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Mouth morphological development of Hilsa (*Tenualosa ilisha*) larvae collected from Meghna Estuary, Bangladesh



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Abstract

Aim: In larval fish, food size is closely related with the length of the fish jaw, as well as mouth gape. So far, no information is available on the larval mouth morphology of Hilsa shad *Tenualosa ilisha*. Hence, observations on the mouth morphological development during different larval stages of *T. ilisha* were examined.

Methodology: Samples of *T. ilisha* larvae were collected weekly from Meghna estuary, Bangladesh between August 2016 and January 2017. The collected samples were transported to the laboratory and the mouth morphology of larvae and early juvenile stages were microscopically examined.

Results: The smallest mouth size was found at 45°, while was largest found was at 90°. There was no evidence of teeth, while the lower jaw was longer than the upper jaw. The four larval stages were observed in this study; viz. yolk-sack stage, pre-flexion stage, flexion stage, post-flexion stage. The mouth was closed at yolk sac stage while in the pre-flexion stage, larval mouth gape ranged between 177 and 367 µm. In the flexion stage, the range was 241 to 497 µm, in post-flexion stage it ranged between 307 and 621 µm and finally in early juvenile stage the range was 393 to 788 µm.

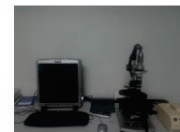
Interpretation: There was a significant relationship with standard length (SL) and upper jaw length ($R^2 = 0.941$, $p < 0.05$) as well as lower jaw ($R^2 = 0.931$, $p < 0.05$). Significant relationship was also found with SL and mouth gap at 90° ($R^2 = 0.938$, $p < 0.05$). Overall, these findings can be considered a base data for mouth morphological development of *T. ilisha*.

Investigate the mouth morphological development during different larval stages of *T. ilisha*

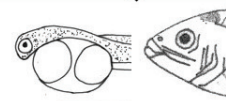


Collection of sample

Identification of larvae



Mouth morphology of larvae and early juvenile stages were examined under Keyence Digital Microscope (VHX-500)



Jaw length and mouth gape at different larval stages

Introduction

Hilsa Shad, *Tenualosa ilisha* is found in the open ocean, coastal areas, estuaries and rivers throughout Asia, including Bangladesh, Myanmar, India, Pakistan, Kuwait, Iraq, Iran, Indonesia and Malaysia. *T. ilisha* is an anadromous species, found to migrate to river as long as more than 1000 km inland for feeding and breeding (Mohanty *et al.*, 2012). It is an accepted concept by most of fishery researchers that maintenance of larval survival and growth, are primarily determined by the availability of food, especially at the early larval stage and transition period of yolk absorption to exogenous feeding (Fraser, 1970; Cushing, 1975). The transition from endogenous to exogenous feeding is one of the most crucial time (Conides and Glamuzina, 2001; Moteki *et al.*, 2001) and, during this weaning period, mass mortalities have been routinely observed (Kamler, 1992; Haniffa *et al.*, 1999).

Physical morphological changes also play a major role in larval transition period, especially during weaning time (Peter, 1996). Mouth and associated organ development are related with the feed and feeding behavior of the fish, and these parameters can vary substantially among different species. The differences among species in mouth development also play a role in the

successful survival of fish larvae (Keast and Webb, 1966). The structure and size of the mouth gap show a close relationship with the ability to consume food particles (Hepher, 1988; Lovell, 1989), and indeed is a limiting factor in larval feeding on either live or artificial diets (Hyatt, 1979). Also, some research findings indicate that mouth gap differences among species is proportionate to the mouth opening and fish length during ontogenic events (Walford and Lam, 1993; Kamali *et al.*, 2006). Hassan and Macintosh (1992) and Dabrowski *et al.* (1983) also reported the differences in the mouth size among larval fish species including common carp *Cyprinus carpio*, grass carp *Ctenopharyngodon idella*, silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis*. Further, they also observed a linear relationship between mouth size and total length of these species.

