MATERIALS AND METHODS

Two fresh water teleost fish, *Clarias batrachus* and *Mastacembelus pancyalus* were selected for the present work. Twenty five adult specimens of each of these fish were collected from Sagar lake, during the last week of every month from July, 1966 to June, 1967 for one complete reproductive cycle. At the time of collection water temperature was noted (Table 3, page 142). The surface bones of the skull were opened and the eyes were removed and a long incision was made in the abdomen to ensure good fixation of both pituitary gland and the gonads. All these preliminary dissections were done within half a minute and then the specimens were immersed in the fixatives on the spot of collection.

Three fixatives, Holland's Modified Bouin (2.5 gms. of copper acetate, 4.0 gms. of picric acid, 10 ml. of 40% formaldehyde, 1.5 ml. of glacial acetic acid and 100 ml. of water), Aqueous Bouin +10% saturated corrosive sublimate and Aqueous Bouin were used. Nine specimens of each fish were fixed in Holland's Modified Bouin, nine in Aqueous Bouin +10% saturated corrosive sublimate and seven in Aqueous Bouin. The fish after fixation were brought to the laboratory where the total length of specimens were measured. The heads of fish were severed and the gonads were dissected out. The surface of the gonads of each fish was dried by blotting paper and the volume of the paired gonads of each fish was measured by liquid displacement method
(Bullough, 1939). The heads and the gonads were, then, kept in their respective fixatives in separate glass tubes for the required period of fixation. An average volume of the gonads for each month is given in Table 3, page 142.

The material was fixed in Holland's Modified Bouin for 72 hours, in Aqueous Bouin +10% saturated corrosive sublimate for 24 hours and in Aqueous Bouins also for 24 hours. The material was, then, thoroughly washed in running tap water for twelve hours and was kept in 70% cellosolve. The pituitary gland was dissected out in 70% cellosolve after washing the material. Both pituitary and gonads were dehydrated in ascending grades of cellosolve (90% one hour, 100% one hour with two changes) and methyl benzoate for one hour (two changes of thirty minutes each) and cleared in benzene for one hour (two changes of thirty minutes each). The material was, then, embedded in paraffin wax of melting point 60°-62°C. Serial vertical longitudinal sections of pituitary were cut at 5 μ thickness and transverse sections of gonads were cut at 6 μ thickness. The sections of gonads were selected from the ribbon with an interval of about hundred sections. Thus on one slide sections from three or four regions representing the entire gonads were stretched.

Following techniques were employed for staining the sections of pituitary gland:

1. Gomori's (1950) Aldehyde Fuchsin (AF) method as recommended by Dawson (1953).
2. Gomori's (1941) Chrome-alum Haematoxylin phloxin (CHPPh) method as recommended by Bargmann (1949).

3. Heidenhain's Azan stain.


Sections of gonads were stained by Heidenhain's Azan and Ehrlich's acid-alum haematoxylin and eosin stains.

For the study of histogenesis of the pituitary gland of *Clarias batrachus* spawns of this fish were collected from natural breeding places from Sagar Lake, Sagar in June, 1968. The fish breeds in June and July immediately after the freshests. The fertilized eggs are non-floating and adhesive. After hatching the larvae were reared in the laboratory until the metamorphosis was completed. All the nine stages described in the development of the pituitary gland in *Clarias batrachus* were reared from one spawn. The developing stages were studied under the microscope for the study of broad morphological details. Few larval stages of *Mastacembelus armatus* were collected from Sagar Lake during June, 1968 for the study of histogenesis of the pituitary gland due to non-availability of eggs and larvae of *Mastacembelus pancerla*. The larval stages of both *Clarias* and *Mastacembelus* were fixed in Aqueous and Alcoholic Bouin fluid for eight to twelve hours. They were then washed in 70% cellosolve and dehydrated in ascending grades of cellosolve, 90% cellosolve (one hour, two changes of 30 minutes each) and 100% cellosolve (one hour, two changes of 30 minutes each). The material was then treated in
methyl benzoate, cleared in benzene and finally embedded in paraffin at 60°-62°C. Vertical longitudinal sections of these blocks were cut at 5 µ thickness and stained after Heidenhain's Azan and Aldehyde Fuchsine staining methods. It has been observed that there is a difference in the duration of the development shown by the larvae which are found in nature and those which are experimentally reared in the laboratory. Hence, the duration has not been taken into consideration while describing a particular stage of development, instead the length of the larva has been considered.

For the study of vascularisation of the pituitary gland heads of advanced stages of larvae (after their metamorphosis has been completed) of Clarias batrachus were fixed in Alcoholic Bouins for 24 to 36 hours. The material was washed in running tap water and decalcified by the electrolytic method of Richman et al. (1947). The electrolyte in this method consists of 8% hydrochloric acid and 10% formic acid in equal proportions. In the present method 10% of acetic acid was used instead of 10% formic acid (Bhargava, 1968). An e.m.f. of approximately 7.5 volts was applied with the object at anode. Decalcification took 12-18 hours. The heads were again washed in tap water for a few hours to remove all hydrochloric acid and acetic acid. The material was then dehydrated in ascending grades of cellosolve, cleared in methyl benzoate and benzene and then embedded in paraffin wax (m.p. 60°-62°C). Serial transverse sections were cut at 8 µ thickness and stained by Heidenhain's Azan and
Mallory's triple stain methods. Vertical longitudinal sections were cut at 5 \( \mu \) thickness and stained by Heidenhain's Azan.