Gut Histology of Malaysian River Catfish, *Mystus nemurus* (C&V) Larvae

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Abstract: For the successful weaning of *M. nemurus* larvae, the development of gut histology was observed. The eggs hatched two days after firtelization (2 daf) and most of the larvae hatched within 2-4 daf. The commencement of external feeding start on the 4 days post-hatch (dph). Fish larvae are charactrized by digestive system and diets that differ from adults. Larvae undergo a pattern of trophic ontogeny, changing with inceasung size, and these changes result in differences in digestive requierments. The histological development of the gut of *M. nemurus* larvae were investigated from hatching untile 21dph using a compound microscopy. During the yolk sac period, the gut is a simple, straight, undifferntiated tube thrghout its length.by 4-5 dph the gut differentiated to the oesophgus, stomach, and intestine. At first feeding, the larval gut is functional, but is structurally and functionally less complex than that of adults.By the 13 dph the larvae attained four tissue layers arrangement.
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Key words: Malaysian river catfish; larvae; gut histology; *Mystus nemerus*.

1. Introduction

Malaysian tropical catfish, *Mystus nemurus* (C & V) or " baung" as locally known, is an edible species preferred by all ethnic races in Malaysia (Khan *et al*., 1990). Eight species of *Mystus* found in Malaysian waters are *Mystus nemurus*, M. *nigriceps*, *M. planiceps*, *M. micranthus*, M. *wyckii*, *M. guhio*, *M. wolffii* and *Mystus bimaculatus* (Lim *et al*., 1993). *Mystus nemurus* is the most popular, and is the largest of local Mystides (Smith, 1945). The species has a wide distribution ranging from the East Indies to Asiatic mainland in Peninsular Malaysia, Indochina and Thailand. The fish is a bottom feeder and feeds extensively on a wide range of food items which include teleosts, crustaceans, benthic invertebrates and detrital materials (Khan, 1987). *Mystus nemurus* are monogamous and sexes cannot be differentiated in fishes less than 18 cm in sizes (Khan *et al*., 1990).

Recently, the interest has been growing rapidly in both its intensive and extensive domestication (Khan, *et a*l. 1990). However, inadequate seed supply coupled with relatively high fingerling prices limit its production. Large scale of rearing of *Mystus nemurus* larvae has yet to be refined in terms of husbandry techniques and nutritional requirements of the larvae.

Fish larvae are the smallest autonomous active feeding vertebrates (Wieser, 1995). The nutrition of larval fish is one of the dominant factors influencing their survival in larviculture (Jancaric,1964 cited in Kolkovski *et al*., 1993).There is still much to be discovered about the way in which fish larvae utilize the food present in an aquaculture environment.

Fish larvae usually are very different from their adult counterparts by having simpler digestive systems, and the development of alimentary canal of fish larvae is morphologically, histologically and physiologically less elaborate than that of adult fish (Govoni *et al*., 1986 and Osman, *et al*. 2008). However, there is a diverse diversity in the morphology and histology of the gut between larvae and adult fish of the same species and between different species of fish. Despite simple characteristics of gut, the larvae require suitable and sufficient food to grow better during this period (Watanabe and Kiron, 1994).

Although live food are still the most reliable, artificial diets have been used in attempts to establish the nutritional requierments of larval fish and to obtain cheap, well defined diets for their intensive rearing (Munilla-Moran *et al*., 1990). At weaning time, the gut of fish larvae is simple and the digistive glands and enzyme production are not fully developed (Holt,1992; Kamali, *et al*. 2006 and camacho, *et al*. 2010). Hogendoorn (1980) and Watanabe and Kiron (1994) reported that poor growth and survival rate are ussually shown when the larvae are exclusively fed on dry diet.

M. nemurus adult fish is an omnivorous, europhagus feeder and indivers ecological regions (Khan, 1987). As a "new" indigenous species in Malaysian aquaculture, much information related to the feeding at larval stage is still unknown. The present study was conducted to observe the histological changes of the gut of *M. nemurus*. This information will provide a good understanding of the fish larval digestive system that could facilitate the determination of suitable larval feed and weaning time.

2. Materials and Methods

The samples for histological study were fixed in Bouin's fixative for 24 hour, then wahed and stored in 70% alcohol. The tissue processing was done by an automatic tissue processor (Shandon Citadel 1000) for 22 hrs (Table 1) and subsequently embedded in paraffin wax. five species/samples were put into blocking and cooling for at least 10 mins. Sample blocks were placed on a microtome holder, trimmed and sectioned at 5 μm thickness. Sections were spread in water bath and collected onto clean glass slides. The slides were dried at 40° C on ahot plate for 10 mins. Slides were stained with haematoxylin and eosin (Harris, 1972) as shown in Table 2. Dry slide were mounted with neutral mounting medium. Samples are then viewed under a compound microscopy (Zeiss Axioskop 50).