Various studies have reported the attainment of specific minimum mouth size, *i.e.*, the jaw length and mouth gap, which are mandatory features for effective catching and feeding on food (Economou, 1991; Munk, 1992; Crockford and Johnston, 1993; Bremigan and Stein, 1994). Hence, this study was conducted to understand the mouth development during different larval stages of *T. ilisha*, which might assist in their aquaculture such as choosing the most appropriate live food.

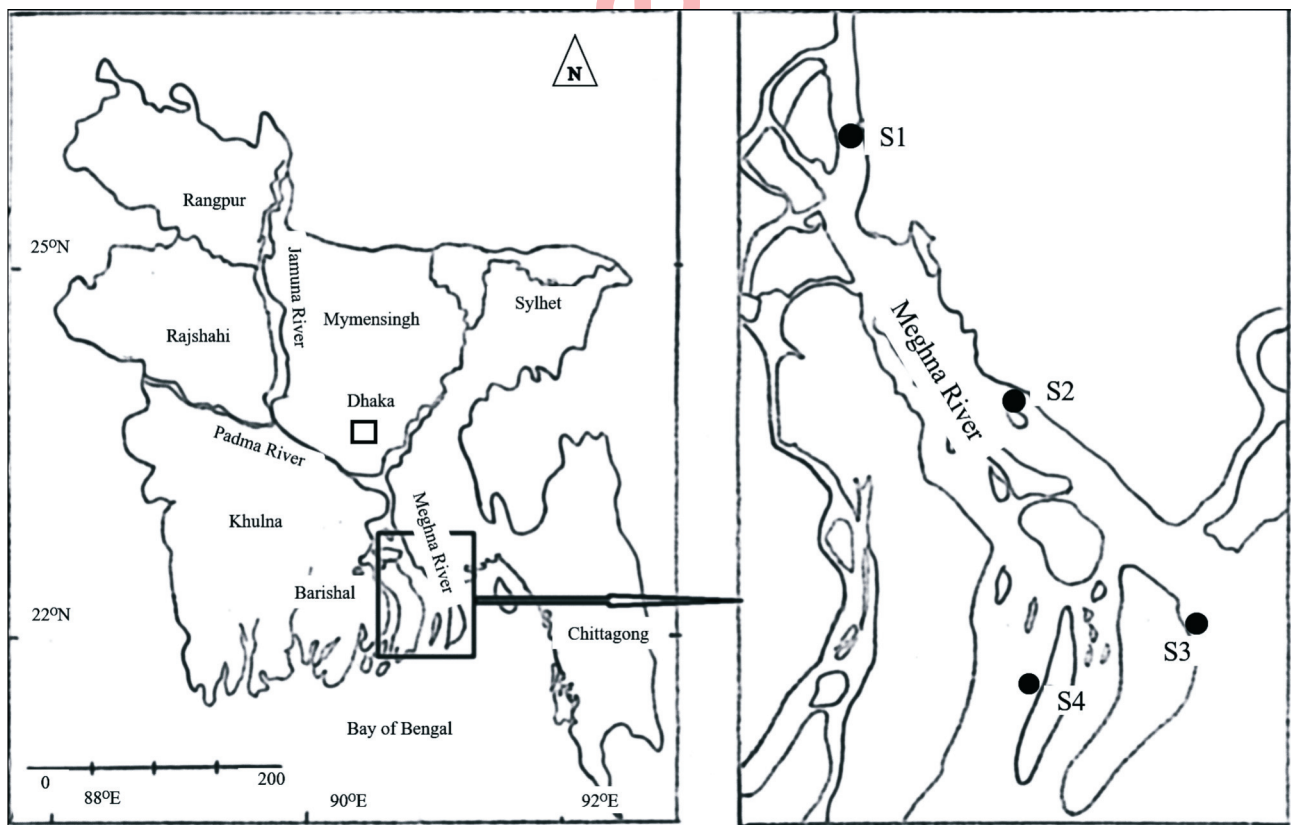


Fig. 1: Location of the sampling stations (dots) in the Meghna river of Bangladesh

Materials and Methods

The larvae of *T. ilisha* used in this study were collected from different sites of Meghna estuary between August 2016 and January 2017 on weekly basis (Fig. 1). The location of the sampling stations were S1 (23.084228 N, 90.643853 E), upper estuary (Char Bhoirobi, Chandpur District); S2 (22.686959 N, 90.932244 E), middle estuary (Ramgati, Laxmipur District); S3 (22.283453 N, 91.124505 E), lower estuary ((Hatiya, Noakhali District); and S4 (22.266470 N, 90.962331 E), lower estuary (Monpura, Bhola District). Water quality parameters of the sampling sites were recorded using a Hach kit from one-meter water depth. Three different types of nets were used for the collection of larvae, they were bongo net (mesh size: 400 μ), plankton net (mesh size: 200 μ) and mosquito net (mesh size: 300 μ). All nets were operated using sub surface tow.

