Chapter – 3
Material and Methods
3. MATERIAL AND METHODS

This study was conducted in the Solan district of Himachal Pradesh and Tehri district of Garhwal region of western Himalayas. The study focuses on streams within three major river basins i.e. The Sutlej, The Yamuna and The Ganges.

Study Design

For the present study 11 sites of 7 streams were selected and delineated a unique catchment for each site. Biological and physical data were collected for 200 m. stretch segment at each site. Sites were sampled between mid morning and late afternoon on the same date every month. Period of very large water release was avoided to maintain sampling constancy. Specific sampling methods were adopted for each set of variables (Table-3.1). Methods included qualitative and quantitative sampling of the fish assemblage, benthic macroinvertebrates and planktonic community, physical habitat assessment and water chemistry.

Physical habitat characteristics

Physical habitat characters were assessed during the base flow conditions along a 200m reach at each site. Variables that are typically used to characterize stream habitat were measured at each site including cross-sectional and longitudinal channel dimensions substrate characteristics, bank conditions, and water current.
In order to classify the streams the methods proposed by Rosgen (1996) were followed. Stream order classification was based on Horton's (1945) approach modified by Strahler (1954). Quantification of mesohabitats was carried out as described by Armantrout (1998).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Method of assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope of the stream (at site)</td>
<td>Determined between two points in the linear fashion with the help of Suunito clinometer</td>
</tr>
<tr>
<td>Order of the stream</td>
<td>With the help of topographic maps</td>
</tr>
<tr>
<td>Substrate composition</td>
<td>Visual examination of the bed material</td>
</tr>
<tr>
<td>Microhabitat/Mesohabitat</td>
<td>A visual estimation of percentage of different habitat types (Riffle, Run, Rapid, Pool)</td>
</tr>
<tr>
<td>Maximum pool depth</td>
<td>Depth of the deepest pool in the stream reach</td>
</tr>
<tr>
<td>Width</td>
<td>Average stream width at wetted perimeter at three transect</td>
</tr>
<tr>
<td>Depth</td>
<td>Average depth at three points along three transects measured with the help of graduated rod. Maximum and minimum depth was considered as deepest and shallowest part respectively in the stream reach</td>
</tr>
<tr>
<td>Rate of water flow</td>
<td>Average flow (cm/sec) of stream channel calculated at 4-5 transects at surface, column and bottom level with the help of EMCON digital current meter</td>
</tr>
</tbody>
</table>

**Measuring the substrate**

The particles of the substrate are measured with a metric ruler or with vernier caliper in centimeter because rocks can be large and narrow. They are measured twice; first the width then the length. Sum of the two values divided by two gives the average size
of the rocks. On the basis of average value the substrate material was classified according to following scheme:

- **Boulders**: 125 cm. or more
- **Small boulders**: 25 - 124 cm.
- **Large cobbles**: 15 - 24 cm.
- **Small cobbles**: 5 - 14 cm.
- **Gravels**: 2 - 5 cm.
- **Small Gravels**: 0.1 - 2 cm.
- **Sand**: < 0.1 cm.

**Water chemistry**

The productive capacity of aquatic system is equated with the chemical richness of the water. Chemical richness of stream environment generally is viewed as the concentration of ions in aqueous medium (Egglishaw and Morgan, 1965). In natural lotic ecosystem chemical richness of water is primarily influenced by geology (Egglishaw and Morgan, 1965; Lee, 1980).

Factors like water temperature (°C), dissolved oxygen (mg/l), pH and conductivity (μS/cm) were measured in the field with the help of multi-line F/SET-3 P-4 water analysis kit. Air temperature (°C) was measured with streamline digital thermometer. Total dissolved solids (mg/l) were determined using Merck's TDS scan meter. For other chemical factors like total hardness (mg/l), calcium and magnesium hardness (mg/l), total alkalinity (mg/l), chlorides (mg/l), nitrates (mg/l), inorganic phosphates (mg/l) and silicates (mg/l) water was brought to the laboratory in 2 l. PVC bottle and assessed according to APHA (1998). ELICO SL 159 UV-VIS spectrophotometer was used to take the optical density wherever required.
Material and Methods

