Gut Morphology of Developing Malaysian River Catfish *Mystus Nemurus* (Cuvier and Valenciennes) Larvae

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Abstract: This study was conducted to monitor ontogenetic changes in the gut morphology of Malaysian river catfish, *Mystus nemurus* during larval development. Fish larvae were reared in three 1 ton fiberglass tanks. During the study, the larvae were fed on *Artemia nauplii* at 5 organisms ml feeding^{-1} from the start of exogenous feeding (4 DAH). The morphological development of the gut in *M. nemurus* larvae was observed using a profile projector and a light microscope for a 21 days period. At hatching and during the yolk absorption period, the gut was a simple, straight, undifferentiated tube throughout its length. By 4-5 DAH, the gut differentiated to the esophagus, stomach, and intestine which coincided to the commencement of exogenous feeding. Strong (GL = 0.3179TL-0.1412, R^2 = 0.9284) relationship was found between gut length (GL) and total length (TL) of fish.
[Ghada Ahmed El Hag, Mohd Salleh Kamarudin, Che Roos Saad and Siti Khalijah Daud. **Gut Morphology of Developing Malaysian River Catfish *Mystus Nemurus* (Cuvier and Valenciennes) Larvae.** Journal of American Science 2012; 8(2): 116-121]. (ISSN: 1545-1003). http://www.americanscience.org. 19

Key words: Malaysian river catfish; larvae; gut morphology; *Mystus nemurus*

1. Introduction

Feeding habits are frequently associated with particular body form and functional morphology of skull, jaws and alimentary tract (**Barrington, 1957; Keast & Webb, 1966; Keast, 1970, 1977; Kapoor *et al*., 1976; Hyatt, 1979**). Based on their feeding habits, fish are broadly classified as herbivorous, omnivorous and carnivorous. The foods utilized in the natural habitat, captivity and pond are different, and so are their feeding habits (**Hepher, 1988**). Fish have special morphological characteristics to adapt to their food and feeding habits. These adaptations are principally characterized by the morphology of the gut (**Kapoor *et al*., 1976**).

In recent years, it has often been reported that the different larval stages of many fish species have different essential requirements for a special kind of live food (**Flüchter, 1982**). The degree of relationship between the morphology of the gut of different fish species and food taken varies, and different species with the same type of diet may differ in the structure of the gut. However, the functional adaptations related to the nature of food and feeding habits usually remain similar (**Hepher, 1988**). Some fish have digestive tracts less than one-half the length of their body, and others have tract sizes up to eight times of their body length. The greatest variation in adaptations to diet can be seen in the structure of stomach and length of intestine (**Lovell, 1989**). The length of the intestine is relative to the length of fish body, and variations among different fish species are seemed to be related to their feeding habits (**Hepher, 1988**).

Despite of intensive efforts during recent years, little progress has been made in developing artificial diets for fish larvae. Many fish species still have to be reared on expensive live food during their larval stages (**Verreth *et al*., 1993**). Live food especially *Artemia*, is the best practical solution to the early feeding problem to most fish larvae. However, in most species prolonged use of live food can be costly and variations in its quality can adversely affect survival and growth of fish (**Watanabe *et al*., 1978; Watanabe, 1982; Dabrowski, 1989**). An early change to the artificial diet is therefore desirable (**Jones *et al*., 1993**). In Malaysia, *Artemia* cysts are imported and are always expensive. In addition, cyst hatching needs extra facility, hatchery space and labor (**Kamarudin *et al*., 1999**). Nutritional problems in rearing fish larvae, especially on artificial diets, have led to studies of the development of the digestive system of some fish species (**Walford & Lam, 1993**). Weaning from live food to artificial food needs the development of a wide variety of methods of weaning fish, and knowledge of gut development and digestive capability which should facilitate the determination of the best artificial diet for larval fish and successful weaning strategy.

