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## Development of digestive tract of hilsa shad, *Tenualosa ilisha* (Hamilton 1822) fry in the lower Meghna estuary, Bangladesh

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### Abstract

This study was designed to describe the morphological development of the gut of Hilsa, *Tenualosa ilisha* from yolk sac to early juvenile stage. Samples were collected from the lower Meghna River, Bangladesh and laboratory analysis was conducted at Universiti Putra Malaysia, Malaysia. Digestive tract was transparent and straight tube in structure at yolk sac stage. After yolk sac absorption, the digestive tract was differentiated into mouth opening, buccopharyngeal cavity, esophagus, stomach, intestines and rectum. Development of digestive tract was almost completed during pre-flexion stage. Gut loop was clearly observed at post flexion stage. Digestive tract was equal to more than three-quarter of standard length during larval development. The percentage of gut length compared with the standard length were  $84.87 \pm 4.87$  %,  $85.64 \pm 4.47$  %,  $82.29 \pm 6.18$  %,  $77.99 \pm 4.98$  %,  $74.02 \pm 3.27$  % at yolk sac, pre-flexion, flexion, post-flexion and juvenile stages, respectively. There was a strong linear relationship between the gut length and standard length ( $R^2 = 0.97$ ). This is the first report on morphological changes of gut and its development of *T. ilisha* larvae, which might be very useful information for aquaculture development of *T. ilisha*.

**Keywords:** *Tenualosa ilisha*, larvae, gut development, juvenile, Meghna river, Bangladesh

### Introduction

*Tenualosa ilisha* is commonly known as Hilsa, belong to the subfamily Alosinae of family Clupeidae. It is found in rivers, estuaries and marine environments. Hilsa is an important commercial fish species in the Indo-Pacific region, especially in Bangladesh, India and Myanmar<sup>[7]</sup>. The annual global average catch shares of Hilsa *Tenualosa ilisha* of Bangladesh increased rapidly from 74.5 % during the 2010 – 2015 periods<sup>[13]</sup>.

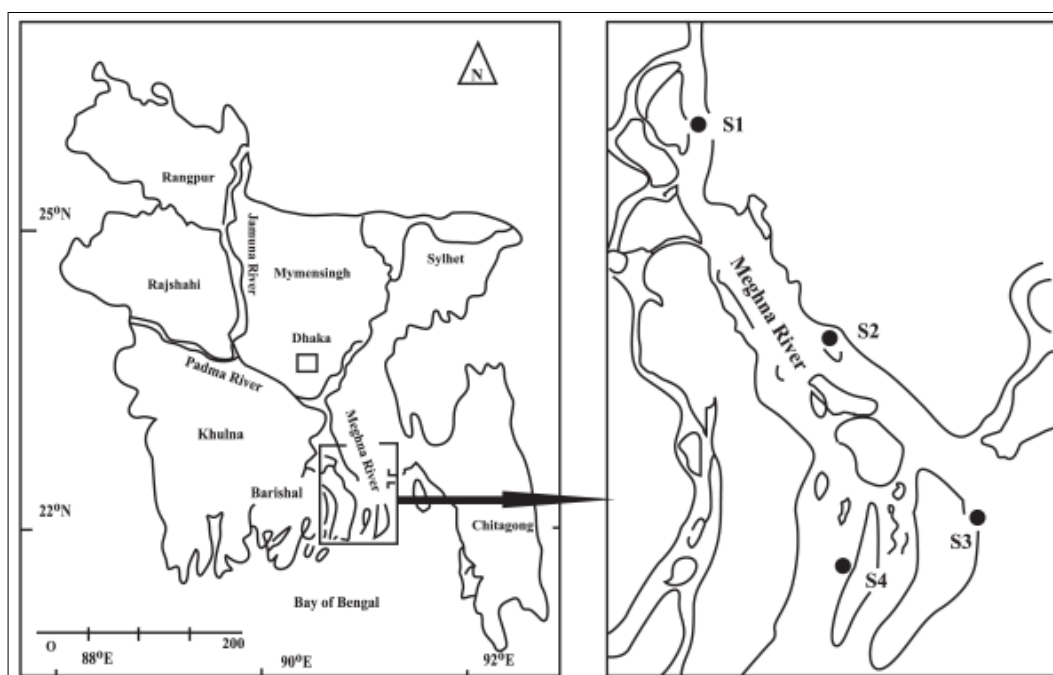
Digestive tract of fish larvae can be divided into buccopharynx, esophagus, stomach, intestine, rectum and anus<sup>[6]</sup>. The buccopharynx was developed as a thin layer stratified epithelium which later made up of mucous cells and taste bud. The formation of buccopharynx took place 3-5 day after hatching (DAH) in *Mystus nemurus* (Valenciennes, 1840)<sup>[6]</sup>, 3 DAH in *Scomber japonicus* (Houttuyn, 1782)<sup>[12]</sup> and 2-5 DAH in *Tor tambroides* (Bleeker, 1854)<sup>[14]</sup>. At hatching, the digestive tract appeared as a straight tube attached dorsally to the large bi-lobed yolk sac and the mucosa was composed of an undifferentiated simple cuboidal epithelium. At 2 DAH, the cells began to differentiate and a few goblet cells were observed among the epithelium in *T. tambroides*<sup>[14]</sup>. Esophagus is a tube like structure after buccopharynx. Its start to fold when the fish takes exogenous feed and the goblet cells was observed to produce mucus. Stomach was appeared at 3 DAH in *S. japonicus* larvae<sup>[12]</sup>. Intestine is the longest part of digestive tract. In the case of *T. ilisha*, the intestine was differentiated at 3 DAH (Kulkarni 1950). In *T. tambroides* juvenile, at 2 DAH, the intestine was began to appear from the primordial cuboidal cells (Ramezani-Fard *et al.* 2010)<sup>[14]</sup>. In *M. nemurus*, the development of intestine started from 3 DAH and continued the process till the larvae reached juvenile stage. In *S. japonicus*, the development of digestive tract was started from 3 DAH (Park *et al.* 2015)<sup>[12]</sup> and 4-5 DAH in *M. nemurus* (Hag *et al.* 2012)<sup>[6]</sup>. Rectum was appear on 1 DAH in *S. japonicus* (Park *et al.* 2015)<sup>[12]</sup>, while it took 4-5 DAH in *M. nemurus* (Hag *et al.* 2012)<sup>[6]</sup>.