The collected fish larvae were sorted out from other zooplanktons based on the common identification characters of Clupeiformes, such as a long and elongated body with high number of myomeres; long and slightly striated gut; posterior anus, anal fin development just behind the dorsal fin and prenatal length covered more than 2/3 of total length (Silva *et al.*, 2010; Leis and Carson-Ewart, 2000; Leis and Trnski, 1989) using a dissection microscope (Olympus Sz51).

Yolk sac stage larvae were avoided for measuring length because those had no evidence of mouth opening. The morphological development of the mouth and jaw of *T. ilisha* larvae were observed from preflexion larvae to early juvenile. A total of 87 larvae of the preflexion, flexion, postflexion stages and early juvenile period were measured during microscopic observation. The number of individuals were taken for analysis as follows: pre-flexion larvae (PF) = 15, flexion larvae (FL) = 24, post-flexion larvae (PO) = 24 and juvenile larvae (JV) = 24. The larvae were taken in a petri dish and its standard length (SL), upper jaw length (UJL) and lower jaw length (LJL) was measured using Keyence Digital Microscope (VHX-500) at 20-30X. A line drawings of the mouth were sketched using the images observed under a microscope. Mouth gape at 45° (MG45) and Mouth gape at 90° (Mg90°) was determined using the following formulae:

$$\text{Mg45} - \sin 45^\circ * \text{UJL} (\sin 45^\circ = 0.71)$$

$$\text{Mg90} - \{(\text{UJL})^2 + (\text{LJL})^2\}^{1/2}$$

All slopes of linear equation were tested using simple t-tests.

Results and Discussion

The results of the water quality analysis ranged in the following manner: air temperature 17-33°C, water temperature 17-32°C, transparency 11-90 cm, dissolved oxygen 4-6.5 mg l⁻¹, free CO₂ 10-29.4 mg l⁻¹, pH 7.5-8.5, total alkalinity 42-121 mg l⁻¹, total hardness 56-142 mg l⁻¹ and salinity 0-2 ppt.

The yolk sac larvae (3.83±0.57 SL) of *T. ilisha* contained a yolk sac consisting of two oil globules. No pigmented eyes were observed and mouth was closed at this stage (Fig. 2a, 2b), but opened during the end of this stage. However, still there was evidence of teeth development at this stage.

Once, the yolk sac was absorbed and pre-flexion appeared (Fig. 2c, 2d), a well-developed esophagus was noticed. Further observations indicated that the upper jaw was shorter than the lower jaw in their well-developed mouth. Their standard length was 8.47 ± 1.58 mm, while their upper and lower jaw length was 0.25 ± 0.04 mm and 0.27 ± 0.03 mm, respectively (Table 1). The jaw length developed in accordance with the standard length of larvae. The size of mouth gape was 177 ± 25 μ m at 45° of the mouth opening, whereas the mouth gape was found 367 ± 47 μ m at 90° of mouth opening.

At the flexion stage (Fig. 2e, 2f), the standard length of larvae was 11.50 ± 1.55 mm and its upper and lower jaw length was 0.34 ± 0.04 mm and 0.36 ± 0.04 mm, respectively (Table 1). At this stage, the digestive system, as well as an active operational mouth of the larvae was well developed. During this stage, the mouth opening of the larvae at 45° and 90° was 241 ± 31 and 497 ± 59 μ m, respectively.

