Mouth Development of Malaysian River Catfish, *Mystus nemurus* (C&V) Larvae

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Abstract: Ontogenetic morphological development for the mouth of Malaysian river cat fish *Mystus nemerus* larvae from hatching to 21 days post-hatch (dph) was studied to facilitate and determine suitable food and food particle size for the growing larvae. The eggs began to hatch 2 days after fertilization (daf) and most of the larve hatched within 2-4 daf. The larval mouth opened at the end of the 1 dph and the commencement of external feeding began on 4 dph following the jaw movement. The barbels appeared on the upper jaw and lower jaw on 3 dph.Two small barbels appeared around the olfactory pits by 5 dph. Free neuomasts were observed below the lower jaw on 7 dph and around the olfactory pits, eyes,and upper jaw operculum by 9 dph. Linear relationships between mouth size (at 45˚and 90˚ opening) and total length of fish were established.

[**Ghada Ahmed El Hag, Mohd Salleh Kamarudin, Che Roos Saad and Siti Khalijah Daud**] Journal of American Science 2012; 8(1):271-276]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Malaysian river catfish; larvae; mouth development; *Mystus nemerus*.

1. Introduction

Functional morphology of fish can be used to provide explanation for differences between species in ontogenic diet switches (Peter, 1996). Mouth structure is related to the feeding type and habit of fish, and is highly variable between fish species, and the variation in mouth structure may partly explain the evolutionary success of fish (Keast & Webb, 1966). The position and size of the mouth show a close relationship to the location and size of food items, and the relative size of the mouth can be used to determine the size of food particles ingested (Hepher, 1988). Particles which are too small may not be detected or captured easily by the fish, while thosewhich are too large may be too difficult to ingest quickly or whole (Lovell, 1989). Moreover, loss of nutrients from large and small food particles after soaking and softening, inevitably lead to wastage, pollution and nutrient leaching. For that mouth size appears to be a limiting factor in larval feeding with both live and artificial diets (Hyatt, 1979).

There are differences between species (both fresh water and marin) in the proportion of mouth opening and fish length during ontogenic growth (Shirota, 1970; 1978a; 1978b; Walford and Lam 1993, Kamali, *et al*., 2006). Dabrowski *et al*. (1983) cited in Hassan and Macintosh (1992) reported the differences in the mouth size between common carp larvae and three cyprinid fish species grass carp (*Ctenopharyngodon idella* Vai.), silver carp (*Hypophthamichthys molitrix* Rich.) and bighead carp (*Aristichthys nobilis* Rich.). They also observed a linear relationship between mouth size and the total length for each of these species. Kourill *et al*. (1981) stated that the growth of common carp larvae was much better when they were fed live zooplankton of 520um rather than 300um in size.therefore,the structure of mouth and mouth size sets the upper limit for prey selection. Klassen and Peake, (2008) stated that the inasitioning larval lake sturgeon from initial died of brin shrimp to exogenous feeding gives good result after 2 week from initial diet.

This study was conducted to monitor the mouth morphological change during larval development of Malaysian river catfish *M.nemerus*.

2. Materials and Methods

The larval were obtain through an artificial spawning from four female and eight males broodstock. In this study, the larval age was based on days post-hatch (dph). Newly hatched Artimia nauplii were given to the larvae at 4 dph at the rate of 5 *Artimia* ml feedin^{-1}. 10 fish larvae were randomly sampled every 48 hrs until 21 dph. The measurements of total body length (TL), upper and lower jaw (UJ &LJ) lengths were made using a profile projector (Nikon V10).

The morphological development of mouth was both observed using aprofile projector and scanning electron microscope (Jeol JS6400), SEM. For SEM the fish larvae were initially preserved in 4% glutaraldehyde for 24 hoursat 4° C, and washed with 0.1M sodium cocodylate buffer (pH 7.2) for 10 minutes, 3-4 times to remove the fixative. The samples were post fixed, larvae were washed again with 0.1M sodium cocodylate: 25%, 50%, 75%, and 95% for 10 minutes in each stage. The procedure was repeated in absolute acetone for 15 minutes and 40° C for 1.5 hours using a critial point dryer (Hitachi, HCP-2) with liquid CO2 at apressure of 800/7.5 µm. The specimens were mounted on stubs with a double sided carbon tape and were gold coated in a sputter coater. The samples were then examined under the scanning electron microscope.