There are mainly two accessory glands in fish digestives system, one is liver and another one is pancreas. The liver appeared during the yolk sac stage and it is developing within the

vacuole of the gut on 3 DAH in *M. nemerus* larvae (Hag *et al.* 2012) [6]. On 1 DAH, the liver cells were composed of small basophilic polygonal cells with a spherical nucleus along the abdominal cavity in *T. tambroides* (Ramezani-Fard *et al.* 2010) [14]. The pancreas was distinguishable from the liver at 4 DAH in *T. tambroides* (Ramezani-Fard *et al.* 2010) [14]. Pancreas grew between swim bladder and the middle part of intestine on 3 DAH in *M. nemerus* larvae (Hag *et al.* 2012) [6]. However, there was no report available on gut morphological development of Hilsa larvae except mouth morphological development (Riar *et al.* 2018) [15]. Therefore, this study was designed to evaluate the morphological development of the gut of Hilsa from yolk sac to early juvenile stage in lower Meghna River, Bangladesh.

## Materials and Methods

The larvae and juveniles were collected from four different sampling points in the lower Meghna River from August 2016 to January 2017 on weekly basis. The specific location of the sampling stations were Chandpur (23°08'42" N, 90°64'38" E), Ramgati (22°68'69" N, 90°93'22" E), Hatya (22°28'34" N, 91°12'45" E) and Monpura (22°26'64" E, 90°96'23" E) (Fig 1). Three different types of nets were used for sampling such as bongo net (400 µm mesh size), plankton net (200 µm mesh size) and mosquito net (500 - 600 µm mesh size). All nets were operated with surface tow. Fish larvae were sorted out from other zooplanktons using a dissection microscope. The sorted larvae and juveniles of *T. ilisha* were differentiated based on common distinguished characteristic features of Clupeiformes (Leis and Trnski 1989; Leis and Carson-Ewart 2000; Silva *et al.* 2010) [10, 9, 16].



**Fig 1:** Four Sampling stations. Sampling points are shown by dot (.)

The morphological development of the gut during early larval stage was observed from yolk sac to early juvenile. Total collection of Hilsa larvae was 100. Among them, yolk sac (YS) larvae = 13, pre-flexion larvae (PF) = 15, flexion larvae (FL) = 24, post-flexion larvae (POF) = 24 and juvenile larvae (JV) = 24. The measurement of SL and GL was taken using Keyence Digital Microscope (VHX-500) under 20-30X. The standard length (SL) and gut length (GL) relationships were analyzed. The line drawings of the gut were prepared from the images generated from the observation of microscope. The water quality parameter was studied using FF2 hach kit, digital water quality multi parameter (HQ40D) and secchi disk for transparency check. For statistical analysis, all the

slopes obtained by the linear equation were tested using simple t-tests (Zar 1974) [18].

