Morphological characteristics of *Tenualosa ilisha* (Hamilton 1822) larvae collected from lower Meghna River of Bangladesh

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Abstract

Aim : The present research was undertaken to describe the morphological development of larvae and early juveniles of hilsa shad, *Tenualosa ilisha*.

Methodology : Samples were collected from the lower Meghna River of Bangladesh. The investigation was based on the ontogenetic development of 100 samples of *T. ilisha*.

Results : Larvae and juveniles were divided into five groups according to morphological features; yolk sac larvae (3.83 mm SL), pre flexion larvae (8.07 mm SL), flexion larvae (11.50 mm SL), post flexion larvae (14.72 mm SL) and juvenile (18.75 mm SL). The number of myomere and vertebrae ranged from 34 – 44 and 40 – 46 respectively. In the case of fin ray, the number of dorsal fin rays were 16 – 20, anal fin rays 18 – 22, pectoral fin rays 14 – 16, pelvic fin rays 7 – 9 and caudal fin rays were found to be 20 – 24, respectively, in different stages. Relationship between the mean values of the body depth and standard length differed significantly among larval and juveniles stages.

Interpretation : This study mainly described the larval morphological development of hilsa, *T. ilisha*. The information would be beneficial for the formulation of effective sustainable management of spawning grounds of hilsa.

Keywords: Hilsa shad, Larvae stage, Meristic characteristics, *Tenualosa ilisha*

Sampling Area

Collection of sample

The relation between the mean values of body depth and body length in early juvenile stage show significant differences compared to larval stages

Absorption of yolk sac and development of different fin base was the main identifying characteristics of pre flexion stage.

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Introduction

Hilsa shad, *Tenualosa ilisha* (Hamilton, 1822) under family Clupeiformes is locally known as Ilish, Hilsa, Ellis etc., in different parts of Bangladesh (Haldar and Islam, 2008). It is anadromous, migrating from sea to freshwater riverine environment to spawn (Amin *et al.*, 2002). Almost same external morphological characteristics is the main difficulty to characterize clupeiformes species at early larval stages. Also, insufficient information is available on *T. ilisha* larvae. On the basis of notochord development, fish larvae can be divided into four larval stages; yolk-sac larvae, pre-flexion larvae, flexion and post flexion (Kendall *et al.*, 1984). Mouth and gut morphology of *T. ilisha* larvae has been reported by Riar *et al.* (2018, 2019).

So far no published information is available that describes the morphological development of *T. ilisha* larvae from Bangladesh. Therefore, the present study was undertaken to describe the larval and juvenile's morphology of *T. ilisha*, which would be consider as the first baseline information in Bangladesh waters.

Materials and Methods

Samples of *T. ilisha* were collected from the lower Meghna River of Bangladesh by weekly sampling from August

2016 to January 2017. Sampling areas were located in the lower portion of Meghna River between Chandpur (23°22' N, 90°65' E) and Hatiya, Noakhali (22°28' N, 91°12' E) (Fig. 1). Three different types of nets a bongo net (400 µm mesh size), a plankton net (200 µm mesh size) and a mosquito net (500-600 µm mesh size) were used for sampling. All nets were towed in the water sub-surface. The collected samples after examination were deposited at the Aquatic Biology and Ecology Laboratory of Aquaculture Department, University Putra Malaysia, Serdang, Selangor, Malaysia.

In laboratory, fish larvae were sorted out from other zooplanktonic organisms using a dissecting microscope. Larvae and juveniles of *T. ilisha* were differentiated from other zooplanktons collected based on common distinguishing characteristics of clupeiformes, such as; high number of myomeres, vertebrae and different fin position compare with other fish families (Silva *et al.*, 2010; Leis and Carson-Ewart, 2000; Leis and Trnski, 1989).

Larval fishes were classified based on the developmental degree of caudal fin and its supporting elements into larval yolk sac (YS), pre flexion (PF), flexion (FL), post flexion (PO) stages] and juvenile (JV) stages (Kendall *et al.*, 1984). The following morphometric characteristics were considered for the analysis:

Fig. 1 : Location of sampling stations (dots) in the Meghna river of Bangladesh.

total length (TL), standard length (SL), pre-anal length (PAL), pre-pelvic length (PPVL), pre-pectoral length (PPCL), pre-dorsal length (PDL), head length (HL), head depth (HD), eye diameter (ED), body depth (BD) and anal to caudal fin length (ACFL). The relationship between the standard length (SL) and various body lengths (PAL, PPVL, PPCL, PDL, HL, HD, BD, ACFL) and the mathematical relationship between HD and ED was also examined for different stages. The variations of BD (Body depth) in relation with SL (standard length) were also analysed. The measurements were taken using Keyence Digital Microscope (VHX-500) at 20-30X. The drawings were made from the images generated from the microscope. The samples were cleaned with distilled water for meristics study and the pectoral (P), dorsal (D), pelvic (V), anal (A) and caudal (C) fin rays were counted.

