MATERIAL AND METHOD

COLLECTION

Fishes were mostly obtained alive for examination and collection of the parasites. Frequent trips were made to different places for the same. Fishes were invariably brought to the Laboratory for detailed examination.

Before dissecting the fish, it was thoroughly examined from outside for any cysts and ectoparasites. Gills, after visual examination were removed for detailed examination. Skin, gills, fins, mouth cavity and eyes were examined under magnifying glass. Eyes were carefully removed and placed in a separate container for detailed examination. Cysts, if found under skin and gills, were carefully removed and placed in separate containers. Subcutaneous musculature and body wall were examined separately for examination of any cysts.

The fish was split open ventrally and body cavity
thoroughly examined for any parasites or cysts. Various organs were next removed and kept in separate containers for detailed examination. Intestine was split open and placed in water in a petri dish to allow parasites to release from the lumen.) It was next examined under binocular microscope, its inner surface carefully scraped or gently teased with the help of a needle on brush to remove any adhering parasite. This procedure was followed in case of other organs also.

The fishes examined were preserved in 4% Formalin for identification. Regular record of collections was maintained.

**FIXATION**

Parasites collected were first of all examined alive before killing and fixing. Larger trematodes were generally fixed in Cornoy's fixative. (Absolute Alcohol 6 parts, Chloroform 3 parts and Glacial Acetic acid 1 part). The material was placed between two cover glasses or a glass slide and gently pressed while the fixative was run through.

Corrosive-sublimate-Acetic (Saturated solution of Corrosive sublimate 100 cc. and Acetic acid glacial 5 cc.) was found equally suitable for fixing the trematodes. The
material was thoroughly washed in distilled water before further processing.

For Diplozoon, Van Cleave's (1953) Fixative, as suggested by Khotenovsky (1974) was used. (85 parts of Rectified spirit - 85 - 90% Ethyl Alcohol, 10 parts of 40% Formalin and 5 parts of Acetic acid). The worms were gently washed in water to remove mucous and placed on a drop of water on a slide and gently heated to kill and relax the parasite which was kept under a cover glass. Fixative was run under it. This fixative has an advantage of quick penetration without any harmful effect on the delicate parasite. The material was next washed and transferred to 70% for preservation or dehydration. For examining unstained preparation, the material was dehydrated with several washes in 90% Alcohol and cleared in Methyl ether of Salicylic acid and mounted in Canada Balsam.

For Metacercariae of Diplostomum, Acetocarmine was directly used for fixing cum staining, as suggested by Schiqin (1968). The metacercariae being not encysted can be directly processed.

Acetocarmine: 40% Acetic acid with 4 gr. of Carmine boiled to make stock solution. For staining the material, the stain was diluted with 40% Acetic acid (1 part stain in 2 parts of Acetic acid).
STAINING

Trematodes of smaller size were generally stained with Acetocarmine. For larger specimens Harris' Haematoxylin gave satisfactory results.

**Haematoxylin:** 2 gms., Rectified spirit 50 ml., Alum sulphate 30 gms, distilled water 500 ml., Mercuric oxide (red) 1 gr., Glacial Acetic acid 5 ml. Alum dissolved in water and boiled, Haematoxylin dissolved in alcohol and two mixtures stirred; Mercuric oxide added while mixture is hot. Acetic acid is next added.

**Borax carmine:** Carmine 2-3 gms., Borax 4 gms; distilled water 100 ml., mixture boiled and equal amount of 70% Alcohol added. Mixture cooled and filtered. Borax carmine was used in case of metacercariae which were earlier treated with silver nitrate for differentiation of calcareous bodies. Material in 70% Alcohol was washed in 1% Silver Nitrate for 2-3 minutes and next thoroughly water in distilled water before proceeding further for staining and dehydration.

Acetocarmine or the other stain was always diluted before use to overstain the material for differentiation (Acid water or Acid Alcohol).
CLEARING:

Cedar-wood oil, xylool or Methyl Benzoate was used for clearing the stained and dehydrated material. The latter clearing agent was found useful for clearing larger specimens. Specimens were placed in a mixture of Methyl Benzoate and Absolute Alcohol for a few hours followed by treatment in pure clearing agent.

DRAWING AND MEASUREMENTS

Mounted preparations were drawn with the help of camera lucid. Larger specimens were drawn in parts under various magnifications. For drawing whole worms which were sufficiently large like Isoparorchis and Euclinostomum, slide projector was used.

Measurements were made with the help of eyepiece micrometer, the divisions of which were already measured with the stage micrometer (standard 1 mm. scale) under different eyepieces and objectives of the microscope.