## Results

### Habitat parameters

The results of water quality parameters in the study areas are given in Table 1. The range of water quality parameters were 17 - 33 °C of air temperature, 17 - 32 °C of water temperature, 11 - 90 cm of transparency, 4 - 6.5 mgL<sup>-1</sup> of dissolved oxygen, 10 -29.4 mgL<sup>-1</sup> of free CO<sub>2</sub>, 7.5 - 8.5 of pH, 42 - 121 mgL<sup>-1</sup> of total alkalinity, 56 - 142 mgL<sup>-1</sup> of total hardness and 0 - 2 ppt of salinity (Table 1).

**Table 1:** Monthly habitat parameters of different sampling stations in the lower Meghna River between August 2016 and September 2017

Parameters	Chandpur (Mean ± SD)	Ramgati (Mean ± SD)	Monpura (Mean ± SD)	Hatiya (Mean ± SD)
Air Temp (°C)	25.66 ± 6.08	25.91 ± 6.10	26.41 ± 5.91	25.41 ± 5.29
Water Temp (°C)	24.50 ± 5.28	23.91 ± 5.58	24.41 ± 5.60	23.75 ± 5.41
Transparency (cm)	43.00 ± 25.17	24.8 ± 15.79	25.83 ± 8.01	28.11 ± 16.45
Dissolved Oxygen (mgL <sup>-1</sup> )	5.24 ± 0.88	5.42 ± 0.74	5.81 ± 0.58	5.28 ± 0.40
Free CO <sub>2</sub> (mgL <sup>-1</sup> )	18.75 ± 3.46	16.09 ± 3.53	16.58 ± 3.47	17.58 ± 6.81
pH	7.91 ± 0.20	7.83 ± 0.40	7.87 ± 0.20	7.75 ± 0.27
Total Alkalinity (mgL <sup>-1</sup> )	83.16 ± 14.71	100 ± 17.73	93.5 ± 19.33	95.66 ± 20.43

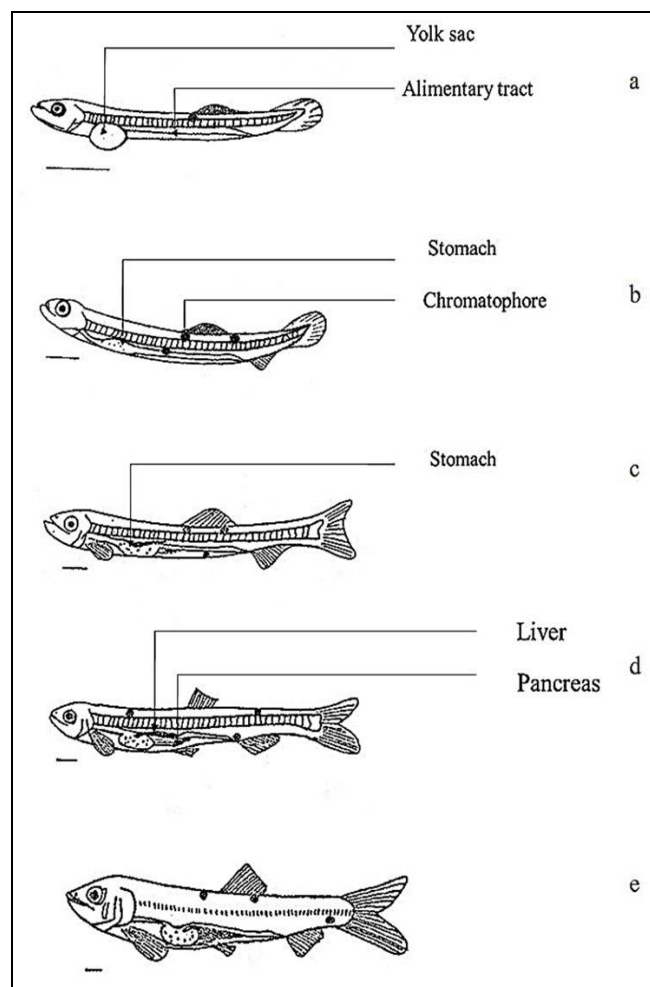
Total Hardness (mgL <sup>-1</sup> )	77.66 ± 12.92	389.82 ± 255.33	82.5 ± 12.77	255.33 ± 201.16
Salinity (ppm)	00	4.40 ± 2.05	1.14 ± 0.78	2.78 ± 1.36