Water quality parameters of sampling area were measured using FF2 hach kit and digital water quality multi parameter (HQ40D). During the study period, the data of water quality parameters collected from different sampling stations were air temperature ranging from 17-33 °C, water temperature from 17-32 °C, transparency from 11 - 90 cm, dissolved oxygen ranged from 4 - 6.5 mg l⁻¹, free CO₂ 10-29.4 mg l⁻¹, pH ranging from 7.5 - 8.5, total alkalinity from 42 - 121 mg l⁻¹, total hardness between 56 - 142 mg l⁻¹ and salinity ranging from 0 - 2 ppt.

Results and Discussion

Yolk sac larvae : Total and standard lengths of yolk sac larvae (Fig. 2a) were 4.20 and 3.83 mm (Table 1). Body depth ranged from 7.43-12.35 % SL with a medium sized head of 14.63-28.70 % SL (Table 1). Functional larval mouth was not yet open, no visible exogenous feeding was found. A large, conspicuous yolk sac with two big oil globules was found and this almost covered

half of the body, which decreased with the larval development. Eyes were not pigmented at early yolk sac stage. One fin fold arose from dorsal portion behind the head and developed around the middle part of the body. No evidence of other fin formation was found during yolk sac stages. The total number of myomere ranged from 42 to 44 (Table 2).

Pre flexion larvae : At pre flexion stage (Fig.2b), total length and standard length were measured as 8.68 and 8.07 mm, respectively (Table 1). Body was characterized by a very elongate (7.02-11.42% SL) shaped, the head was small to medium (7.74-19.05% SL) with a fully pigmented eye, measuring from small to large (18.02-56.00% HL) fishes. Total myomere and vertebra ranged from 37 to 43 and 44 to 46 (Table 2). The notochord did not started to flex yet. The mouth was found open and active, with the presence of exogenous food particle in the gut. The finfold showed no identified difference compared with yolk sac stage. The pectoral fin base started to develop and was visible as finfolds without any rays. The initial formation of opercula was observed. Larvae observed between 5.0 and 7.0 mm SL were found to exhibit punctiform chromatophores, which randomly developed above the gut and on the dorsal part of the larval fish. Some fishes had transparent dorsal and anal fin formation. Larvae within 10 and 13 mm SL, some rays were visible in the dorsal fin (Table 2).

Flexion larvae : Flexion stage larval (Fig. 2c) total length was 12.86, while the standard length was 11.50 mm. The body ranged from 6.93-14.05% SL, the heads were small to medium in size (13.33-24.39% SL) and the eyes were medium to large in size (18.45-38.16% HL) (Table 1). Total myomere ranged from 36 to 40 and 42 to 46, respectively (Table 2). This stage was identified by notochord flexion (Fig. 2C). The finfold was not observed at this

Table 1 : Morphological measurement of *Tenuulosa ilisha* larvae in the yolk-sac (YS), pre-flexion (PF), flexion (FL), post-flexion (PO) stages and juvenile period (JV)

