19. Head Depth (HD)
20. Pre-Orbital distance (PreOr)
21. Post-Orbital distance (PostOr)
22. Eye Diameter (ED)
23. Inter-orbital Distance (IoD)

The morphometric characters were studied after Jayaram (2010) and a brief description of the characters is given below (Fig. 4.2):

Total Length (TL)
The greatest distance between the anterior projecting part of the head to the posterior most tip of the caudal fin. The measurement is a straight line and is not taken over the curves of the body.

1. Standard Length (SL)
The straight distance from the anterior part of the head to the end of the vertebral column is taken as the standard length.

2. Pre-dorsal Distance (PreD)
A straight measurement from the midpoint of snout or upper lip or the anterior most part of the head to the structural base of the anterior most dorsal fin ray is the pre-dorsal distance.

3. Post-dorsal Distance (PostD)
A straight line measurement from the structural base of the dorsal fin to the flexure line of the body over the caudal peduncle or the end of the vertebral column is post dorsal distance.

4. Length of Dorsal Fin (LDF)
The greatest distance of the dorsal fin measured in a straight line from its anterior most to the posterior most junctions with the body.

5. Depth of Dorsal fin (DDF)
It is measured from the anterior point of junction of dorsal fin with the body to the anterior tip of the fin or spine even if other rays do not reach this point.
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6. **Length of Anal Fin (LAF)**
   The greatest distance of anal fin measured in the straight line between the anterior most and posterior most points of junction with the body.

7. **Depth of Anal Fin (DAF)**
   It is measured from the anterior point of junction of anal fin with the body to the anterior tip of the spine or fin even if other rays do not reach this point.

8. **Pre-Anal Distance (PreA)**
   A straight line distance from the base of anterior most fin ray of the anal fin to the anterior most point of the head.

9. **Length of Pectoral Fin (LPF)**
   It is measured between the origin and place of insertion of pectoral fin into the body to its extreme tip.

10. **Length of Pelvic Fin (LP’F)**
    It is measured between the origin and place of its insertion of pelvic fin into the body to its extreme tip of the fin or spine even if other rays do not reach this point.

11. **Minimum Body Width (MBW)**
    Minimum perpendicular distance from the dorsal side to ventral side

12. **Maximum Body Width (MB’W)**
    Maximum perpendicular distance from the dorsal side to ventral side.

13. **Distance between Pectoral and Pelvic fins (DPP)**
    It is the distance between the anterior most point of the pectoral fin to the anterior most point of the ventral fin taken across the length of the fish.

14. **Distance between Pelvic and Anal fins (DPA)**
    It is the distance between the anterior most point of the pelvic fin to the anterior most point of the anal fin taken across the length of the fish.

15. **Length of Caudal Fin (LCF)**
    It is measured from the anterior point of junction of caudal fin with the body to the extreme tip of the fin or the spine even if the other rays do not reach this point.
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16. **Length of Caudal Peduncle (LCP)**
   An oblique measurement from the last point of contact of anal fin posteriorly to the end of the vertebral column or the flexure of the body over the caudal peduncle.

17. **Length of Rostral Barbel (B)**
   It is the measurement from base to the tip of barbel extended from nostrils.

18. **Length of Maxillary Barbel (B)**
   It is the measurement from base to tip of barbel present on either side of the mouth.

**Head Length (HL)**
A straight line measurement of distance from the tip of the snout to the most distant point on the opercular membrane on the upper angle of the gill opening.

19. **Head Depth (HD)**
   The perpendicular distance measured from the midline at the occipital vertically downwards to the ventral contour of head or breast.

20. **Pre-Orbital Distance (PreOr)**
   The distance from the most anterior midpoint on the snout or upper tip to the front margin of the orbit.

21. **Post-Orbital Distance (PostOr)**
   The greatest distance from the posterior edge of the orbit to the posterior tip of the fleshy operculum.

22. **Eye Diameter (ED)**
   The maximum distance between margins of the cartilaginous eyeball across the cornea.

23. **Inter-orbital Distance (IoD)**
   Least distance of the bony rims between inner margins of eyes.
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Fig. 4.2 Diagrammatic representation of fish showing various morphometric characters.
Analysis of the morphometric data

The morphometric data was analyzed by the method of the least square. A graph was plotted based on the regression and correlation of each morphometric character. Regression equation is given by the formula

\[ Y = a + b(X) \]

Where
- \( Y \) = Dependent variable
- \( a \) = Constant
- \( b \) = Slope of line
- \( X \) = Independent variable

The morphometric readings were then converted into percentages and have been expressed with respect to total fish length and head length. These percentages were then used to compute mean, standard deviation (S.D) and range. The actual values were used to calculate correlation coefficients (r), regression equations between independent and dependent parameters and for plotting the regression lines. For computation of mean, standard deviation (S.D), range difference, correlation coefficient, regression equation and plotting of the regression lines, the standard version of computer program SPSS (version 16.0) was used.