### Yolk sac larvae

Yolk sac stage was considered just after hatching from egg. At this stage, the larval mouth and anus was completely closed in condition, eyes were open but no color was observed. Yolk sac was cover almost half of the larval body (Figure 2a). The digestive tract was simple, straight, transparent tube like structure along with the dorsal wall of the yolk sac; and in length it is more than three-quarter of the body. At the end of the yolk sac stage ( $4.00 \pm 0.04$  mm SL) (Table 2), the gut lumen was slightly enlarged. The anus in the digestive system of the *T. ilisha* was opened at the end of yolk sac absorption stage. But the mouth was still remains closed. Digestive tract measured about  $3.39 \pm 0.21$  mm in length (Table 2). Percentage of the gut length compared with the standard length was  $84.87 \pm 4.87$  % (Table 2).

**Table 2:** Mean standard length (SL) and gut length (GL) of *T. ilisha* larvae and juveniles and percentage of GL

Stages	SL (mm)	GL (mm)	GL%
Yolk sac	$4.00 \pm 0.40$	$3.39 \pm 0.21$	$84.87 \pm 4.87$
Pre flexion	$8.07 \pm 1.83$	$6.95 \pm 1.52$	$85.64 \pm 4.47$
Flexion	$11.50 \pm 1.55$	$9.40 \pm 0.95$	$82.29 \pm 6.18$
Post flexion	$14.72 \pm 1.70$	$11.47 \pm 1.43$	$77.99 \pm 4.98$
Juvenile	$18.76 \pm 3.02$	$13.88 \pm 2.33$	$74.02 \pm 3.27$



**Fig 2:** Development of the digestive tract with growth of *T. ilisha* larvae. Yolk sac stage (a), pre-flexion stage (b), flexion stage (c), post-flexion stage (d) and juvenile (e) (bar = 1mm)

### Pre flexion larvae

The pre flexion stage was started with fully opened mouth and anus. The eyes were fully pigmented in this larval stage ( $8.07 \pm 1.83$  mm SL) with a long gut ( $6.95 \pm 1.52$  mm) (Table 2). The dorsal and anal fin fold observed in this stage was formed in the external structure of main body, which helped to search, and intake of food. The digestive tract was increasing in size as the body enlarges. In this pre-flexion larval stage, stomach was developed between the esophagus and intestine (Figure 2b). The formation of the digestive tract can be divided into pharyngeal cavity, esophagus, rudimentary stomach, intestine and rectum. In this stage, food particles were first observed in the stomach. Also with the preliminary structure of the digestive system, the larvae were capable to ingest and digest exogenous feed component. The yolk was completely disappeared, and the intestinal wall was developed. Rectum of larvae was observed at the end of the intestine just before anus opening. Pancreas and liver were also noticed at the end of the pre-flexion stage. The percentage of the gut length of the larvae is  $85.64 \pm 4.47$  % at this stage compared with its standard length (Table 2).

### Flexion larvae

In this stage, the larvae head was became bigger. The body length was elongated at this stage. The end point of notochord started to fold and the bones were differentiated at this flexion stage ( $11.50 \pm 1.55$  mm SL) with a well-developed gut ( $9.40 \pm 0.95$  mm) (Table 2). Further, in this flexion stage, the accessory gland, liver, and pancreas were clearly noticed. Development of the digestive tract is almost in the well-developed stage. The pancreas and liver development was also completed at this stage (Figure 2c). The percentage of the gut length compared with the standard length was  $82.29 \pm 6.18$  % (Table 2).

### Post flexion larvae

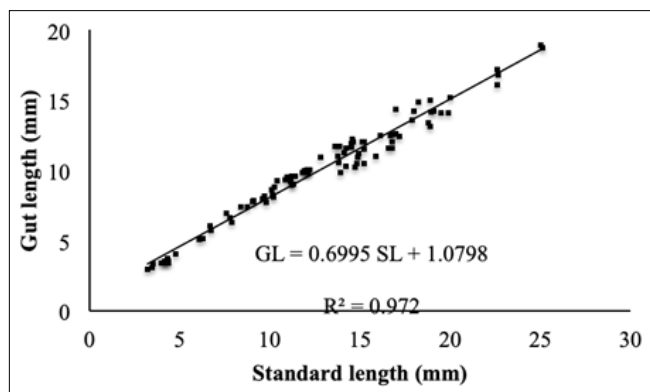
At this stage, the standard length of the larvae was  $14.72 \pm 1.70$  mm and its respective gut length was  $11.47 \pm 1.43$  mm (Table 2). The notochord tip folding was completed along with the completed caudal fin (Figure 2d). Most of the fin was well developed in this stage; also the gut length increases along with the body length and it became more complex at this stage. The percentage of the gut length compared with the standard length and it was  $77.99 \pm 4.98$  % (Table 2).