Table (I): Tissue processing. by an Automatic Tissue Processor.

	Solution	Time
1	70% Acohol	2 hrs
2	90% Alcohol	2 hrs
3	Isopropyl Alcohol I	1 hrs
4	Isopropyl Alcohol II	1 hrs
5	Isopropyl Alcohol III	2 hrs
6	Isopropyl Alcohol IV	2 hrs
7	Isopropyl Alcohol V	2 hrs
8	Chloroform I	5 hrs
9	Chloroform II	30 mins
10	Paraffin Wax I	3 hrs
11	Paraffin Wax II	1h-30 mins
	Total time	22 hrs

Table. **(2)**: H & E staning procedure.

	Solution	Time
1	Xylene	2 mins
2	Xylene	2 mins
3	Alcohol 100%	2 mins
4	Alcohol 100%	2 mins
5	Rinse in water	30 sec
6	Hemotoxylin	15 mins
7	Rinse in water Acid Alcohol%	3 dips
8	Rinse in running water	7 mins
9	Alcohol 100%	2 mins
10	Alcohol 100%	2 mins
11	Eosin	5 mins
12	Alcohol	2 mins
13	Alcohol	2 mins
14	Alcohol	2 mins
15	Xylene	2 mins
16	Xylene	2 mins

3. Results

Newly hatched *M. nemurus* larvae have straight simple, undifferentiated tube-like gut was dorsally located to the yolk sac. The gut was histologically undiffrentiated along its length. The anus was opened at 3 days post harvest (dph). At 4-5 dph, the digestive tract was diffrentiated into the oesophagus, stomach, and intestine. On 13 dph it attained the four tissue layers arrangement in adult vertebrates which is a characteristic of the digestive tract lining.

Oesophagus

At 5 dph, the oesophagus (Fig.1) can be distingushed from other regions by the appearance of epithelial cell with numerous goblet cells(G). The layers of the oesophagus consist of lumen, mucosa(M), submucosa (S), muscularis (MU) and serosa(SE). The muscularis was formed from the striated circular muscle. The goblet cells were also arranged in the posterior and mid region of the epithlial fold. The development of the mucosal was first observed on the 7 dph (Fig 2). In various stage of development, the mucosa was not well separated from the submucosa. Both lamina propria and submucosa were made up of fibrous conective tissue. The mucosa of the esophagus cosist of stratified squamous epithelium with goblet cells and this became fully developed on 7 dph. From 7 dph, the epithelium fold increased in length and size with the increase in the number of goblet cells. These cells were scttered all over epithelium witn the growth of larvale and increased in fold length (Fig. 3). On 13 dph, the musclaris has two layers; the inner longitudinal muscle layer and outer circular layer. The longitudinal muscle layer was connected to the submucosa more than to the musclaris. Four tissue layers of the oesophagus appeared on the 13 dph (Fig. 4). Some blood vessels appeared in submucosa and serosa at 15 dph (Fig. 5).

Stomach

At 5 dph, the stomach became differentiated from the other region of the gut. The mucosa which was attached to the connective lamina propria was formed by an epithelium. The epethelium was made up of columnar cells and arranged in a single layer (Fig 6). By 9 dph (Fig. 8-9)these cells began to differentiate into glandular cells and became well developed by 13 dph(Fig. 10).

Anterior stomch

On 5 dph the larval gastric epithelium formed small folds. The mucosa consisted of simple columnar cells arranged in single layer (Fig. 6). A number of gastric glands lied between the tunica propria and the gastric epithelium. Submucosa consist of fibrous connective tissue and muscularis formed in circular layers. The serosa comprised a thin layer of loose connective tissues. On the 7dph, the

stomach (Fig. 7) appeared to be more fully developed and the thickness of the muscularis increased. The fold and numbers of gastric glands increased with growth.
In this region, there was no gastric gland and the mucosa was thinner than that of the fundic stomach. The lamina propria was made of thick connective tissue. By 5 dph, the muscularis was formed in thick circular layers. The thickness of the muscularis increased on the 7 dph. On 9 dph, the lamina propria (L) appeared to be more distinguished on the posterior region of the stomch (Fig. 9). The serosa was composed of a thin layer of connective tissue fibers. On 13 dph, the stomch had four tissue layers and some blood vessels appeard in the serosa and submucosa.