In fish larvae, the total activity of digestive enzyme is generally low but increases with age (**Dabrowski, 1979; Hepher & Schimer, 1981**). **Kitamikado and Tachino (1960)** found that amylolytic activity in young rainbow trout is quite

high and increases as the fish grows, reaching a peak at the weight of 100g. **Jones *et al.* (1997)** reported that while factors such as gut morphology, genetic control and trophic level act to constrain enzymatic response to diet, abundant evidence indicates that diet may be used to manipulate enzyme activity, at least during larval stage. **Kamarudin *et al.* (1996)** observed higher activities of amylase and proteolytic enzymes (except trypsin) in *Clarias gariepinus* larvae, when they were fed on an artificial 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 morphological 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

Fish larvae were obtained through an artificial spawning from several broodstocks (eight males and four females). Three 1-ton fiberglass tanks filled with 300 liter filtered freshwater, were used for rearing of the fish larvae. Larvae were fed twice a day with newly hatched *Artemia nauplii* of 5 organisms ml feeding^{-1}. Experimental tanks were daily cleaned by the siphoning of all unconsumed feed and fecal materials. 50% of water was also changed daily. The physio-chemical parameters of the water (dissolved oxygen concentration, temperature, NH_3 - N, and pH) were also monitored daily using a dissolved oxygen meter (YSI Model 57), a mercury thermometer (Globe brand), Hach fish farmers test kits, and pH meter (Model SP-9) respectively.

The morphological development of the gut of *M. nemurus* larvae during the early larval stage was daily observed up to three days after hatching. Thereafter, the development was observed at two-day intervals up to 21 days after hatching. 10 larvae were taken at every sampling into petri dishes containing a little water. The measurements of the total length (TL) and gut length (GL) were taken from the images generated by profile projector (Nikon V10) under 10-20 X magnification. The drawings of the gut were made from the images generated from the microscope.

Statistical analysis:
All slopes of linear equation were tested using simple t-tests (Zar,1974).

3. Results

Dissolved oxygen and NH_3-N concentrations, pH, and temperature in rearing tanks, during the study period were 5.9-8.7 mg L, 0.21-0.22 mg L^{-1}, 7.2-7.8 and 25-27.3°C, respectively. The initial total length of the *M. nemurus* larvae during this study was 5.69±0.033 mm and reached 21.93±1.41mm at 21 days after hatching (DAH). At hatching, the gut was a simple, straight and undifferentiated tube throughout its length. The larvae survived on the yolk sac from the 1 to 3 DAH. The mouth was opened at the end of 1 DAH while the upper and lower jaws started to move and the anus started to open at 3 DAH (Fig.1). The yolk sac was completely absorbed and the exogenous feeding started at 4 DAH. Ingested *Artemia* could be seen in the larval digestive tract from 4-5 DAH while contraction began at 5 DAH. From 4-5 DAH, the digestive tract distinctively developed into the esophagus, stomach and intestine.

A pronounced increase in the gut length of *M. nemurus* larvae was observed once the exogenous feeding began (Table I). A true functional stomach was completed by 5-7 DAH. Strong correlations between GL and TL and between GL and larval age were observed (Figs.1. and 2). The equations estimated were:

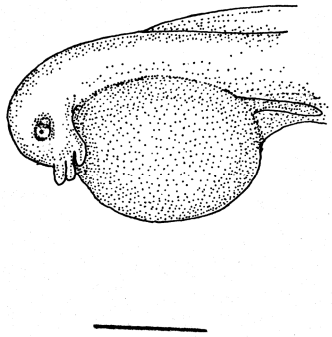
$GL = 0.3179TL - 0.1412$ $r^2 = 0.9248$

$GL = 0.2512\ DAH + 1.2549$ $r^2 = 0.8968$

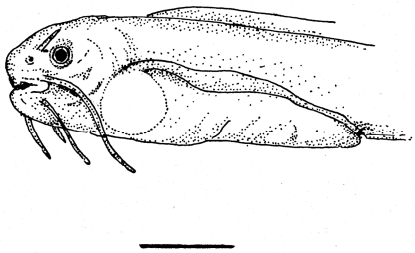
Table I. Mean TL and GL of developing *M. nemurus* larvae.

DAH	*TL (mm)*	*GL (mm)*
1	5.69±0.03	1.9±0.037
3	7.55±0.04	2.07±0.07
5	8.05±0.26	2.28±0.09
7	9.99±0.61	2.57±0.31
9	10.64±0.84	3.7±0.25
11	12.98±0.68	4.04±0.45
13	14.41±0.57	4.3±0.46
15	15.88±1.19	4.95±0.71
17	17.14±1.83	5.39±1.08
19	19.48±0.85	6.06±0.27
21	21.93±1.41	6.86±0.79

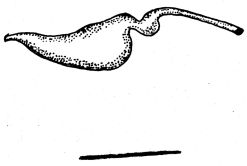
Fig. 1 shows the development in the gut morphology with growth of *M. nemurus* larvae.