3. **Results**

During the study, the water tempereture, dissolved oxygen (D.O),pH, and NH3 in the larval tanks ranged 25-27°C, 3°C, 5.9-8.9 mgL^{-1},7.2-7.8, and 0.2 to 1.22 mgL^{-1},respectively. *M.nemerus* eggs hatched at the end of one day after firtilization (40 -48 hrs daf) .The newly hatched larvae were characterized by a large yolk sac, unpigmented eyes and the mouth was closed (Fig.1). The barbles were not differentiated and no free neuromasts were observed in the newly hatched larvae .The mouth opened at the second daf (65-80 hrs AF).

Two thicker barbels and four others appeared in each side of the upper and lower jaw, respectively, by 3 dph. The average legth of the larvae was 7.55± 0.433 mm. the jaws started to move, while the eyes were pigmented and almost the entire yolk sac was obsorbed. Teeth appeared on the lower and upper jawes at 4 dph when the exogenous feeding began. By 5 dph, the barbles on the upper jaw were thinner and longer than those on the lower jaw. Two small barbles also appeared on the upper side of the mouth beside the olfactory pits. On the 7 dph, fully developed free nuromasts (4 pairs) were found under the lower jaw and by the 9 dph, the free nuroma.sts were also found around the olefactory pits, the eyes, and upper the operculum of each larval side. Thereafter the number of free nuromasts increased on the head (Fig 1. (f)).

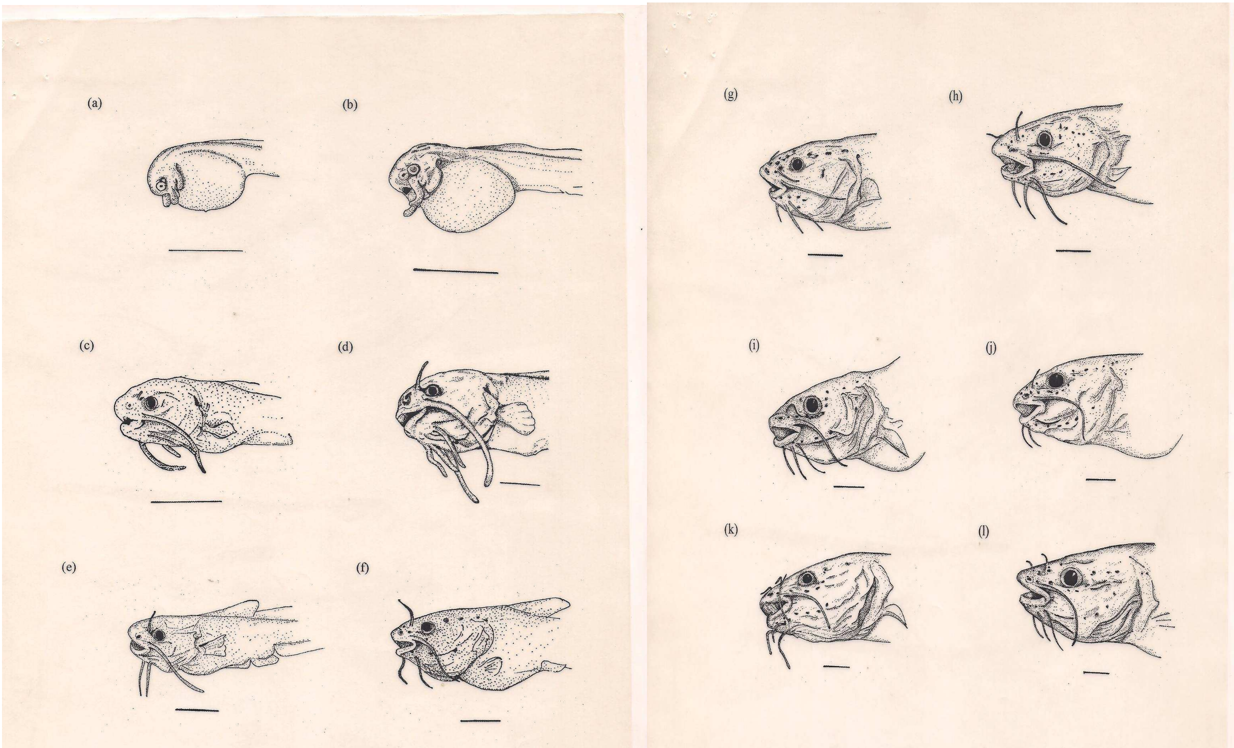
The mouth morphological change of *M. nemerus* larvae are illustrated in (Fig 1). The mouth structure of the larvae was subterminal in position. The shape of the mouth change from blunt to sharply (triangular) during 1-7 dph and from 8-21 dph, respectively. Table1 summarizes the TL, UJ, LJ,mouth size and number of free nuromasts of *M. nemerus* during larval development up to 21 dph. The mouth size of *M. nemerus* larvae increased from 114.9um for 1 dph to 786.29um for 21 dph at 45° oppening and from 195.13um for 1 dph to 1512.98um for 21 dph at 90° oppening (table 1). Strong correlations between TL and UJ (upper jaw), TL and LJ (lower jaw), TL and mouth size (at 45° and 90° oppening) were observed during this study (Fig.2, Fig.3 and Fig.4).