Intestine

The intestinal region became differentiated from the the rest of the digestive tract by the presence of tall simple columnar cells with goblet cells at 5 dph (Fig. 11). The muscularis was made of a smooth circular layer. The mucosa of interior intestine consisted many unbranched villi, each villus contains two types of cells, the simple columnar epithlial cells with a distinct centrally basal nucleus and goblet cells (Fig. 12-13). The folds of the mucosa of posterior intestine were less branching. More goblet cells were located on the anterior part. The circular layer was more distinguished on 13 dph (Fig. 14) and the intestine became fully developed.

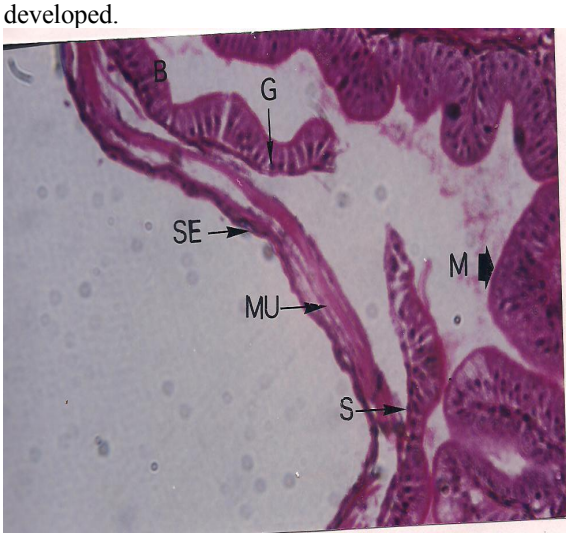

Fig. 1: Longitudinal section of the oesophagus on 5 dph. (Bouin's; H&E; X320).

M=Mucosa Mu=Muscularis S=Submucosa SE=Serosa G=goblet cell.

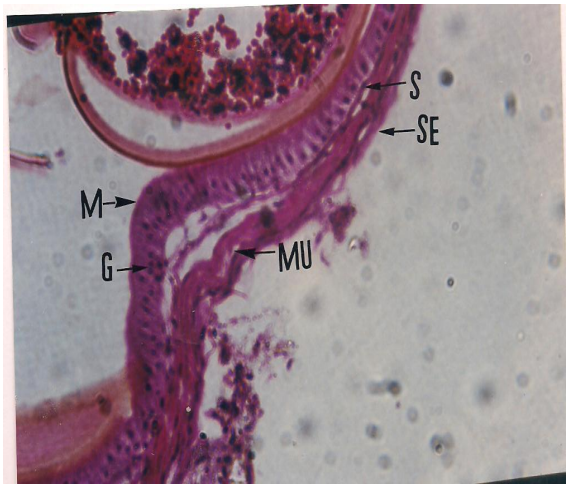

Fig. 2: Longitudinal section of the oesophagus on 7 dph. (Bouin's; H&E; X285).

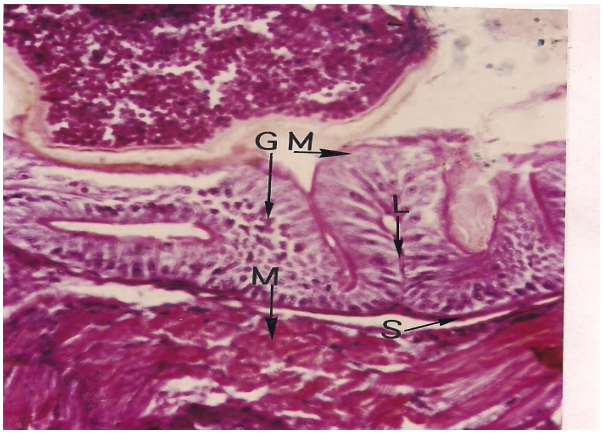

Fig. 3: Longitudinal section of the oesophagus on 9 dph. (Bouin's; H&E; X322).
L= Lamina propria

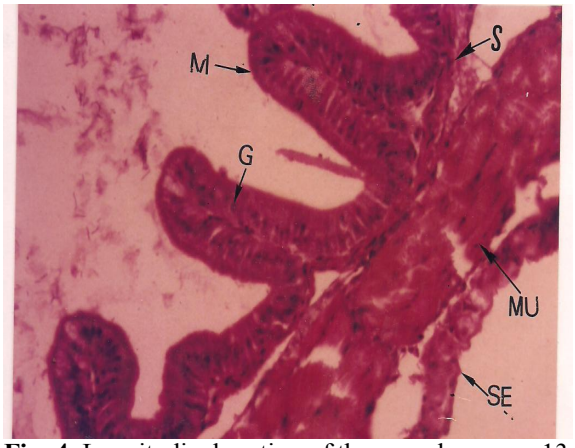

Fig. 4 :Longitudinal section of the oesophagus on 13 dph. (Bouin's; H&E; X311).