Measurements	YS (n=13)		PF (n=16)		FL (n=24)		PO (n=24)		JV (n=24)	
	Mean ± sd	Proportion (%)	Mean ± sd	Proportion (%)	Mean ± sd	Proportion (%)	Mean ± sd	Proportion (%)	Mean ± sd	Proportion (%)
TL	4.20±0.64		8.68±1.93		12.86±1.58		17.39±2.00		22.27±3.45	
SL	3.83±0.57		8.07±1.83		11.50±1.55		14.72±1.70		18.75±3.02	
HL	0.75±0.14	19.69±3.50	1.25±0.35	15.50±2.87	2.20±0.48	19.11±3.27	3.42±0.44	23.30±2.51	4.95±1.28	26.23±3.62
HD	0.47±0.10	12.17±1.77	0.81±0.19	10.00±0.89	1.37±0.28	11.87±1.46	1.65±0.21	11.26±1.51	4.59±1.34	24.36±7.80
ED			0.38±0.09	31.28±8.13	0.69±0.16	31.50±5.26	1.22±0.18	35.83±3.71	1.14±0.30	23.95±7.80
BD	0.37±0.05	9.63±1.01	0.78±0.17	9.70±1.24	1.19±0.24	10.40±1.72	1.87±0.45	12.81±3.00	5.10±1.11	27.17±3.49
PDL					6.53±0.73	57.36±6.00	6.90±0.83	47.05±5.04	9.34±2.57	49.26±6.27
PPCL					2.81±0.47	24.60±3.53	6.43±0.76	23.91±2.24	4.72±1.36	24.94±4.00
PPLL					5.80±0.84	50.65±5.20	3.51±0.44	43.88±4.76	8.60±1.82	45.64±3.82
PAL					9.56±1.15	83.56±6.63	11.00±0.85	75.21±5.88	15.00±2.31	73.91±2.54
ACFL					0.78±0.12	6.87±1.33	0.82±0.12	5.62±0.74	1.36±0.26	7.39±1.60

All proportions are related to standard length, except for eye diameter, in relation to head length. Amplitude expressed in mm, as mean values ± sd (Standard deviation). Total length (TL), standard length (SL), head length (HL), head depth (HD), eye diameter (ED), body depth (BD), pre-dorsal length (PDL), pre-pectoral length (PPCL), pre-pelvic length (PPVL), pre-anal length (PAL) and anal to caudal fin length (ACFL).

Fig. 2 : Larvae and Juvenile of *T. ilisha*: Yolk sac larvae (a); pre flexion larvae (b); flexion larvae (c); post flexion larvae (d) and juvenile (e).

stage. Dorsal fin, anal fin and pectoral fin formations were observed as well as the development of head with the development of gill filaments and rays. At this stage, nasal pores development was also observed. In more than 12 mm SL larvae, the dorsal, anal and caudal fin rays began to develop. At this stage, all paired and unpaired fin were visible and the fin rays could be differentiate easily. The number of dorsal fin rays ranged from 16 to 20. The number of anal fin rays ranged from 18 to 22, while caudal fin rays ranged from 20 to 24. There were three punctiform chromatophores in the middle part of the caudal fin over the gut; and the third chromatophores were observed on the base of pectoral fin (Fig. 1c).

Fig. 3 : Body depth (BD) percentage with the standard length (SL) of *T. ilisha* larvae in different stages *Yolk sac (YS), pre-flexion (PF), flexion (FL), post- flexion (PO) and juvenile (JV). All stages expressed by mean value $\pm$ S.D.

Post flexion larvae : Total length and standard length ranged between 17.39 and 14.72 mm ($n = 24$) (Table 1) in post flexion stage (Fig. 2d). Total myomere number ranged from 34 to 38 and vertebrae from 40 to 44. At this stage, all paired and unpaired fin rays could be counted under a microscope and it was observed that dorsal fin ranged from 16 to 20, pectoral from 14 to 16 pelvic from 7 to 9, anal fin from 18 to 22 and caudal ranged from 20 to 24, respectively (Table 2). The body depth was 8.56-18.28 % of SL, head was 18.56-28.44 % of SL and the eyes were 18.8-30.0 % of HL (Table 1). This stage was characterized by pelvic fin development, complete ray formation and starting of squamation.

Juvenile stage : The most critical changes were related with body depth (BD) at this stage. The body depth was 27.17 of body length and was higher as compared with larval stages. Three punctiform chromatophores were found in the middle part of the caudal fin, over the gut and last one chromatophores was found at the base of the pectoral fin. No changes were found in the number of fin rays for all paired and unpaired fin as compared with post flexion stage (Table 2). *T. ilisha* measured at the juvenile stage (Fig. 2e) had 22.27 mm TL and 18.75 mm SL ($n = 24$). (Table 1). At this stage, body was 22.15-33.24 % of SL, the head was 19.57-32.6 % of SL and the eyes were 14.29- 44.32 % of HL (Table 1). Some *T. ilisha* with visible myomeres had a total number ranging from 34 to 36. Scale formation was observed from the juvenile stage. Punctiform chromatophores were found throughout their life and their pigmentation moved on the base of the anal fin and later. Dorsal region pigmentation was same as post flexion stage; pigmentation was found at the base of caudal fin and chromatophores were also found on its base (Fig 2e). Body relations and respective proportions in different developmental stages are detailed in Table 1.

