4. MATERIAL AND METHODS

A total of ten study sites were selected for the present investigations from Western Himalayas. The streams of Himachal Pradesh belonging to the rivers Ghaggar (Baddi and Markanda), Yamuna (Giri Gaura, Giri YN) were visited once a month from August/September 1998 to July/August 1999 whereas, the streams of Garhwal region [Nailchami (U), Nailchami (D), Balganga (U), Balganga (D), Gular (U) and Gular (D)] were visited at quarterly intervals from December 1999 to September 2000. The following methodology was adopted to record the observations from the study sites:

1. Fish fauna

A. Collection and identification

Random sampling was done over a stretch of 500 mts at each site with the help of cast net of 1 cm mesh size having a diameter of 1-2 mts. Fishing gears such as hand net, scoop net, dip net and other local gears were also employed, wherever necessary. The representative specimens of different fish species were preserved in 10% formaldehyde solution and then identified in the laboratory using the standard reference of Day (1875-1878), Tilak and Husain (1977), Johal and Tandon (1979, 1980), Talwar and Jhingran (1991) and Jayaram (1999). The generic and specific spellings of fishes mentioned in the text are after Jayaram (1999).

B. Indices

- Fish species richness (FSR)

It is the total number of species present in a community at a site.
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❖ Abundance

It is the total number of individuals recorded from a given stream.

❖ Shannon index (1949)

The Shannon index, $H'$ (Shannon and Weaver, 1949) assumes that all the species are represented in the sample and are randomly sampled

$$H' = - \sum_{i=1}^{n} p_i \ln p_i$$

Where

$p_i = \frac{n}{N}$ i.e. the proportion of individuals found in the $i^{th}$ species

$\ln = \text{the natural logarithm.}$

The maximum diversity ($H'_{\text{max}}$) is found when all the species are equally abundant.

$$H'_{\text{max}} = \ln S$$

Where

$S = \text{the total number of species present at a site}$

❖ Shannon’s equitability ($E_h$)

We can compare the actual diversity value to maximum possible diversity by using a measure called Evenness or Shannon’s equitability. Evenness is also defined as the relative abundance of a species in a community.

$$E_h = \frac{H'}{H'_{\text{max}}} = \frac{H'}{\ln S}$$

$E_h$ is constrained between 0.00 to 1.00 with 1.00 being complete evenness. An evenness measure approaching zero would mean that the species abundances differ greatly while an evenness of one would mean they were all the same.

C. Similarity coefficients

One way to compare diversity between different areas is to simply compare diversity indices. Another method is to compare the diversity of the
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sites using indices called as similarity coefficients. These coefficients compare the numbers (qualitative) and sometimes abundances (quantitative) of species common to both the sites.

❖ Qualitative coefficients

Two commonly used similarity coefficients are the Jaccard measure (Jaccard, 1912) and the Sorensen measure (Sorensen, 1948) probably for their ease of calculation.

➢ Jaccard measure (1912)

\[ C_j = \frac{a}{a + b + c} \]

➢ Sorensen measure (1948)

\[ C_s = \frac{2a}{2a + b + c} \]

Where

\( a \) = the number of species common to both sites.

\( b \) = the number of species in site B, but not A.

\( c \) = the number of species in site A, but B.

The range of these coefficients varies from 0.00 (no matching or complete dissimilarity) to 1.00 (complete similarity).

❖ Quantitative coefficients

These indices take into consideration the number of individuals of each species at the two sites.

➢ Czekanowski coefficient (1909, 1913)

It is very similar to Jaccard measure except that it takes into consideration the abundance of individuals common to both sites.

\[ \text{Percent Similarity (PS}_{jk} = \left( \frac{2W}{A+B} \right) \times 100 \]

Where \( W = \sum_{i=1}^{n} \min (X_{ij}, X_{ik}) \)
Material and methods

\[ A = \sum_{i=1}^{n} X_{ij} \quad \quad B = \sum_{i=1}^{n} X_{ik} \]

\( X_{ij} \) = abundance of species \( i \) in the sampling unit \( j \).

\( X_{ik} \) = abundance of species \( i \) in the sampling unit \( k \).

\( S \) = total number of species at a site.

Thus, the percent similarity between sampling units \( j \) and \( k \) is the numerator of twice the sum of minimum (min.) of the paired observations of sampling units \( j \) and \( k \) divided by denominator of the total of the species abundances for the two sampling units.

