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Embryonic and Larval Development of River Catfish, *Hemibagrus nemurus* (Valenciennes, 1840)

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ABSTRACT

The aim of this study was to characterize embryonic and larval developmental stages of the river catfish, *Hemibagrus nemurus*. Fertilized eggs were spherical, adhesive and demersal with a mean egg diameter of 1.5±0.3 mm. Seven embryonic periods were characterized for timing and features: zygote, cleavage, blastula, gastrula, segmentation, pharyngula and hatching. Mean hatch was 23±1 h post fertilization at 27°C. The newly hatched larvae measured 3.0±0.2 mm in total length. Morphogenesis was completed in a day. The yolk sac was completely absorbed in three days. *H. nemurus* has a short embryonic developmental period in comparison with other catfish species. The information obtained from this study will be useful for egg incubation and larval rearing during the culture of *H. nemurus*.

Key words: Catfish, early development, embryology, *Hemibagrus nemurus,* larval development

INTRODUCTION

It is pertinent to have knowledge of embryonic and larval development in different fish species to better understand the biology of different fish species in the areas of organ development, nutritional needs and environmental preferences (Koumoundouros *et al*., 2001; Borcato *et al*., 2004). Egg and larval developmental studies of fish are also useful for understanding systematic or genetic relationship useful for identification of spawning sites (Meijide and Guerrero, 2000). Disruption or abnormalities in developmental stages of embryos and larvae are often considered as indicators of disturbances in the environment. These stages are also useful in ontogenic and phylogenetic studies (Legendre and Teugels, 1991; Verreth *et al*., 1992). Embryology and larval development in cultured fish is important and evident in aquacultural practices and fish production. Specifically, needs of each larval stage and use of such information are key steps for increasing larval growth and maximizing survival (Puvaneswari *et al*., 2009).

Early life of fish is characterized by various cellular and morphological changes. Fish larvae are dependent on adequate and proper nutrition, especially when the yolk sac is absorbed affecting the survival rate of larvae. Linking larval quality to feeding can be obtained through embryonic and larval developmental studies. Larval developmental studies are therefore useful for linking favourable culture conditions to morphology at larval stages (McFadzen *et al.,* 1994; Wittenrich *et al.,* 2007). Similarly, relationships are apparent between embryonic development,

such as cleavage patterns and mortality in fish (Avery *et al.*, 2009). Abnormal embryonic development during early developmental stages can increase mortality up to hatch and a few days after hatching. Morphology during embryogenesis has been shown to be an indicator of embryo quality (Penney *et al.*, 2006; Avery *et al.*, 2009). As well, useful information on eco-toxicology could be obtained from early life history because fish species are sensitive to changes in dissolved oxygen, salinity, water contaminants, turbidity, alkalinity and pH at these early stages of development (Pereira *et al.*, 2006). Thus, it is demonstrated that early life history information can reveal problems associated with embryonic and larval development in various habitats and from breeding and culture practices (Jafari *et al.*, 2010).

Hemibagrus nemurus is a river catfish found in most Asian countries. It is economically important for commercial fisheries and aquaculture and it has high food value because it is rich in omega 3-ployunsaturated fatty acid (Rainboth, 1996; Mesomya *et al.*, 2002). Embryonic and larval development studies on some catfish species have been documented but no information is available for *H. nemurus*. This will be the first study on embryonic and larval development in *H. nemurus*. The aims of this study were to provide information on early life history, first feeding and ontogeny. Fish farmers can utilize the information to improve culture of *H. nemurus*.

MATERIALS AND METHODS

Egg collection and environmental condition: Eggs and milt used for this study were obtained from F1 generation broodstocks caught from the wild in Pahang river, Pahang, Malaysia. Three females and five males with body weights 1.0-1.2 kg were used. Females were induced to spawn using 0.5 mL kg^{-1} ovaprim hormone. Artificial fertilization was done using the dry method, by spreading the milt over the eggs and thorough mixing with addition of 10-20 mL of salt water (35 g L^{-1}) (Cerqueira, 2005). Milt from all males was pooled and used to fertilize the eggs from each female separately. Three female fish were used for this study (n = 3). After fertilization, 900 eggs from each female were washed with 0.9% NaCl solution (saline solution) and 300 were incubated in each of nine 5 L plastic aquaria. Thus, the experiment was done in triplicate (i.e., three replicate aquaria for each female) and also included a control group that consisted of 300 fertilized eggs that were studied without a temperature control chamber. Eggs were incubated at 27°C and water was changed every two h by siphoning the water and replacing it. Timing of development and morphological features were used to identify development stages according to Kimmel *et al.* (1995).

Sampling: Twenty eggs were sampled in triplicate from each aquarium and transferred to petri dishes (n = 600). Sampled eggs were observed every 15 min until hatching and after hatch, larvae were sampled twice a day for observation. Observation of eggs was done under a light microscope in a standardized temperature control chamber maintained at 27°C. After observations, sampled eggs were placed in a 1000 L aquarium.