At the post-flexion stage (Fig. 2g, 2h), the standard length of larvae was 14.72 ± 1.70 mm, while its corresponding upper jaw and lower length was 0.43 ± 0.05 mm and 0.45 ± 0.05 mm, respectively (Table 1). Similar to the flexion stage, the length of the mouth opening at 45° and 90° was 307 ± 38 and 621 ± 74 μ m, respectively.

Table 1: Mean standard length (SL), upper jaw (UJ), lower jaw (LJ) and mouth gape (MG) at 45° and 90° of *T. ilisha* larvae and juvenile*

Development Stage	SL (mm)	UJL (mm)	LJL (mm)	MG 45° (μ m)	MG 90° (μ m)
Yolk sac	3.83 ± 0.57	**	**	**	**
Pre-flexion	8.47 ± 1.58	0.25 ± 0.04	0.27 ± 0.03	177 ± 25	367 ± 47
Flexion	11.50 ± 1.55	0.34 ± 0.04	0.36 ± 0.04	241 ± 31	497 ± 59
Post-flexion	14.72 ± 1.70	0.43 ± 0.05	0.45 ± 0.05	307 ± 38	621 ± 74
Juvenile	18.80 ± 3.12	0.55 ± 0.09	0.56 ± 0.08	393 ± 61	788 ± 119

* SL, UJL and LJL expressed in mm, while MG expressed in micron (μ m) as mean values ± S.D.; ** = Not opened