Table 1: Mean TL, UJ, LJ, mouth gape (45º and 90º openings) and number of neuromasts of developing *M. nemurus* larvae.

DAH	TL (mm)	UJ (mm)	LJ (mm)	45º opening (µm)	90º opening (µm)	No. of neuromasts (Pairs)
1	5.69±0.030	0.21±0.02	0.14±0.01	105.71±0.10	195.13±0.190	_
3	7.55±0.040	0.33±0.03	0.23±0.03	166.22±0.06	306.84±0.120	_
5	8.05±0.260	0.41±0.02	0.28±0.05	205.29±0.43	378.95±0.790	_
7	9.99±0.610	0.50±0.04	0.36±0.04	269.63±0.34	497.73±0.630	4
9	10.64±0.84	0.54±0.04	0.37±0.04	281.12±0.31	518.94±0.570	13
11	12.98±0.68	0.65±0.05	0.52±0.04	398.32±0.49	735.28±0.910	21
13	14.41±0.57	0.86±0.03	0.72±0.06	544.63±0.51	1005.35±0.94	24
15	15.88±1.19	0.94±0.03	0.75±0.40	571.44±0.35	1054.84±0.65	27
17	17.14±1.83	1.02±0.06	0.86±0.04	654.16±0.31	1207.56±0.59	29
19	19.48±0.85	1.15±0.42	0.95±0.12	705.49±0.92	1305.12±0.17	30
21	21.93±1.41	1.22±0.14	1.22±0.64	786.29±0.20	1512.98±0.26	32

Figure 1: Development in the Mouth morphology with growth of *M. nemurus* larvae.

(a) 1 dph (b) 3 dph (c) 5 dph (d) 7 dph (e) 9 dph (f) 11 dph (g) 13 dph (h) 15 dph (I) 17 dph (j) 19 dph (k) 21 dph.
Bar= 1mm.



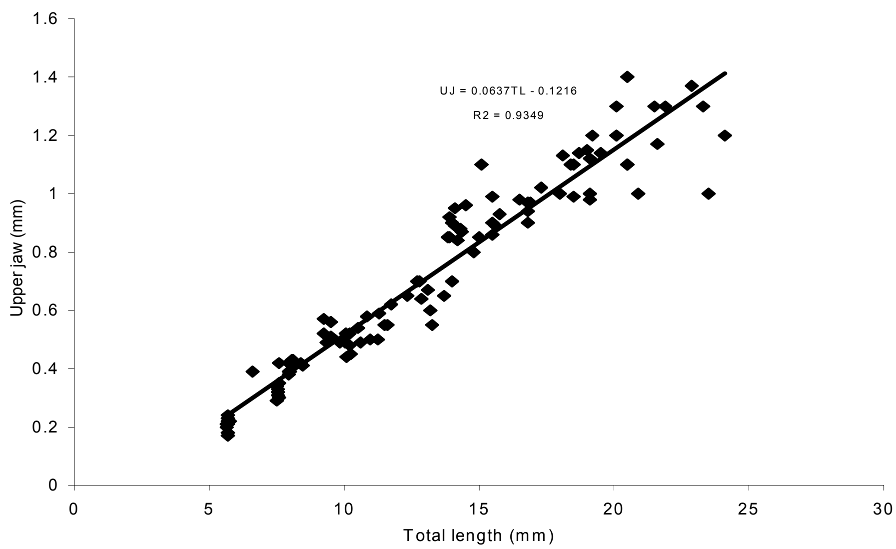


Figure 2: A linear relationship between upper jaw and total length of *M. nemurus* larvae