### Juveniles

At the juvenile stage (Figure 2e), fish were mostly like to be an adult individual. All the component of the digestive tract was well developed and the fish mostly dependent on the exogenous food sources. The standard length and gut were observed for *T. ilisha* as  $18.76 \pm 3.02$  mm and  $13.88 \pm 2.33$  mm, respectively and the percentage of the gut length was compared with the standard length and it was  $74.02 \pm 3.27$  % (Table 2).

A strong correlation ( $R^2 = 0.97$ ) was observed between GL and SL as estimated below:  $GL = 0.6995 SL + 1.0798$  (Figure 3).





**Fig 3:** Linear relationship between gut length (GL) and standard length (SL) of *T. ilisha* larvae

### Discussion

The digestive system of the *T. ilisha* larvae was transparent, straight tube like structure during yolk-sac stage as observed in the larvae of *M. nemurus* (Hag *et al.* 2012) [6], *T. tambroides* (Ramezani-Fard *et al.* 2010) [14] and *Sarda orientalis*, (Kaji *et al.* 2002) [8]. During the yolk sac stage mostly the larvae used its yolk sac for its energy consumption. At the end of yolk-sac stage and the beginning of pre-flexion stage, the digestive tract can be differentiated visibly into mouth opening, buccopharynx, esophagus, intestines and rectum. This observation was in accordance with the findings of Kaji *et al.* (2002) [8] in *S. orientalis*. In *M. nemurus* larvae, the differentiation of digestive tract took at least 4-5 day after hatching or just after the yolk absorbed (Hag *et al.* 2012) [6]. Similarly, the differentiation of gut was noticed in milkfish *Chanos chanos* just after the yolk sac absorbed (Ferraris *et al.* 1987) [4].

In this study, the food particle in the digestive tract was first observed just immediately after the yolk sac absorption. Also, it was observed that when the mouth was active and the anus was completely opened. Avila and Jurio (1987) [1] reported the ability of the larvae to ingest exogenous food when the gut was developed after the yolk sac absorption. (The stomach development was observed as a bulge at the end of the esophagus during the start of the exogenous feeding in some fish larvae, for example *Seriola lalandi*, (Chen *et al.* 2006) [3]. Hag *et al.* (2012) [6] described a similar finding in *M. nemurus* fish larvae. At the beginning of pre-flexion stage, the size of the stomach was small and increasing with the increasing body length. Based on the observation of Hag *et al.* (2012) [6], *M. nemurus* larvae was ingested a small amount of food after yolk sac stage, however the size of the gut increases as the body increased in size.

Busch (1996) [2] described that all herring fish larvae started their first exogenous feeding on the same day immediately after yolk absorbed. Also, he noticed that at the end of the yolk sac larval stage, the esophagus started to open due to the reduction in the yolk sac pressure or yolk absorption. Whereas, in the case of *T. ilisha* larvae, the first event of exogenous feed was noticed at the end of yolk sac stage or at the beginning of the pre-flexion stage. At the first feeding of *Clupea harengus* larvae, Busch (1996) [2] observed a correlation between the gut length and standard length; both increased accordingly, for example 8.1 and 8.7 mm of gut length and SL, respectively. In this observation, the standard length and gut length of *T. ilisha* during first feeding was  $8.07 \pm 1.83$  mm and  $6.95 \pm 1.52$  mm, respectively.

Govoni (1980) [5], O'Connell (1981) [11] and Watanabe and

Sawada (1985) [17] described that the two accessory glands including liver and pancreas (with their ducts) was developed after yolk absorption. In the case of *T. ilisha* larvae, the liver and pancreas were noticed during the pre-flexion stage and they were clearly differentiated at flexion stage.

### Conclusion

Studies on the development of digestive tract were an important parameter of larval management. For introduction of a species from wild to captive condition, it is very important to study the food behavior of the species. This study provided potential knowledge about the development of digestive system of *T. ilisha* larvae; this information would help to manage the larval rearing of *T. ilisha* for aquaculture development.

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