CHAPTER V

HISTOLOGY
INTRODUCTION

The alimentary tract of teleosts has attracted considerable interest because of its diversity of form, related to diet. Therefore, the gross morphology and histology of the tract has long been a topic of investigation, either simply descriptive (Al-Hussaini, 1946; Hale, 1965; Sis et al., 1979; Clarke and Witcomb, 1980; Morrison and Wright, 1999), or related to feeding habits (Girgis, 1952; Sinha, 1976; De Silva et al., 1980)

The anatomy, morphology and histology of the digestive tract of many teleostean fish species have been studied (Cinar and Senol, 2006; Daiet, et al., 2007; Domeneghini, et al., 2002; Kuperman, and Kuzmina, 1994; MacDonald, 1987; Park, and Kim, 2001; Petrinec, et al., 2005). The digestive apparatus of fishes show marked diversity in its morphology and function. This is related to both the taxonomy and different feeding habits, as well as to body shape. The gross anatomy and histology of the alimentary tract of fish have been well documented by Smith (1989) and Domeneghin et al., (1998)

There are considerable differences in macroscopic and microscopic features and also functions of alimentary canal among fish species, although it shows some basic structural similarities. The overall gastrointestinal morphology is related to different feeding habits including the nature of the food and frequency of food intake, as well as to taxonomy, body size and shape (Cinar, and. Senol, 2006; Murray, et al., 1996). Along with the general histological structure of the digestive tract of the mucous layer also have been studied in some fish (Murray, et
The main mucus secretors in the digestive tract are goblet cells, surface epithelium of intestine and some cells in intestinal glands, if present. The fundamental similarity between the ultrastructure of the intestinal epithelium in fish and other vertebrates has been noted. In some studies it was demonstrated that there may be some differences in fine structure of intestinal epithelium in fish of various species (Kuzmina, 1978).

The stomach varies in size. Its function is to churn contained material, mixing it thoroughly with the digestive juices that it secretes. Some absorption occurs on a limited basis. Typically it is a sigmoid, highly distensible, sac with numerous folds in its inner lining. The stomach can be divided into 3 sections: cardiac (anterior), transitional (mid), and pyloric (posterior). All sections are highly muscular with the cardiac demarcating the change from the striated muscle of the anterior digestive tract to the smooth muscle occurring distally. There are a number of layers of muscle, including a muscularis mucosa with adjacent layers of connective tissue often containing large number of eosinophilic granule cells.

Oppel (1896-1897), Sullivan (1907), and Biedermann (1911), have given historical review to provide an appropriate background for the present study. Dawes (1929) described the histology of the gut of the Plaice and defined the pharynx, oesophagus, and rectum, pointing out certain changes in both cytoplasm and nucleus of the columnar epithelial cells which he associated with secretory activity. Sarbahi (1940) differentiated a caeca from a pyloric portion of the intestinal bulb and described conical cells which hang freely into the lumen of the
rectum in *Labeo rohita*. McVay and Kaan (1940) investigated the goldfish *Carassius auratus* and discerned the changes which occur in the cells of the intestinal epithelium. Attempts to correlate the structure of the alimentary tract with the feeding habits of the fish have been reviewed by Al-Hussaini (1945).

Many descriptive reports have been written on the teleost fish intestine. The morphology of the gut has been studied with both the light and electron microscope, principally in species that are important sport (Reifel and Travill, 1977, 1978, 1979), food (Sis *et al.*, 1979; Clarke and Whitcomb, 1980; Kuperman and Kuzmina, 1994; Murray *et al.*, 1996) and aquarium fish (Hale, 1965). These studies reveal that the teleostean intestine, though basically simpler than those of higher vertebrates displays numerous species variations related to diet as well as phylogeny and body form (Kuzmina, 1978; Kapoor *et al.*, 1975).

The histology and ultra-structure of the oesophagus and stomach of the tilapia, an omnivorous warm-water teleostean fish that is a polyhybrid species of notable economic importance has been described by Gargiulo *et al.*, (1996a).

The work done on the histology of the alimentary canal of fishes of Europe and America has been summarized by Dawes (1929). Valuable work has been done by Al-Hussaini (1946, 1947, 1949 a, 1949 b) on the histology, cytology and physiology of the gut of teleost fishes from the Red Sea.