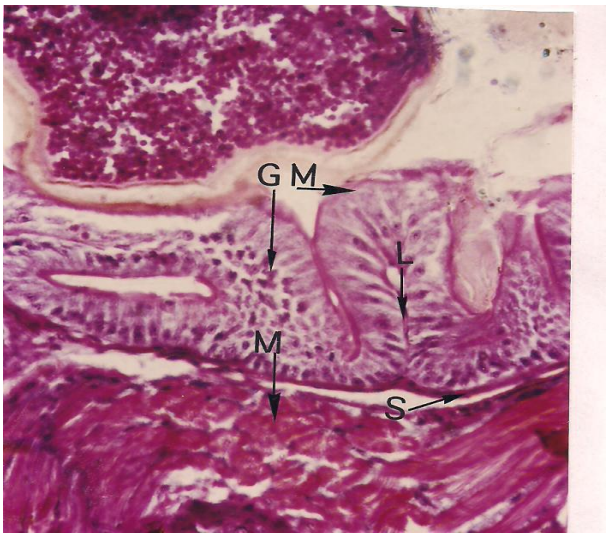

Fig. 5 :Longitudinal section of the oesophagus on 15 dph. (Bouin's; H&E; X281).

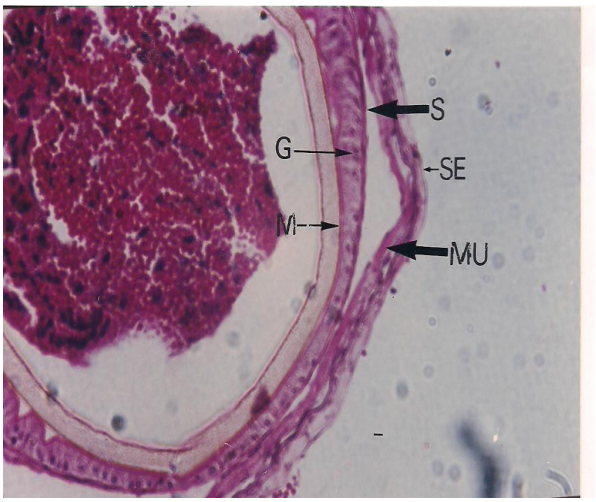

Fig. 6: Longitudinal section of the stomach on 5 dph. (Bouin's; H&E; X285)
GS= Gastric gland

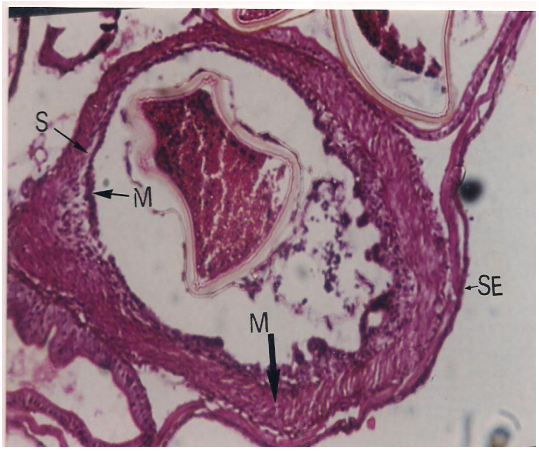

Fig. 7:Longitudinal section of the stomach on 7 dph. (Bouin's; H&E; X400).

4. Discussion

The development of the digestive system of *M. nemerus* larvae during the yolk sac stage similar to most larval fish (Mahr *et al*., 1983; Ferraris *et al*., 1987; Verreth *et al*., 1992; Zambonine and Cahu, 2001; Guimaraes, *et al*. 2009 and Ramezani-Fard, *et al*. 2011) probably because larval fish require only the most rudimentary system for yolk resumption. This system would be developed to adapt to the feeding habits of the fish during different development stages (Ferraris *et al*., 1987; Klassen and Peake, 2008 and Guimaraes, *et al* 2009). The differentiation of *M. nemerus* gut occurred after the yolk completely resorbed in 3 dph.