Image capture and analysis: Embryonic and larval development were observed by taking digital images using Leica DM 750 light microscope attached with a Leica digital camera ICC 50 and analyzed with Leica Application Suite Software (LAS EZ Version 2.0). Measurement of egg and larvae were done by using ocular micrometer. The time of appearance of each developmental stage was recorded. Data were statistically analysed by using Statistical analysis software SAS 9.2. Data of eggs in different incubation tanks and the control tank were compared using Students t-test and chi-square test and $\alpha = 0.05$ chosen as the level of significance.

RESULTS

There was no significant difference ($p>0.05$) from the observed fertilized eggs and the control group in development rate to hatching in the fish samples used (n = 600). The different stages of embryonic and larval development of *H. nemurus* were described using according to Kimmel *et al.* (1995). Table 1 shows the summary of developmental timings and diagnostic characteristics of *H. nemurus* fertilized egg during embryonic and larval developmental stages. Seven embryonic developmental stages were observed. These were: zygote, cleavage, blastula, gastrula, segmentation, pharyngula and hatching stages. Each stage had unique anatomical and physiological characteristics. The ontogenic events observed in *H. nemurus* were similar to most catfishes (Islam, 2005; Osman *et al.*, 2008; Puvaneswari *et al.*, 2009).

Table 1: Embryonic and larval developmental features of river catfish, *H. nemurus*

Stage of development	Period of development	Time of development (h post fertilization)	Features
Embryonic	Zygote	0:15	Blastodisc formed, single cell embryo
	Cleavage	0:30	First cleavage, 2-celled embryo
		0:45	Second cleavage, 4- celled embryo
		1:00	Third cleavage, 8 celled embryo
		1:15	Fourth cleavage, 16- celled embryo
		1:30	Fifth cleavage, 32-celled embryo
		1:45	Sixth cleavage, 64-celled embryo
	Blastula	2:00	Morula, 128-celled embryo
		2:15	Early disco-blastula, 256 celled embryo
		2:30	Yolk syncytial layer appeared
		2:45	Mid-blastula, blastodisc compressed yolk
		3:00	Late blastula, 30% epiboly
	Gastrula	3:30	Early gastrula, germinal ring formed
		4:15	Embryonic shield formed
		6:15	Mid-gastrula, 70% epiboly
		7:25	Late -gastrula, 90% epiboly, brain rudiment thickened, notochord rudiments distinct
		8:30	Neurula, 100% epiboly, blastopore closure
		9:00	Bud stage
	Segmentation	9:35	First somite appeared, tail appeared, early movements, primary organogenesis
		10:50	Cephalic differentiation and organization, optic vesicle, neural tube visible, olfactory placodes
		18:30	Tail development, yolk mass reduces, movement, 20-somite
		18:35	25-somite, vigorous movement, heart rudiment
	Pharyngula	18:40	Movement, heartbeat, blood circulation, eye lens
		18:50	Separation of caudal section from the yolk
		21:40	Free trunk tail
	Hatching	23:00	Hatching, chorion shell was broken, free larvae
Larval	One day	48:00	Completion of morphogenesis, eye pigments small head, mouth not yet opened
	Three days	96:00	Larvae fully developed, mouth opened, yolk sac completely absorbed

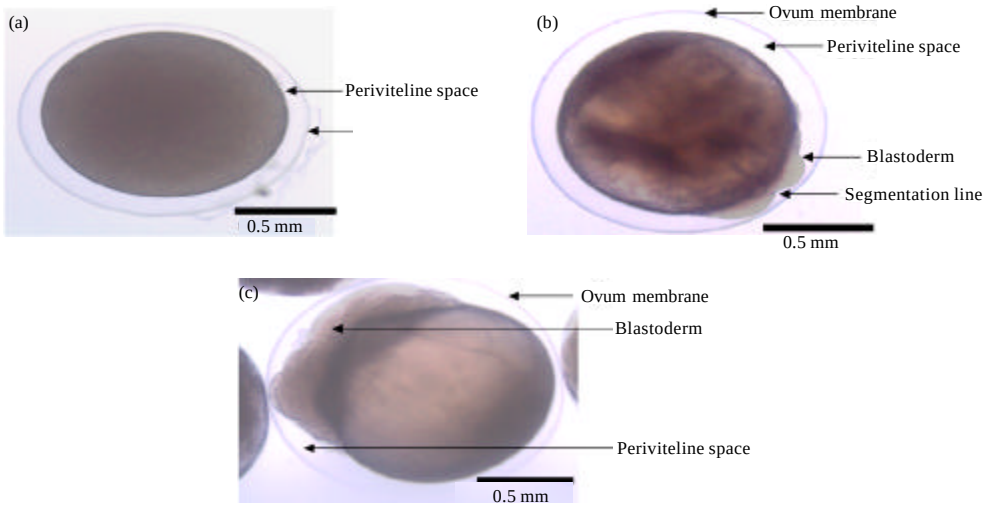

Fig. 1(a-c): Embryonic developmental stages of river catfish, *H. nemurus*; (a) Fertilized egg, (b) Two-celled stage and (c) Discoblastula

Zygote and cleavage stages: The eggs of newly spawned *H. nemurus* were brownish in colour and had diameters that ranged from 1.0 to 1.4 mm. The fertilized eggs of *H. nemurus* were spherical in shape, adhesive and demersal with perivitelline space. The eggs increased in size after fertilization. In this study, the mean diameter of fertilized eggs was 1.5±0.3 mm (Fig. 1a). In the zygotic stage, the egg had a blastodisc which formed at the animal pole. Afterwards, different cleavage stages were observed with timings provided in Table 1. The formation of the blastodisc is noticeable at this time (Fig. 1b).