The aim of the present study is to establish the normal histological structure of alimentary tract of the three fishes i.e *Channa punctatus* (carnivorous fish) and Tilapia (omnivorous fish) and *Cyprinus carpio* (herbivorous fish) have been
carried out. The information gathered could offer baseline knowledge for future studies on the digestive tract whereas being relevant for understanding the nutritional physiology of freshwater fishes.
MATERIALS AND METHODS

Live specimens of *Channa punctatus*, *Cyprinus carpio* and *Oreochromis mossambicus* at Kaigaon toka Dist Aurangabad (M.S) India, were obtained from fishermen. These fish samples were brought to Fishery Research laboratory, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for histological studies. Pieces of stomach and intestine of the alimentary canal were fixed in Bouin's fluid for 16 to 18 hours as the tissue undergoes autolysis rapidly after death immediate fixation is essential. The fixed material was transferred and processed through ascending grades of alcohol, dried in a wax miscible agent and impregnated in wax (M.P 58º to 60ºC). Sectioning was carried out on a rotary microtome at 6µm. Sections were floated on warm water at 48ºC and mounted on chemically cleaned slides coated with egg albumin. The mounted, unstained sections were dewaxed in three stages of xylene at 1 minute each and stained with standard haematoxylin and eosin stain. Staining was carried out using haematoxylin and eosin staining technique (Bullock, 1978). In this method nuclei of cells are stained by haematoxylin and cytoplasm is coloured by eosin.

Stained mounted sections were examined under light microscope for good ones, and the selected slides were used for photomicrography.

RESULTS AND DISCUSSION

*Channa punctatus*
Stomach

The stomach of *Channa punctatus* consists of the cardiac and the pyloric region. It was observed that the histological section of stomach of *Channa punctatus* consists of the outer layer serosa which is 101.5 μm in thickness, then mucosa layer which is 187.4 μm in thickness, and the inner most submucosa layer which is 9.0 μm in thickness.

The mucosa of the stomach is formed by a single layer of columnar epithelium with folds. The mucosal folds of *Channa punctatus* are shallow. There are numerous gastric pits formed by the invaginations of the mucosal layer into the lamina propria of stomach. The mucosa of lamina muscularis was inconspicuous, a few muscular fibers are present. The submucosa is formed by loose connective tissue. Serosa is the outer layer and it is 101.5 μm in thickness (Plate 13a).

Intestine

The histological section of intestine of *Channa punctatus* shows that it consists of the outer layer serosa which is 24.0 μm in thickness, then mucosa layer which is 74.1 μm in thickness, and the inner submucosa layer which is 3.2 μm in thickness.

The mucosal surface in the intestine has numerous elongated and deep folds lined by simple tall columnar cells and goblet cells. The muscularis mucosa is absent. The tunica muscularis shows two distinct layers of smooth muscles, inner circular and outer longitudinal layer of muscles. Serosa is the outer layer and it is 24.0 μm in thickness (Plate 13b).
Cyprinus carpio:

Stomach

It is observed that stomach is absent in *Cyprinus carpio* (Chapter IV).

Intestine

The histological section of intestine of *Cyprinus carpio* shows the outer layer serosa which is 87.2 µm in thickness, then mucosa layer which is 106.5 µm in thickness, and the inner submucosa layer which is 6.2 µm in thickness.

Histological section of intestine shows tunica mucosa with a loose connective tissue lamina propria, tunica submucosa, tunica muscularis (inner circular and outer longitudinal smooth muscles) and tunica serosa layers. The muscularis mucosa, submucosa, between the lamina propria and mucosal tubular glands are absent in the tunica mucosa. A thick layer of connective tissue, the stratum compactum, separates the mucosa from submucosa. The mucosal surface has numerous projections (villi), decreasing in length towards the posterior intestine and are lined by simple epithelium madeup of single-layered tall columnar cells. Serosa is the outer layer and it is 87.2 µm in thickness (Plate 13a and b)

Oreochromis mossambicus

Stomach

The histological section of stomach of *Oreochromis mossambicus* shows the outer layer serosa which is 30.4 µm in thickness, then mucosa layer which is 55.5 µm in thickness, and the inner submucosa layer which is 3.2 µm in thickness.
The section of stomach shows wide well defined long folds and connective tissue covered with columnar cells. In between the folds alveolar glands open at the bottom of the lamina propria. The lamina propria contains tubular glands followed by submucosa and narrow tunica muscularis. The stomach of *Oreochromis mossambica* show wide submucosa with thick bundles of collagen fibers beneath the smooth muscles and outer serosa. The mucosa is composed of a single layer of epithelial columnar cells. The muscularis is made up of two layers, an inner layer of circular muscles and an outer layer of longitudinal muscles. The section of stomach shows number of mucous cells. The tunica muscularis appears as scattered longitudinal bundles in between the collagen fibers. Serosa is the outer layer and it is 30.4 µm in thickness (Plate 14a).

**Intestine**

The histological section of intestine of *Oreochromis mossambicus* shows the outer layer serosa which is 15.1 µm in thickness, then mucosa layer which is 47.1 µm in thickness, and the inner submucosa layer which is 2.6 µm in thickness.

The epithelium lining of sub mucosa consist of three common types of columnar cells. Each columnar cell is long and slender.