An early development of oesophagus may be important at the onset of the first feeding and is essential for other functions such as osmoregulation. If the function of esophagus is primarily digestive, the mucus may facilitate the movement of food and the mucosal folds would allow for extension during food consumption or increase the area for digestive activities. The developmet of oesophagus folds increased from 7 dph with the larval growth. This may be due to the start of exogenous feeding. Increase in the luminal area of the oesophagus is due to development of coplex folds (Meister *et al*., 1983).

Taste buds were not detected in the oesophagus of *M. nemerus* larvae. This suggested that the larvae may not be as selective on their food as other species (Reifel and Travill, 1977; Sis *et a*l., 1979; Hirji, 1983). The arrangment of inner longitudinal and outer circular layers of the musclaris in the oesophagus of *M. nemerus* (13 dph) different from the rest of the digestive tract similar to those of other larval fish (Lien, 1967; Ferraris *et a*l., 1987 Guimaraes, *et al*. 2011). In *M. nemerus* larvae, the inner longitudinal muscle seemed to be embedded in the submucosa similar to that seen in the perch larvae (Hirji,1983). In various stages of development, the mucosa was not well separated from the submucosa because the lamina propria cannot be differentiated from submucosa.

The development of the stomach occurred following the complete yolk resorption 4-5 dph, and continued to developed by 7 dph. The differentiaton of both fundic and byloric region was similar to those of most fish larvae (Tanaka, 1973; Lopez, 1982; Verreth *et al*., 1992 and Osman, *et al*. 2008). The appearance of the gastric glands on 7 dph indicated the presence of gastric enzymes.

The mucosa of the anterior or fundic stomach composed primarily of two functional layers. Fundic stomach is the only region in the digestive tract where non-acidic mucopolysaccharides are aecreted (Kapoor *et al*., 1976). The authors noted that the mucopolysaccharides may serve to protect these cells from auto-digestion and enzymes produced by the gastric glands. The number of gastric glands increased in *M.nemurus* with the larval growth. This means the secretion of enzymes from this region should also increase with larval development.The development of the posterior or pyloric stomach was clearly observed on 7dph, suggesting that the onset of mechanical

digestion may be related to the beginning of exogenous feeding. The mucosal cell lining on the lumen of the pylorus was much thicker than those in the fundic stomach. The absence of gastric gland in the pylorus of milkfish may be due to the pylorus may not conteribute enzymes or hydrochloric acid for further chemical digestion but may only serve to facilitate food digstion by mechanical means (Reifel and Travill, 1977).

The appearance of pepsin in *M.nemurus* larvae was only observed from 4dph i.e.at the start of exogenous feeding (Kamarudin, 1999). The absence of structure and function of a fully developed stomach during the first 3 days of exogenous larval feeding of African catfish, *Clarias gariepinus* (Burchell) which will limit the amino acid availability of protein sources commonly used in fish feeds (Verreth *et a*l., 1992).

Since the earliest weaning time of *M.nemurus* larvae founf by Eguia (1998) coincides well with the onset of gastric acid secretion, the poor survival and growth rates of *M.nemurus* larvae when fed with artificial diets at earlier feeding stages seem to be directly related to the absence of afunctional stomach and suficient pepsin digestion.

The intestine, which starts from the pyloric sphincter, is the longest single part of an alimentary canal. In *M.nemurus* larvae, there was no pyloric caeca present. The epithelium of the intestine differed from that of the stomach and the histological characteristics of intestinal epithelial cells were very similar to many other fish larvae (Ozaki, 1965; Yamamoto, 1966; Kapoor *et al*., 1976 and Carmona, *et al*. 2010). In *M.nemurus* larvae, the intestinal epithelium has numerous microvilli at the luminal surface forming the brushborder, and the intestinal mucosa formed from a simple columnar epithelial cells. The appearance of brushborder which is particularly rich in enzymes would facilitate the digestive process (Welsch and Storch, 1976). The four layers of intestine appeared on 13 dph and this pattern indicated the end of the larval stages Chainabut *et al*., (1991).

The results of this study indicated that *M.nemurus* larvae are able to accept and digest artificial feeding diets as early as at 5-7 dph i.e at the earlier developing stage of the stomach and this should lead to successful weaning. The differentiation that occurred soon after hatching would allow an early exogenous feeding. The appearance of four tissue layers of the gut on the 13 dph indicated the end of the larval period of *M.nemurus*.

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