For any pair of the sampling units with identical species abundances, their similarity is complete i.e. \( PS = 100\% \). The percentage dissimilarity (PD) can be calculated as

\[ PD = (100 - PS). \]

**Morisita-Horn measure (Wolda, 1981)**

This index is calculated from the equation

\[ C_{MHI} = \frac{2 \sum (a_{n_i} \times b_{n_i})}{(d_a + d_b) aN \times bN} \]

Where

\( aN \) = the number of individuals in site A.

\( bN \) = the number of individuals in site B.

\( a_{n_i} \) = the number of individuals in the \( i^{th} \) species in site A.

\( b_{n_i} \) = the number of individuals in the \( i^{th} \) species in site B.

\[ d_a = \frac{\Sigma a_{n_i}^2}{aN^2} \quad \text{and} \quad d_b = \frac{\Sigma b_{n_i}^2}{bN^2} \]

It was originally developed to measure niche overlap and is Horn’s (1966) modification of an index of similarity proposed by Morisita (1959).

**D. Scanning Electron Microscopy (SEM) of adhesive organs**

To study the details of the adhesive organs of some fishes, SEM studies
were carried out. The following procedure was adopted for the preparation of specimen for SEM.

Adhesive apparatus was removed with the help of a sharp blade and was fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH = 7.2–7.4 for 24 hours. After several washings in the rinsing buffer 0.1 M sodium cacodylate buffer containing 7% sucrose was added and further dehydration was carried out in various grades of acetone.

The specimens after acetone treatment were transferred into emylacetate solution. Then the specimens were dried in a Polaram Critical Point Dryer (CPP) and mounted on metal stubs with double adhesive tape. The specimens were coated with 100A" thick layer of Gold/Palladium in JEOL sputter ion coater. The specimens were examined with JEOL TSM 6100 SEM at 20k, and the images were observed on the screen. Negatives were prepared for photography.

2. Water quality

Fish assemblage depends on the physico-chemical environment, hence, changes in the composition of a fish community often indicate a variation in pH, salinity, temperature regime, solutes, flow, D.O., substrate composition or pollution level. Therefore, abiotic factors play an important role in determining fish communities.

For the assessment of water quality, the samples were collected in 2 litre PVC bottles from each site and brought to the laboratory for further analysis. All water quality parameters were estimated by using the standard methods following APHA (1998).

Factors like water temperature (°C), dissolved oxygen (mg/l), pH and conductivity (μS/cm) were measured in the field with the help of “Multiline F/SET - 3 P-4 water analysis kit”. Air temperature was measured with the help of streamline thermometer. The total dissolved solids were determined using E-merck's TDS Scan-meter.

Other parameters such as turbidity (NTU), chlorides (mg/l), total alkalinity (mg/l), total hardness (mg/l), nitrates (mg/l), phosphates (mg/l) and silicates (mg/l) were determined in the water samples according to the methods described in APHA (1998).
Material and Methods

Inorganic phosphate (mg/l) and nitrate (mg/l) were determined by taking O.D. on “ELICO SL-159 UV-VIS SPECTROPHOTOMETER” and were based on the methodology described in APHA (1998).

3. Biota

For the collection of biota a ring type terricota net (24 meshers/mm²), fitted with a wide mouthed glass bottle was kept in the flowing water to enable the water flow through it. The samples collected were preserved in 5% formaldehyde solution on the spot for counting of plankton. For living study and identification of the biota, separate water samples were collected in similar manner.

The standard references of Pennak (1953), Kundu (1986) and Ward and Whipple (1992) were consulted. 50 litres of stream water was filtered for plankton collection.

For the identification of phytoplankton, zooplankton, benthos, Bryophytes, Pteridophytes, Gymnosperms and Angiosperms (herbs, shrubs, trees) both in the stream zone (if any) and riparian zone; standard references were consulted : Day (1875-1878), Smith (1950), Pennak (1953), Mellanby (1963), Pangtey and Joshi (1989), Talwar and Jhingran (1991), Ward and Whipple (1992) and Jayaram (1999).

The aquatic insects and other benthic life were collected by enclosing one square meter of stream bottom with square-meshed cloth. The bottom stones, gravel and sand were upturned to dislodge the aquatic life. Each animal was then brush picked and preserved in 5% formalin.