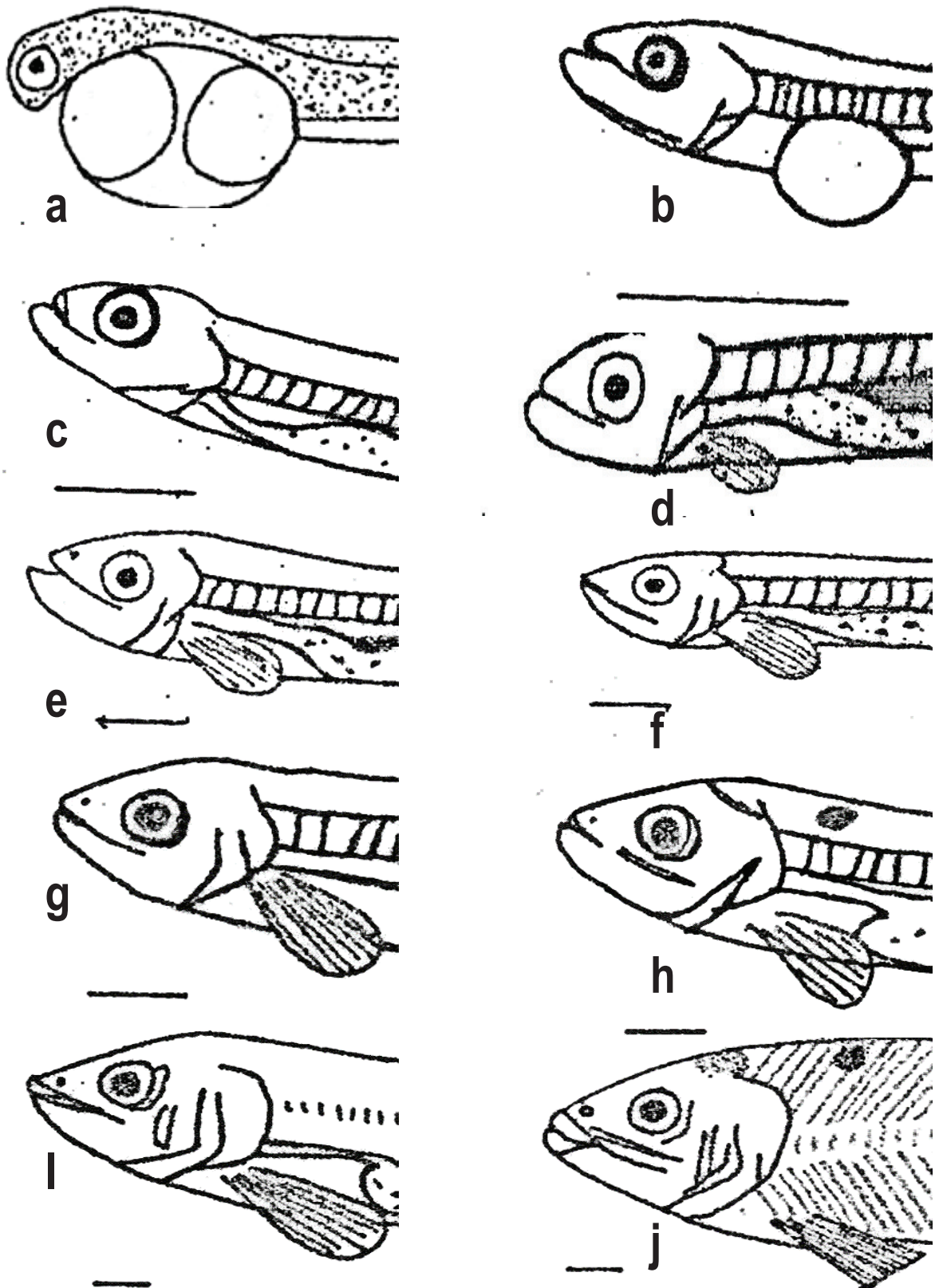


Fig. 2 : Mouth morphological development of *T. ilisha* larvae and juvenile. Yolk sac stage (a,b), pre-flexion stage (c,d), flexion stage (e,f), post- flexion stage (g,h) and juvenile stage (i,j). Bar = 1mm

At juvenile stage (Fig. 2i, 2j), the larval external morphology was almost similar to an adult individual and its SL = 18.80 ± 3.12 mm. The upper (0.55 ± 0.09 mm) and lower (0.56 ± 0.08 mm) jaws, as well as its mouth were shorter in size (Table 1). The jaw extended upto in front of eye. In this stage, the mouth opening length at 45 and 90° was 393 ± 61 and 788 ± 119 μ m, respectively. Overall, from the data analysis, there was a strong relation between standard length (SL) with the upper jaw length (UJL), lower jaw length (LJL), mouth gape at 45° (Mg45°) and mouth gape at 90° (Mg90°). The established liner relationship models are given below:

$$\text{UJL} = 0.281 + \text{SL} \times 0.0329 \quad (R^2 = 0.941, p < 0.05; \text{Fig. 3})$$

$$\text{LJL} = 0.0274 + \text{SL} \times 0.0634 \quad (R^2 = 0.931, p < 0.05; \text{Fig. 4})$$

$$\text{MG}_{45^\circ} = 19.965 + \text{SL} \times 23.394 \quad (R^2 = 0.941, p < 0.05; \text{Fig. 5})$$

$$\text{MG}_{90^\circ} = 39.21 + \text{SL} \times 68.658 \quad (R^2 = 0.939, p < 0.05; \text{Fig. 6})$$

In the case of yolk-sac larvae, the total length were 4.20 ± 0.64 mm while Kulkarni (1950) observed 2.3 to 3.1 mm total length for hatchlings. There were two oil globules observed at this stage, while Kulkarni (1950) observed three oil globules for newly

hatched larvae. In this study, there was no pigmentation in eye and mouth was close at the yolk sac larvae and the same observation was found with *Anchoviella vaillanti* larvae (Silva *et al.*, 2010).

Just after yolk sac absorption, the mouth of the larvae opened and the larvae started exogenous feeding, which is an essential transition to support faster growth (Scharf *et al.*, 2000; Aljettawi *et al.*, 2004). Jaw length and mouth gape has a close relationship in picking the food particles (Hepher, 1988). This study found that smallest food size equal to the mouth gap 45° while the largest size equal to the 90° of mouth opening (Hag *et al.*, 2012). In this study, the size of mouth gap was 177 ± 25 μ m at 45° of mouth opening whereas the mouth gap was found 367 ± 47 μ m at 90° of mouth opening at pre flexion stage. So the minimum food size was equal to 177 ± 25 μ m, while the maximum size of food particle equaled 367 ± 47 μ m for *T. ilisha* larvae at the pre-flexion stage. With increasing growth, and thus mouth gape, the size of the food particles can also increase in *T. ilisha* juveniles (Hasan *et al.*, 2016). The consistently longer lower jaw indicates larvae are surface feeders, which is in agreement with the suggestion of Hasan *et al.* (2016). However, because there was no evidence of teeth it is assumed that *T. ilisha* is a non-predatory