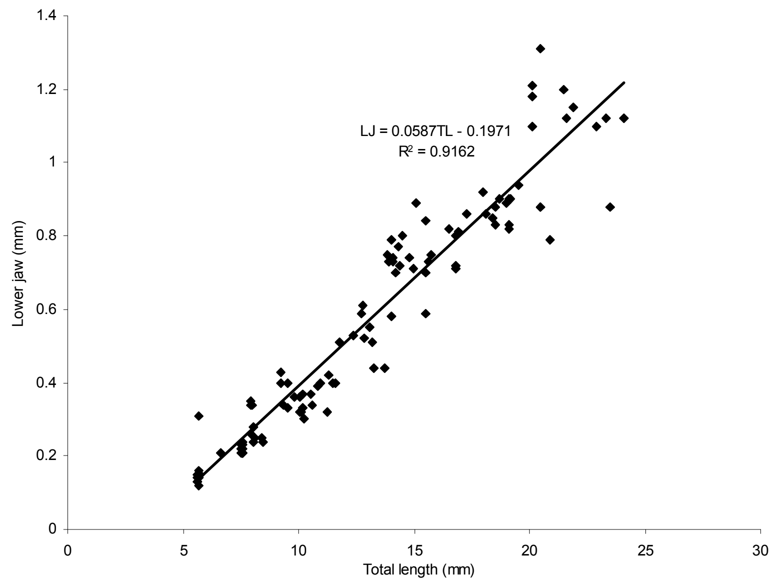


Figure 3: A linear relationship between lower jaw and total length of *M. nemurus* larvae

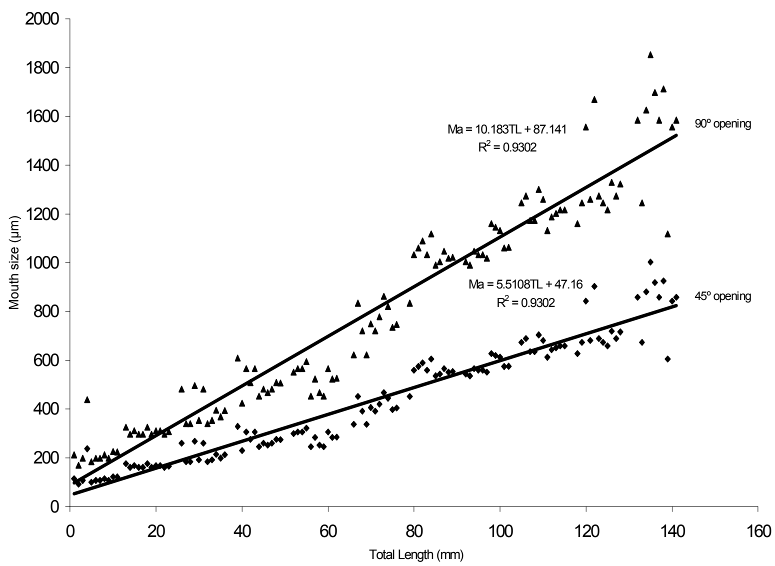


Figure 4: A linear relationship between mouth size and total length of *M. nemurus* larvae