Collection of plankton

For the collection of samples for the planktonic study 50 lt. of water was filtered in the wide mouthed 500ml PVC bottle with the help of plankton net made up of bolting silk (No. 25, 0.3 mm mesh). Lugol’s iodine solution and 4% formaldehyde solution were added to these bottles to fix and preserve the plankton. Lugol’s solution speed up the rate of sedimentation and provide stain to the phytoplankton. Such samples were kept undisturbed for a week or so as to allow sedimentation and then supernatant was removed with the help of a pipette avoiding agitation leaving behind the phytoplankton. The supernatant was examined under the microscope to ensure that it was plankton free. The diatoms were cleaned in 100ml. of boiling 20% nitric acid containing 0.25g. of potassium-di-chromate (K₂Cr₂O₇) and mounted in DPX for the permanent preservation. Identification of phytoplankton was made following Ward and Whipple (1992) and Anand (1998). The plankton cells were counted with the help of Sedgewick rafter counter. Density was calculated following APHA (1998) and expressed as units x 10³/l.

Collection of benthic macroinvertebrate population

Macro-invertebrates were collected from three riffle habitats arbitrarily selected in each stream based on current velocity and substrate. Distance between two riffles was more than 20m. For the collection 1m² area was marked and all the stones in the marked area were washed in a bucket and then the contents were transferred into a small bottle. All the samples were preserved in 5-10% formaldehyde solution. Invertebrates were then sorted from debris and placed in 70% ethanol. Aquatic insects were identified upto generic level with the help of Ward and Whipple (1992); Pennak, (1978); Kudo, (1986); APHA, (1998).
Material and Methods

Collection of Ichthyofauna

Different habitat like pools (edge pool and middle pool), rapids, riffles and run were sampled with the help of cast net of 1 to 2 meter diameter and mesh size of 1 cm. knot to knot. In addition hand net, scoop net and drag net were also used. All the fishes trapped were counted and released back in the water. Unidentified specimens were preserved in 10% formaldehyde solution and brought to the laboratory and then identified using standard references (Day, 1878; Johal and Tandon, 1979, 1980; Talwar and Jhingran, 1991 and Jayaram, 1999).

Analyses of Data

Total density and density of each species/genus of different groups (viz. fishes, phytoplankton and benthic macroinvertebrates) at all the collection sites were determined. For the statistical analysis of the data Microsoft Excel and SPSS - 10(1999) statistical programs were used. Fish habitats were grouped for describing the relationship between the fish community and available habitat. The purpose of the study was also to make comparison between the two streams on the basis of fish/biota density within habitat categories.

Species diversity indices

Species diversity indices were calculated for fishes, phytoplankton and benthos separately for each water body. These include those provided by Shannon and Weaver (1949) and Margalef (1958).

A. Shannon-weaver species diversity index

\[ H' = -\sum Pi \ln Pi \]

Where \( Pi = n_i / N \)

\( n_i = \) No. of individuals of the species in a particular habitat.

\( N = \) Total number of individuals in the sample.
B. Margalef species diversity index

\[ D' = \frac{S-1}{\log N} \]

Where \( S \) = Total number of species
\( N \) = Total population of all the species

C. Jaccard similarity coefficient (Jaccard, 1912)

\[ C_j = \frac{j}{(a+b-j)} \]

Where \( j \) = Number of species common at both sites A and B
\( a \) = Number of species at site A
\( b \) = Number of species at site B

\( C_j \) ranges between 0 to 1. The habitats are completely similar if \( C_j = 1 \) and completely dissimilar if \( C_j = 0 \).

Hydrological Variables

A. Froude number (a dimensionless number expressing the ratio of internal and gravitational forces in a fluid) was calculated for the water current.

\[ Fr = \frac{\bar{U}}{gD} \]

Where:
\( \bar{U} = \) Mean water current
\( g = \) acceleration due to gravity
\( D = \) Average depth

\( Fr < 1 \) = Sub critical flow
\( Fr = 1 \) = Critical flow
\( Fr > 1 \) = Super critical flow

B. Hydraulic discharge

\[ Q = Wdu \]

Where \( W, d \) and \( u \) are average width, average depth and water velocity respectively at a particular point.