For the identification of plants on the sides of the streams, the references of Pangtey and Joshi (1989), Chopra and Verma (1992), Chopra (1998) and Srivastava (1992a,b) were consulted. Some experts were also consulted from the Department of Botany, Panjab University, Chandigarh to identify the preserved flora of these streams. The plants were preserved in newspaper pages and brought to the laboratory for identification.
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4. Stream morphology

Channel type classification is useful for defining sampling strata by reaches when making habitat and fish population measurements, for rating a stream's sensitivity to land disturbance and erosional potential for identifying the potential effectiveness of various fish habitat improvement structures for a particular channel type (Myers and Swanson, 1992; Rosgen, 1994).

In order to classify the streams the methods of Rosgen (1996) were followed. The following methodology was adopted for different parameters.

1. Longitude, latitude and altitude (m.s.l.) were determined with the help of Magellan Trailblazer XL GPS system.

2. The stream gradient (both in percentage and degree) was determined between two points in the linear fashion with the help of Suunto Clinometer.

3. The stream width (mts.) was measured with Bushnell Laser Range Finder Yardage Pro 400. It is a horizontal distance (average of 5-6 points having different widths) along the stream perpendicular to the stream flow from wetland to wetland to the nearest 0.1 mm.

4. Water current (cm/sec) was calculated with the help of EMCON current meter. Readings were recorded from 3-4 points, having different depths by placing the propeller of the EMCON current meter at the desired spot. Average of all these values was considered as the mean water current (cm/sec) of the entire stream.

5. The stream depth was calculated with the help of a graduated iron bar. It is the vertical distance from the bottom to the upper surface layer of water. Depth was recorded from at least 7-8 points having different depths and the average of all these points was considered as the mean depth.

5. Habitat structure

The habitat type and the substrate material were classified after Armantrout (1999). A brief description of the substrate material and the habitat type used by Armantrout (1999) is given below:
**Material and methods**

A segment of the stream with reduced current velocity and with depth exceeding other surrounding habitats.

**Riffle**

A relatively shallow area with gradient less than 4% with swift flowing water completely or nearly covering obstructions (except at very low water) and substrates of smaller rock gravel or bedrock having surface or subsurface agitation.

**Rapid**

A relatively deep stream section with swift current and gradients exceeding 4% resulting in series of short drops, considerable surface agitation, pocket pools and rocks and boulders exposed at all but high flows.

**Run**

An area of swiftly flowing water with gradient over 4% with minor surface agitation and in which slope of the water surface is roughly parallel to the overall gradient of the stream.

In the present study, the habitat with water velocity ≤ 30cm/sec. and deeper than their counterparts are considered as pools. Different types of pools (viz. alcove, backwater, corner, dammed, plunge, pocket and underscore) were sampled during the present study. Habitat with velocity >

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<table>
<thead>
<tr>
<th>Name of the Particle</th>
<th>Size in Millimeters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large boulder</td>
<td>Over 1024</td>
</tr>
<tr>
<td>Small boulder</td>
<td>256–1024</td>
</tr>
<tr>
<td>Cobble</td>
<td>64–128</td>
</tr>
<tr>
<td>Coarse gravel</td>
<td>32–64</td>
</tr>
<tr>
<td>Fine gravel</td>
<td>2–34</td>
</tr>
<tr>
<td>Sand</td>
<td>0.062–2.0</td>
</tr>
</tbody>
</table>
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30 cm/sec but < 100 cm/sec. were taken as runs/riffles and the habitat with velocity > 100 cm/sec were taken as rapids. In each habitat, the water velocity was recorded with the help of Environmental Measurements and Control (EMCON) digital current meter.

The sampling was done between mid-morning and the late afternoon on a fixed day every month in the four habitat types at each of the ten study sites. The sampling was conducted fifteen times in each habitat type. Sampling during heavy rains was avoided to maintain the sampling consistency in all the habitat types.

The fish diversity for each site was calculated using Shannon–Weaver diversity index (1949) as already described above. Of the 26 species recorded, Barilius barna, Botia lohachata, Botia birdi, Salmostoma bacaila, Glyptothorax brevipinnis and Channa punctatus were not included because of their rare occurrence. The differences between different sites were analyzed using paired t-test by SPSS programme version 7.5.

6. Status of the fishes

The conservation status of the fish species was based on the criteria given by International Union for Conservation of Nature (IUCN) by Sanjay Molur and Walker (1998).
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