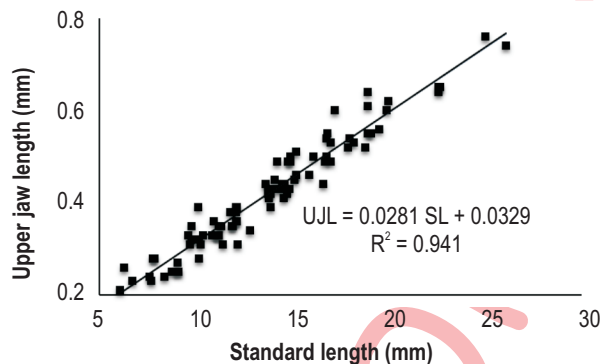


Fig. 3 : A linear relationship between upper jaw length (UJL) and standard length (SL) of *T. ilisha* larvae ($\text{UJL} = 0.281 \text{ SL} + 0.0329$)

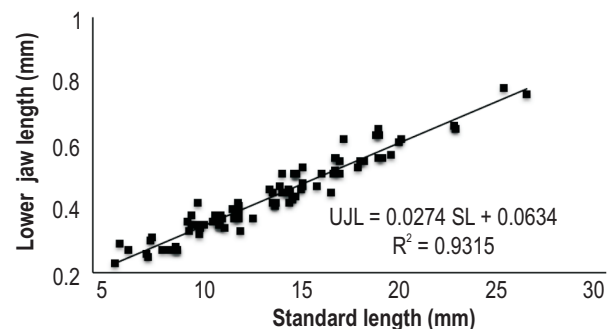


Fig. 4 : A linear relationship between lower jaw length (LJL) and standard length (SL) of *T. ilisha* larvae ($\text{LJL} = 0.0274 \text{ SL} + 0.0634$)

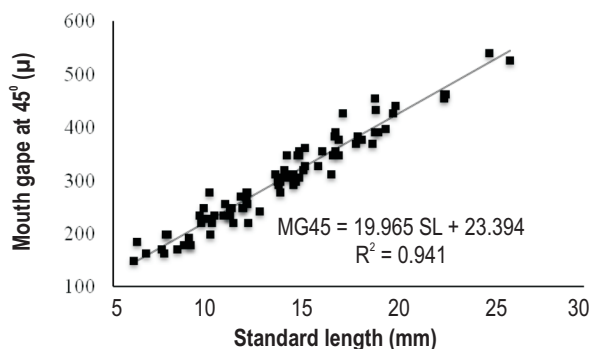


Fig. 5 : A linear relationship between mouth gape at 45° (Mg45°) and standard length (SL) of *T. ilisha* larvae ($\text{Mg}_{45^\circ} = 19.965 \text{ SL} + 23.394$)

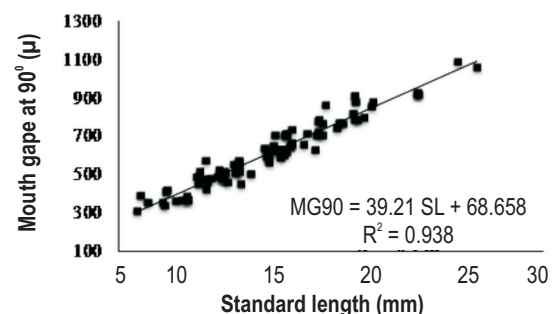


Fig. 6 : A linear relationship between mouth gape at 90° (Mg90°) and standard length (SL) of *T. ilisha* larvae ($\text{Mg}_{90^\circ} = 39.21 \text{ SL} + 68.658$)

fish. Indeed, Hasan *et al.* (2016) described *T. ilisha* as herbivorous in nature and depend mainly on phytoplankton for feeds.

Overall, this study showed that exogenous feeding only began after yolk-sac absorption and their first live food should be small such as rotifers or *Daphnia* sp. Considering the high commercial importance of *T. ilisha*, further research into their larviculture techniques should be conducted. This can include optimal environmental conditions and feeding regimes.

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