4.4. Water quality parameters

Fish community structure often depends on water quality and therefore any variation in the water parameters result in the corresponding changes in the fish community structure and abundance. Therefore, it is of paramount importance to study the abiotic factors of any study site. For the assessment of the water quality, the water samples were collected in a 2 litre PVC bottle and brought to the laboratory for further analysis using standard references of Trivedi and Goel (1986) and APHA (2005). The following water parameters were analyzed:

a. **Color of the water:**
   - The color of the water was observed visually.

b. **Water temperature (°C):**
   - Water temperature was measured with the help of centigrade thermometer.

c. **pH:**
   - pH was determined using E-merck’s pH meter.
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d. Total Dissolved Solids (ppm):
Total Dissolved Solids was determined using E-merck’s TDS meter.
e. Carbon-dioxide (mg/L):
Carbon-dioxide was determined titrimetrically using strong alkali (NaOH) as titrant and phenolphthalein as an indicator.
f. Dissolved oxygen (mg/L):
Dissolved oxygen was determined by using modified Winkler’s method using sodium thiosulphate as titrant and fresh starch solution as indicator. Initially manganous sulphate and alkali azide was used for fixation.
g. Biochemical oxygen demand (mg/L):
Biochemical oxygen demand was determined by finding the Oxygen requirement of organism in the sample in 5 days at 20 °C.
h. Hardness (mg/L):
Hardness was determined by titration method, using EDTA as titrant and Eriochrome Black T as indicator.
i. Calcium (mg/L):
Calcium was determined by titration method, using EDTA as titrant and murexide as indicator.
j. Magnesium (mg/L):
Magnesium was calculated as difference between total hardness and calcium hardness multiplied by factor (0.244).
k. Alkalinity (mg/L):
Alkalinity was estimated by titration method, using sulphuric acid (0.02N) as titrant and phenolphthalein and methyl orange as indicator.
l. Chlorides (mg/L):
Chlorides were determined titrimetrically using silver nitrate as titrant and potassium chromate as an indicator.
m. Nitrates (mg/L):
Nitrates were estimated by Brucine method using sulphuric acid, sodium arsenite as reagents. Nitrate and brucine reacts to produce a yellow colour. The optical density was measured at 410 wavelength using
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spectrophotometer. Standards were run simultaneously so as to estimate the concentration of nitrates.

n. Phosphates (mg/L):

Phosphates were estimated by stannous chloride method. Ammonium molybdate and stannous chloride were used as reagents which react to produce blue colour and the optical density was measured with help of spectrophotometer at 410 wavelength. The amount of phosphate was calculated in mg/L from the standard curve.

o. Sulphates (mg/L):

Sulphates were estimated by using barium chloride, NaCl-HCl solution and Glycerol-ethanol solution as reagents and optical density was measured with help of spectrophotometer at 420 wavelength. The amount of sulphates was calculated in mg/L from standard curve of sulphates.

p. Silicates (mg/L):

Silicates were estimated by using molybdosilicate method wherein HCl, ammonium molybdate solution and oxalic acid were used. The optical density was measured with help of spectrophotometer at 410 wavelength. The amount of silicates was calculated in mg/L from the standard curve.

4.5. Biota

(i) Collection and Identification

For the collection of planktons, a ring type terricota net (24 meshes/mm2) fitted with a wide mouthed plastic bottle were used. The water samples (50 L) were filtered through it to collect the plankton sample. The standard references of Pennek (1953), Kudo (1986), Ward and Whipple (1992) was consulted for identification of biota. The planktons were assessed qualitatively.

The benthic life was collected by enclosing one square meter of the area of the wetland. The stones and gravel will be scrubbed off gently to dislodge any aquatic life. These were preserved in standard formaldehyde solution and were also identified using the references of Ward and Whipple (1992).

(ii) Photography

Further more, after identification; photography of water samples for planktons
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was done. For this purpose, two or three drops of sample was put on the slide and covered with a cover slip. After wards slide was kept to rest for ten to fifteen minutes so that planktons can settle down. Then the slide was observed under phase contrast microscope and photographs were taken using Leica camera attached to microscope.

(iii) Scanning Electron Microscopy

SEM was done for categorization of various members of Bacillariophyceae. The procedure followed was as under:

- Two or three drops of water sample were put on the slide. Kept it parallel to surface for fifteen minutes.
- After that extra water was dried with the help of conical edges of whatmann filter paper no. 1. Care must be taken to prevent wiping of off the diatoms itself.
- Then the remaining material was transferred with the help of brush onto the metal stub on which silver tape was already fixed.
- The stubs were then first dried under lamp for ten to fifteen minutes and then properly dried in dessicator for two hours.
- The stubs were coated with 100 Angstorm thick layer of Gold or Pallidium in JEOL sputter ion coater.
- The stubs were observed in JEOL JSM 6100 SEM at 20 Kv and photographs were taken.
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