Chapter IV.

Material and Methods.
A. Collection of material:

1. Collection of fishes from different localities.

Salmo trutta fario, Schizothorax osocinus, Oreinus plagiostomus, Crossochilus latius diplochilus, Cyprinus carpio communis, Cyprinus carpio specularis, Bettia hirta, Lebia sp., and Gymnaniella affinis: the species of fresh water fishes studied in the present thesis, are found in large numbers in suitable habitat such as lakes, rivers, and streams throughout Kashmir Valley. The fishes used for study were collected from lakes, rivers, and hatcheries in the Valley. The majority of fishes were however obtained from lakes such as Nagin lake, Mansbal lake and Dal lake, and the rest either from river Jehlum and Telbal Nallah or from various hatcheries.

For purposes of the study of gross anatomy of scales and confirmation of results by otoliths, opercular bones and vertebrae associated with investigations, a number of fishes were bought from the fish market at Srinagar as well. The collection of fishes was done periodically for four consecutive years from 1963 to 1967. In all about 1,000 fishes were examined in the entire study.

2. Recording of data:

Immediately after capture, about 90% of each catch was preserved in 10% formalin and the rest maintained
alive. The entire catch was transported to the labora-
tory. The lengths (standard & total) of the weighed fishes
were recorded according to the method given by Lagler (1962),
while the total weight was taken inclusive of gut contents.
The dates of capture were—from Nov; 1964 to April; 1967.

3. Preparation of Scales:

The scales reported in present investigations were
removed from both sides of the body. The datum scales
were removed mainly from the regions posterior to the
attachment of the pectoral fin and below the lateral line.
A few were also taken from comparison from other regions
of the body. Llewellyn (1966) has calculated the percent-
tage of clear annuli scales and finds 75 % of the datum
scales (of the thesis) to show clear annuli; while dorsal
thoracic shows 66 %, dorsal abdominal show 58 %, ventral
abdominal 56 %, and 45 % and 40 % scales with annuli.

About 10 scales from each fish were selected and
rubbed between the moist thumb and the order to remove the
epidermis and the slime. The selected scales were then
placed in 10 % formalin, washed with water, & dried till
required for examination, placed in envelopes bearing the
data required for the fish concerned. The scales of some
species were rather small and these were mounted in bal-
sum, after processing and in some cases staining with Eosine.
The bigger scales were soaked in water for five to ten minutes and flattened between two slides before each examination.

4. Preparation of Otoliths:

Otoliths from the selected specimens from which scales were taken, were extracted by giving an incision on the sides of the head in a dorso-ventral direction. After extraction, the otoliths were ground to decrease their thickness by abrasion between two pieces of emery paper. After abrasion, these were put in a solution of one part of absolute alcohol and one part of Glycerine for about 7-10 days to increase their transparency. The bigger otoliths were seen to require a longer duration (about a fortnight) to acquire sufficient transparency. Coarse transparencies were not very satisfactory. After clearing process, they were dehydrated and mounted permanently in Canada balsam for study. None of the methods as given by Johnston (1938), Dakin (1939), Arora (1951) and Ling (1953) gave good results. Staining of otoliths also gave indifferent results.

5. Preparation of Opercular bones:

Opercular bones were removed by giving an incision above the Opercular region and in the attaching ligament of the hyomandibula. The Branchio-stegal rays were removed
by means of forceps and the opercular bones were cleared of the skin covering and muscles by placing in hot water for half an hour. Then they were rubbed between forefinger and the thumb, washed with water and dried. The numbering was done by the method given by Chugunov (1926). These were placed in proper envelopes along with the scales which were also numbered.

Studies on Opercular bones after alizarin staining were also carried out, but as the unstained bones showed markings better than the stained ones, most of the data pertaining to opercular bones were from unstained material.

6. Preparation of Vertebrae and their sections:

A few vertebrae after the first four were removed and placed in 10% potassium-hydroxide in order to remove the attached muscles, ligaments etc. Then after clearing them in soap water, their sections were cut by a fret-saw. After proper cleaning the sections of the vertebrae were placed in a solution of one part of absolute alcohol and one part of Glycerine for about a week. Then they were mounted on slides in Canada Balsam after proper dehydration and studied. It was seen that dehydrated, unstained sections of vertebrae gave good results than undehydrated ones. Alizarin preparations were also unsatisfactory for age studies.
B. Different methods employed for determination of fish age:

The main methods for the study of the scale structure and the fish age employed during the course of the present investigations were:

1. The Scale method.
2. The Otolith method.
3. The Operculum method.
4. The Vertebra method.
5. The known age method.
6. The scale annuli fish-length calculation method.

The details of scale, Otolith Operculum and Vertebra methods, used in the present investigations are given in item (C).

The known age method was adopted in as many fishes as possible by obtaining various age-groups from hatcheries and confirming the annuli counted by the scale and bone methods.

The scale-annuli fish-length calculation method of Hiner Lea (1929) has been followed in the present work and has given consistent results.

C. Methods employed for examination of Scales, Otoliths, Opercula and Vertebrae:

1. Enlarged image method:

The larger scales of fishes were placed between
two glass plates in an enlarger. The image was properly projected and drawn by tracing over the image, then studied. The photographs of these enlarged images were also taken for permanent record. Such photographs were also taken for Opercular bone of some species of fishes studied in present investigations as well.

2. **Camera-lucida microscopic method**

Hand drawings of Otoliths, Vertebrae sections and Opercular bone of some species were made to scale with the help of a camera-lucida, coupled with different powers of eye pieces and objectives under the microscope and the Stereoscopic Binocular.

3. **Transparency and Opacity method by reflected light**

As a result of increased transparency by abrasion of Otoliths, preservation in Glycerine and alcohol and dehydration, the study of mounted Otoliths and Vertebrae sections were highly instructive in reflected light under the stereoscopic binocular and the microscope. Studies were also made by the opacity method as given by Llewellyn (1966). The opacity method gives better results as it renders the annuli much clearer.

4. **Diffused light method**

While examining Scales, Otoliths Opercular bone of Crossochilus only and vertebrae sections under the Stereoscopic binocular and the microscope, filters (both
blue oranged and were used. As a result of diffused light clear rings were easily seen in all the above mentioned materials. Thus, the results were confirmed and the proper age groups recorded.

5. **Photographic methods**

After properly numbering and studying different age groups, microphotographs of some representative scales, otoliths, opercular bone and vertebrae sections were taken by a "Zeiss microphotographic Camera". Many of these microphotographs are recorded in the thesis, while the rest were used to confirm the results obtained by other methods.

**D. Experimental methods:**

Live fishes obtained from the Haseembagh fish market and specimens of different sizes for different species were selected and kept in separate aquaria with following variations in food supply:

1. Aquarium with fish fed moderately.
2. Aquarium with fish starved.
3. Aquarium with fish fed abundantly.

As the temperature did not fall below 5°C and rise above 15°C in the laboratory and it remained same for all the fishes, this factor can be ruled out of the consideration. The period of experimentation was from 10th Sep. '66- 10th Dec. '66. after which the fishes
died. Scales were removed from these fishes after one month from the start of the experiments, after two months and after three months. The dates being 10th Oct. 1966, 10th Nov. 1966 and 10th Dec. 1966. The scales from each of these three experimental fishes were removed, examined and recorded.