Overall, the relationship of prefin length with SL were observed from 34.34 to 64.94% for PDL; 13.25 to 33.2% for PPL; 36.69 to 60.12% for PPVL and 59.04 to 91.79% for PAL. HL measured from 7.74 to 33.0%; HD from 8.75 to 32.6%; BD from 6.93 to 33.24 %; and ED from 14.29 to 56.0% HL. Total myomere

Table 2: Meristic data for different larval and juvenile stages of *T. ilisha*

Stages	D	P	PV	A	C	M	V
YS	ND	ND	ND	ND	ND	43 ± 01	NC
PF	NC	ND	ND	ND	ND	40 ± 03	45 ± 01
FL	18 ± 02	NC	NC	20 ± 02	22 ± 02	38 ± 02	44 ± 02
PO	18 ± 02	15 ± 01	8 ± 01	20 ± 02	22 ± 02	36 ± 02	42 ± 02
JV	18 ± 02	15 ± 01	8 ± 01	20 ± 02	22 ± 02	NV	NV

*Pre-flexion (PF), flexion (FL), post-flexion (PO) larvae and juveniles of *T. ilisha*. Fins: pectoral (P), pelvic (PV), dorsal (D), anal (A) and caudal (C). Myomeres (M) and Vertebrae (V); *ND = Not Developed; *NV = Not visible; *NC = Not counted.

number in *T. ilisha* counted from 35 to 46 (Table 2). Relations between mean values of the body depth and standard length, JV stage showed significant ($p < 0.05$) difference with all other stages.

Tenuulosa ilisha larvae were generally identified with the same characteristics as Alosinae fish larvae described by Leis and Carson-Ewart (2000). There were 41- 46 myomere observed in this study but Leis and Carson-Ewart (2000) found 43-46 myomere for Alosinae.

In the case of yolk-sac larvae, the total length was 4.20 mm while Kulkarni (1950) observed the total length for hatchlings to be 2.3 to 3.1 mm. In this study, there were two oil globules observed at this stage, while Kulkarni (1950) observed three oil globules in hatched larvae. For this sampling, the larvae were collected from nature but Kulkarni studied on artificially hatched larvae. The age of the larvae used in this study was more than 1 day. The length increased, while the oil globule decreased with development.

Absorption of yolk sac and development of different fin base was the main identifying characteristics of pre flexion stage. The eyes were open and pigmented. An open and active mouth was observed at this stage. Silva *et al.* (2010) observed same characteristics for *Anchoviella vaillanti*. He, however, observed myomeres from 31 to 45 while this study observed 42 to 44 myomeres.

The flexion of notochord was the main identifying character of flexion stage. Three punctiform chromatophores developed on the dorsal base of larvae and fin ray were developed at each fin base. Leis and Carson-Ewart (2000) described same identifying characteristics for alosinae subfamily.

Post flexion stage was the last stage of larvae, characterized by complete flexion of notochord and completely developed fin with rays. There were 35-38 myomeres and 41- 44 vertebrae counted at this stage. The same identifying characteristics were observed in alosinae subfamily (Leis and Trnski, 1989), however, in the Clupeinae subfamily there were 34-37 myomeres and 35-36 vertebrae (Silva *et al.*, 2010).

Formation of scales was the major identifying characteristic of early juveniles. From the data analysis, it was

found that the body depth increases more than the body length at metamorphosis from larvae to juveniles. So, the percentage of body depth with body length in early juvenile stage show significant differences compared to larval stages. The same findings were observed by Leis and Trnski in Clupeiformes larvae (1989). Two *Tenuulosa* species have been observed in freshwaters of Bangladesh; they are: *T. ilisha* and *T. toli*. However, no larvae and juveniles of *T. toli* have been found in freshwaters (Blaber *et al.*, 1996). This report also supports the presence of *T. ilisha* larvae in the Meghna River of Bangladesh.

This observation on morphological study on *T. ilisha* larvae and early juveniles is the first attempt of its kind. Thus, more attention should be focused on identifying the *T. ilisha* larvae from Bangladeshi water bodies. These results might be helpful for future detail study of *T. ilisha* larvae for detecting spawning grounds of hilsa in Bangladesh waters.

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