4. Discussion

In this study hatching of *M.nemerus* began at 40-48 h after fetilization (2 daf) and most of larvae hatched within 2-4 daf. While the mouth opened at the end of the first days post-hatch (dph) and the eggs yolk was completely absorbed in about 72h (3 dph).Similer finding was found in catfish (*lophiosilurus alxandri*) by Guimaraes, *et al*. (2009) they found that the stage of yolk sac extend from 1 to 2 dphin this species. The larval mouth of Malaysian mahsser opend at one day after hatching (Ramezani-Fard, *et al*. 2011).The initialize time of exogenous feeding beginning at The absorbtion of yolk sac and according to Cloutier *et al*. (2011), the larvae cannot close their mouth, as their yolk-sac resorbs, so the detemination of the mouth size is very important thing during the larval stage. *M.nemerus* larvae attained an average total length of 8.05±0.25mm at the time of their first exogenous feeding. The eyes of *M.nemerus* larvae were not pigmented at 1 dph.This result is similar to many larval studied Cloutier *et al*.(2011), for marble goby, Machinandiarena *et al*., (2003) for red porgy *Pagrus pagrus* and Bagarinao(1986) for seabass,rabbitfish and milkfish. In case of *M. nemerus* the eyes were fully pigmented at 3 dph. The position of the mouth was inferior with teeth appeared on the lower and upper jaw at 4 dph with increase in total length of the larvae.

The increase in mouth size of *M.nemerus* larvae was found to be the fastest at the initial (a few days after commencement of exogenous feeding) larval stage. These findings agreed with other fish larvae studied for example in silver carp, grass carp and big head (Dabrowski and Bardega, (1984)) grow faster within the first few days with the commencement of exogenous feeding. The correlation and marked infelection on the curves of the upper jaw length to total length were found.

In an experiment with *M.nemerus* larvae, Eguia (1998) reported that (without measuring the mouth size) the feed particles sizes that gave optimum growth and survival for weaning larvae were 425 to 600 µm and 600 to 700 µm from 4-12 (dph) onwards, respectivly. These ranges were generally agreed with those estimated in this study. Lakhdar (1998) showed that the best growth of perch larvae was obtained when the particle sizes smaller than 125um and larger than 125 µm were given during the first and the second week after hatching, respectively. In *M.nemerus* larvae a significant and positive correlation of mouth gap with larval total length were found.

The prey size is related to the mouth size of fish larvae and mouth gap increase with larval age so theoptimum and maximum prey size must be in the rang of mouth gap (size). A positive relationship between the total length and mouth gap was found in this study. Kohinoor *et al*. (1995) found that the average diameter of the mouth opening increased from 0.48 mm (TL 4.55 mm) at 2 dph to 1.88 mm (TL 12.75 mm) at 15 dph and 0.67 mm (TL 5.4 mm) at 2 dph and 2.36 mm (TL 26.05 mm) at 15 dph for *Anabas testudineus* (Bloch) an *Clarias gariepinus* larvae, respectivly. In common carp larvae (13 to 31 mm TL) prefer larger food size as fish size increase, the food particle size in the ranges of 125-300 um, 300-500 µm, 300-790 µm, and 500-1000 µm diameter were found to be the most appropriate for carp larvae with TL 13-18,17-22 and 24-31 mm, respectively (Hasan and Macintoch, 1992).

The faster development of barbles in *M.nemerus* larvae indicates its importance in feeding. In a number of fish species, such as Cyprinids and catfishes,barbles serve as important sense organs where taste buds are found,the buds are generally more numerous on the distal part of the barbles (Hepher, 1988). Hepher also reported that barbles with dense sensory buds are sensetive to touching. The fish with barbles find their food with a combination of taste and touch of the taste buds (Peter, 1996).

In this study free nuromsts were not observed in the newly hatched larvae, the reason may be this lavae depend on the commencement of exogenous feeding, free nuromasts have tow functions as mechanoreceptor and chemorec eptor (Welsch and Storch, 1976). Fully developed free bnuromasts were found under the lower jaw by 7 dph and increase in number day after day as the fish grow.

This study showed that the mouth size showed a postive correlation with larval length. With the curve, the determination of suitable diet with appropriate praticle size for the growing larvae become easier. The barbles and free neroumasts developed most possibly as important sensory organs for the larvae. Information on the development of mouth structure should facilitate a better understanding of feeding mode and type of this speciess.

Acknowledgment

We thank the Government of Malaysia through Intensification of Research in Priority Area (IRPA) project No. 49-01-02-04 and International Foundation Science, Sweden (IFS) grant No. A/2204-1 through University Putra Malaysia, for funding this study. Thanks also go to the staff of Aquatic Resources Technology Laboratory, Institute of Bioscience, UPM for their valuable assistance and cooperation.

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