MATERIAL AND TECHNIQUES

_Hphasora daniconius_ (Ham. Buch.) is a small cyprinoid fish, measuring a maximum of 20.0 cm. in length and commonly found in hill streams and tanks of Sagar, Madhya Pradesh. In the rainy season, the fish ascends the smaller, shallower and swiftly flowing tributaries of the main streams. During the breeding season, the female lays under the stones numerous transparent rounded eggs, each measuring about 2.0 mm. in diameter. The eggs are fertilized by the male and guarded by one of the parents till hatching.

_Clarias magur_ (Cuv. & Val.) is a moderate sized siluroid fish attaining a length of 45.0 cm. and commonly found in muddy waters of Sagar, Madhya Pradesh. During the breeding season, the male and female bury themselves in the mud where the female lays numerous eggs. These are fertilized by the male and also guarded till hatching by one of the parents.

The eggs of _Hphasora daniconius_ (Ham. Buch.) and _Clarias magur_ (Cuv. & Val.) were collected along with their parents with the help of a close-meshed net in the early hours of morning during the months of July and August. The eggs of _Hphasora daniconius_ (Ham. Buch.) were stored in earthenware pots in the fields and quickly brought to the laboratory where these were transferred to glass petri-dishes of 15.0 cm. diameter each. The eggs of _Clarias magur_
(Cuv. & Val.) were also transferred to small glass troughs in the laboratory. Water of the glass troughs and the petri-dishes containing the eggs was kept constantly aerated by means of an electrically operated mechanism.

The eggs of *Rasbora daniconius* (Ham. & Buch.) and *Clarias magur* (Cuv. & Val.) were observed to hatch out within forty eight hours after bringing them in the laboratory. For the purpose of the present study, seven stages represented by embryos of the following lengths have been selected:

*Rasbora daniconius* (Ham. & Buch.) (Division Cypriné, family Cyprinidae)*

(1) 4 mm. (2) 5 mm. (3) 6 mm. (4) 8 mm. (5) 10 mm. (6) 15 mm. (7) 18 mm.

*Clarias magur* (Cuv. & Val.) (Division Siluri, family Clariidae)*

(1) 5 mm. (2) 7 mm. (3) 8 mm. (4) 10 mm. (5) 12 mm. (6) 15 mm. (7) 18 mm.

The embryos were directly transferred into fixing fluid. Fixatives such as a mixture of corrosive-formol with acetic acid, alcoholic Bouin’s fluid, 90% Ethyl alcohol, and 5% Formalin were tried. However, the best results were

* Classification according to Berg (1940).*
obtained with the following formula of Bouin's fluid:

- Saturated alcoholic picric acid solution - 15 parts.
- Formalin 40 - 5 parts.
- Glacial acetic acid - 1 part.

Embryos were fixed for 12 to 18 hours in alcoholic Bouin's fluid. After fixation and washing, the embryos measuring up to 10 mm in length were dehydrated in ascending alcohol series, cleared in xylene and embedded in paraffin wax (melting point 56°C - 60°C). Embryos measuring more than 10 mm long were first decalcified either by 3% nitric acid in 70% alcohol or by the electrolytic method of Richman et al. (1947) as modified by Shergava (1965-66). The electrolyte consisted of 8% hydrochloric acid and 10% acetic acid in equal proportions. An a.c. of approximately 7.5 volts was applied to the object at anode. Decalcification took place in 12 to 14 hours. The embryos were first washed thoroughly with water to remove all traces of hydrochloric acid and acetic acid and then dehydrated in ascending alcohol series. The clearing was done in benzene and embedding in paraffin wax (m.p. 56°C - 60°C).

Serial sections of the embryos were cut, 8-10 microns thick, on a Beck's Rotary Microtome. The sections were stained either with Mallory's triple stain or with Delafield's haematoxylin and eosin. The Mallory's triple stain was prepared as follows:
Stain A - 1% aq. solution of Acid Fuchsin.

Stain B - Aniline Blue. - 0.5 Gm.
Orange G. - 2.0 Gms.
Phosphotungstic acid. - 1.0 Gm.
Distilled water. - 100 ml.

The sections were first stained in stain A for 4 minutes and then transferred directly to stain B in which they were left for 10 minutes. These were then washed thoroughly with water and differentiated in 90% alcohol. The slides stained with Mallory's triple stain showed the nuclei coloured as red, cartilage, bones and fibrous connective tissue - ranging from light blue to dark blue, nerves and glands - various shades of violet, muscles - red, erythrocytes and keratin - orange. It has been found out that the Mallory's triple stain gave the best differentiation of the developing cartilages and bones.

The study of the development of the vertebral column has been made by means of serial sections and by the graphical reconstruction method of Bhargava (1965-67). The magnification of a particular set of eye-piece and objective lenses at stage height (for the sake of uniformity) under a camera lucida was determined. This magnification multiplied by the thickness of the section, gave the thickness of each
section for graphical reconstruction. The eye-piece scale was calibrated with the stage micrometer scale and thereby the equivalent value under camera lucida magnification at stage height was calculated. This equivalent value of the eye-piece scale was used for all measurements in graphical reconstruction and it corresponded with the magnified thickness of each section. An arbitrary line was drawn on the graph paper. Starting from one end, the points of structures in consideration were plotted on either side of this line. Moving to the next section, the points were again plotted for the structures in accordance with the calculated magnification. This process was repeated. The points of the same structure were joined to construct it on the graph paper.

Diagrams given in the thesis were sketched at the stage level of Neopta research microscope. The photomicrographs were made on "Fortepan 34" panchromatic photographic film with a speed of 230 DIN by using a Rectaflex (Junior) camera.