The tunica propria, an extension of sub mucosa runs into the folds. The submucosa is a thin stratum of the connective tissue fibers.

The muscularis consist of an inner circular and outer longitudinal layer of muscles. Muscle fiber is smooth and is bound together by strands of connective tissue. The serosa is the thin outer covering.
The intestine of *Oreochromis mossambicus* was observed to be long and thin walled with varying diameter that was histologically uniform except in anterior portion separated from the central part with intestinal mucosa developed longitudinal folds and high villi which are 323.7 µm thick. The anterior portion of intestine of *Oreochromis mossambicus* is characterized by mucosal folds which appear to be swollen and bulging towards the lumen. The small intestine of *Oreochromis mossambicus* is characterized by narrow tunica muscularis. The mucosal epithelium of the entire intestine has simple columnar cells with goblet cells. The number of goblet cells increases towards the posterior part of the intestine. The thick circular smooth muscles constrict the lumen. It probably acts as a sphincter. The serosa is wide and arranged circularly (Plate 14b).

Similar results are observed by Rahimullah (1945), Dharmarajan (1936), Vanajakshi (1938), Sarbahi (1940) and Mohsin (1944-46) while studying the histology of alimentary canal of certain teleost fishes.

According to Kapoor (1957, 1958b); Chaudhary and Khandelwal (1961); Sehgal (1966); Khanna and Mehrotra (1971); Sinha (1976) and Sinha and Maitra (1975c, 1976) intestinal bulb is provided with high mucosal folds which are the characteristic features of the intestine proper.

According to Maitra *et al.*, (1989) gastric glands are present in the intestinal bulb.

Similar result are observed by Banan Khojasteh *et al.*, (2009) on rainbow trout showing that the basic organization of intestinal wall formed by tunica
mucosa with a loose connective tissue lamina propria, tunica submucosa, tunica muscularis (inner circular and outer longitudinal smooth muscles) and tunica serosa layers.

Recently Raji et al., (2010) described the histological features of digestive tract in carnivorous fish *Serrasalmus nattereri* (piranha), were he observed the mucosa of the stomach was formed by simple columnar epithelium with folds and gastric pits formed by the invagination of the mucosal layer into the lamina propria. The mucosal surface of the intestine has numerous folds lined by simple tall columnar cells, along with goblet cells.

Malihezaman Monsefi et al., (2010) observed in *Aphanius persicus* that the outer end of the mucosa consists of numerous villi that show simple high columnar epithelium. It is covered with simple columnar epithelium in villi and short simple branched tubular glands.

Raji et al., (2010) described the histological features of digestive tract in fresh water fish *Clarias batrachus*. He observed that the mucosa of the stomach of Walking catfish included the cardiac, fundus and pyloric region. The mucosa of the cardiac and fundus were formed by a single layer of columnar epithelium with folds.

Although there are great differences in the histology of intestinal tract among different fish species, gut of *Channa punctatus*, *Cyprinus carpio* and *Oreochromis mossambicus* is made up of tubular portion of the digestive tract (stomach, pyloric caeca, intestine and has four layers mucosa, submucosa,
muscularis and outer most serosa. These layers are present in different thickness throughout the gastrointestinal tract (Kumar and Tembhre, 1996). According to the results obtained from present study, the intestine of *Channa punctatus* contains numerous goblet cells in the mucosa, whereas goblet cells are absent in the mucosa of *Cyprinus carpio* and *Tilapia mossambica*.

According to Banan Khojasteh (2009) the intestinal wall of rainbow trout is composed of the tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. Intestinal mucosa shows many villi and a simple columnar epithelium is associated with goblet cells and intra epithelial lymphocytes.
Plate 13

a) Transverse section of intestine of *Channa punctatus* showing (SE) serosa, (MM) mucosa, (LP) lamina propria, (E) epithelium and (LU) lumen.

b) Transverse section of stomach *Channa punctatus* showing (SE) serosa, (MU) mucosa, (SM) sub mucosa (LP) lamina propria, (E) epithelium and (LU) lumen.
Plate 14

a) Transverse section of large intestine *Cyprinus carpio* showing (SE) serosa, (MM) mucularis, (LP) lamina propria, (E) epithelium and (LU) lumen.

b) Transverse section of small intestine *Cyprinus carpio* showing (SE) serosa, (MU) mucosa, (SM) sub mucosa (LP) lamina propria, (E) epithelium and (LU) lumen.
PLATE - 14

a

b
Plate 15

a) Transverse section of stomach of *Oreochromis mossambicus* showing (SE) serosa, (MM) mucosa, (LP) lamina propria, (E) epithelium and (LU) lumen.

b) Transverse section of intestine of *Oreochromis mossambicus* showing (SE) serosa, (MM) mucosa, (LP) lamina propria, (E) epithelium and (LU) lumen.
PLATE - 15

a

b