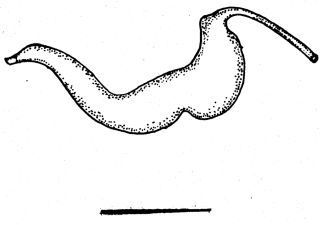
A. 1DAH



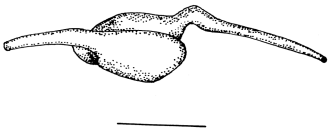
B. 3 DAH



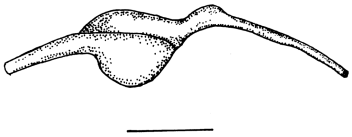
C. 5 DAH



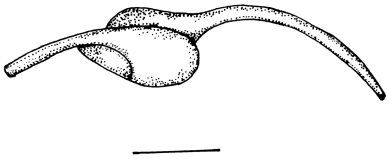
D. 7 DAH



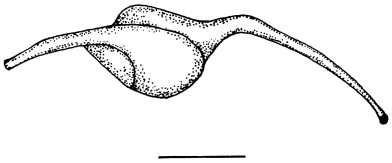
E. 9 DAH



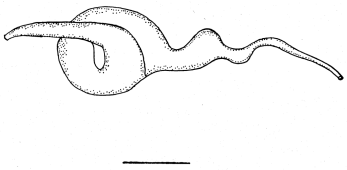
F. 11 DAH



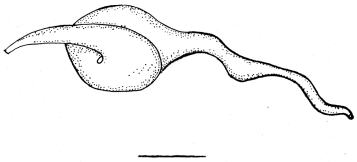
G. 13 DAH



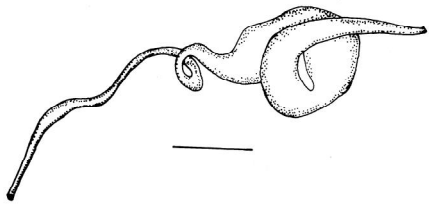
H. 15 DAH



I. 17 DAH



J. 19 DAH



k. 21 DAH.

Fig. 1 Development in the gut morphology with growth of *M. nemurus* larvae Bar= 1mm

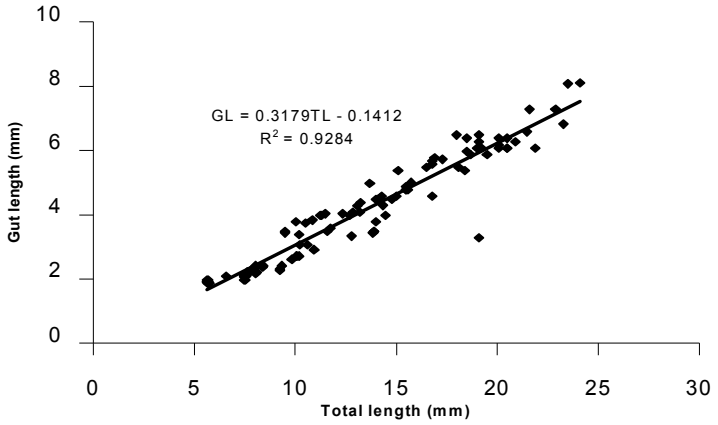


Fig. 2 Linear relationship between gut length and total length of *M. nemurus* larvae

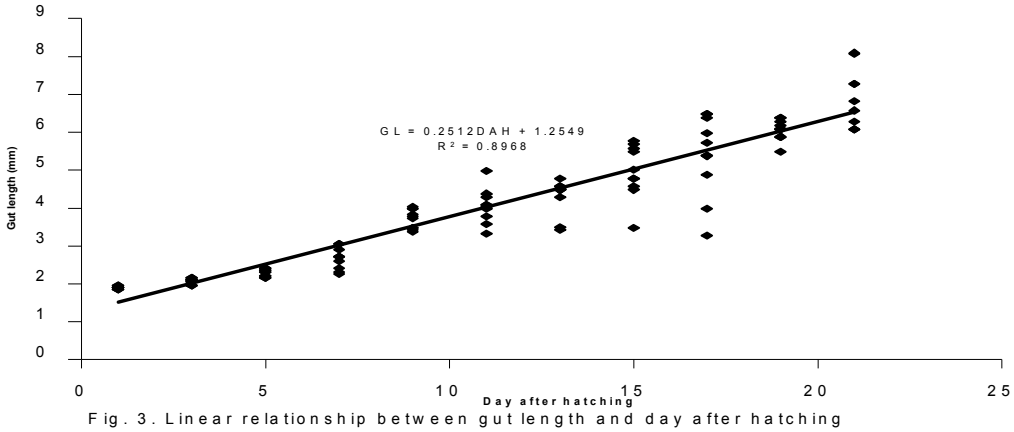


Fig. 3. Linear relationship between gut length and day after hatching of *M. nemurus* larvae

4. Discussion

The morphology of the gut and feeding behavior of fishes vary and the most pronounced distinction among fishes with different digestive processes is the fact that some fish have gastric section (stomach) and some have none (**Lovell, 1989**). The results of this study indicated that by 5 DAH, *M. nemurus* larvae possessed a distinct gastric section without a pyloric caeca this may be during newly stage the digestive systems are poorly developed in newly hatched larvae, with the digestive tube undifferentiated and the anuses are still closed.Many marine and freshwater fishes have pyloric caeca and various functions have been suggested for the pyloric caeca such as an accessory food reservoir, digestive function supplementing that of the stomach and the intestine, absorption of carbohydrates and fats, resumption of water and inorganic ions, and increase intestinal surface area. Absence of pyloric caeca was also reported for walking catfish, *Clarias batrachus* **(Chainabut *et al.*, 1991**).

The differentiation of the gut took place (4-5 DAH) when the yolk has been completely exhausted. **Kamarudin (1999)** reported that some enzyme are not found in the larvae of *M. nemurus* at hatching while pepsin only appears from the 4 DAH and remains low until day 8. The appearance of digestive enzymes at different stages seems to coincide to the development of the larval gut (**Yufera and Darias, 2007**). In milkfish, *Chanos chanos* (Forskal) the gut differentiation is begins just when the yolk sac has been depleted, **Ferraris *et al.* (1987)**. This coincides with the appearance of phosphatase, aminopeptidase and esterase. Comparative studies on the early development of different fish larvae showed that the period of yolk sac absorption coincides with the development and differentiation of certain organs especially the digestive tract (**Watanabe, 1982; 1984**).

A fully functional stomach for *M. nemurus* larvae was completed by 5-7 DAH, after the start of exogenous feeding (4 DAH). *M. nemurus* stomach differentiated and was fully developed on 4-5 and 5-7 DAH, respectively.This result supported when we observe that *M. nemurus* larvae readily accept artificial diets by 4-6 day after the onset of exogenous feeding (4 DAH) which coincides with the differentiation and development of the larval stomach, also when, the morphological differentiation of the gut has occurred, the capacity of the larvae to use exogenous energy sources could be realized. **Dabrowski (1979)** reported that there are several indications that even a simple morphological structure of digestive tract allows the production of digestive enzymes. And in other study **Avila and Jurio (1987)** suggested that, the capacity of the larvae to use exogenous energy sources was begin when the morphological differentiation of the gut has occurred.

At 5 DAH the stomach of *M. nemurus* larvae was not capable of taking large amount of food, but the capability of taking large amount of food increased with the larval growth. This could be due to the total stomach contents increases with the increase of body length in *M. nemurus* larvae. **Eguia (1998)** noted that *M. nemurus* larvae readily accept artificial diets by 4-6 day after the onset of exogenous feeding (4-6 DAH) the different result in our study may return to different in tempreture.

Similar to most fish larvae the gut of *M. nemurus* larvae is initially simple. This means that the fish larvae may face some problems in utilizing artificial diets at first feeding, and therefore require special diets during its early larval stage. Based on the results of this study, the size and the amount of food should be increased according to the increase in length and capacity of the gut.

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 Universiti 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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