Blastula stage: The blastomeres were smaller in size, yolk syncytial layer began to form, the animal-vegetal axis of the blastula shortened, the blastodisc compressed the yolk cell and the blastoderm began to cover the yolk cell moving from the animal pole to the vegetative pole (Fig. 1c). The germinal ring and embryonic shield began to form.

Gastrula stage: The germinal ring and embryonic shield were evident at the early stages of gastrula. The early gastrula had blastoderm which covered about two-third of the yolk. The germinal ring was clearly visible and the embryonic shield was evident. At the mid-gastrula stage; the blastoderm was 70% of the entire distance between the animal pole and the vegetal pole. In the late gastrula stage (Fig. 2a) 90% of the entire distance between the animal pole and the vegetal pole was covered by the blastoderm, brain rudiments thickened and notochord rudiment was distinct from segmental plate. The neurula stage was reached. Blastopore had already closed and the bud stage was observed. The tail bud was now distinct. The notochord rudiment was separated from the neural keel and 100% epiboly was observed at this stage.

Segmentation stage: The embryo began to differentiate, the first somite appeared, neuromeres developed, early movements of tail were observed, primary organogenesis began and the pharyngeal arch primordia were developed. Cephalic organization and differentiation were

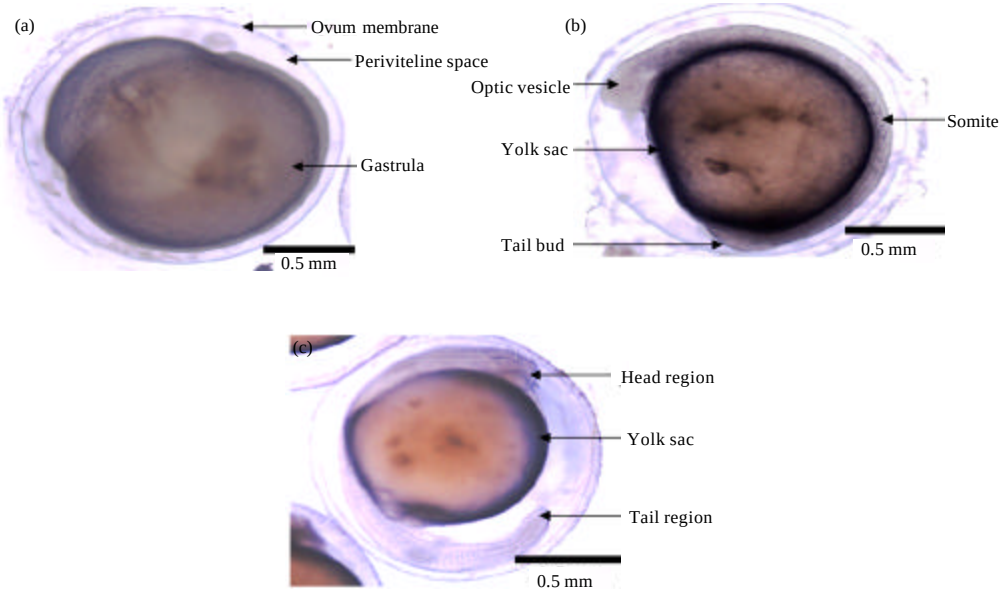

Fig. 2(a-c): Embryonic developmental stages of river catfish, *H. nemurus*; (a) Late gastrula, (b) Six to eight somite stage and (c) Tail separation stage

observed. Notochord and somites were visible, olfactory vesicle, optic vesicle, tail vesicles, neural tubes and brain neuromeres were developed (Fig. 2b). The head and tail regions of the embryo were distinct at this stage. Following this development, growth of the caudal area was evident. Tail and caudal development was initiated, yolk mass was reduced, caudal tail started to separate from the yolk (Fig. 2c), about 20 somites was observed, movement of embryo was also observed. This was the first sign of embryo movement. Spontaneous movement of the embryo was observed as a result of repeated tail contractions, number of somites increased to about 25. Heart rudiment was observed at this stage and the embryo occupied almost all the space in the egg.

Pharyngula stage: The pharyngula was formed, movement of the embryo was more frequent and vigorous, heart beat and blood circulation were observed even though the blood plasma was colourless, the eye lens and auditory vesicles were developed, fin buds were also observed, the egg space was fully occupied by the embryo and free trunk tail was observed at the end of the pharyngula period.

Hatching: Hatching occurred at the end of the pharyngula period at 23:00±1 h post fertilization (pf). Figure 3a shows a newly hatched *H. nemurus* larva. The heart was fully developed; heart beats and blood circulation were observed. The larva had a transparent slender body with a roundish yolk sac. Cartilage developed in the head and pectoral fin. The larva had completed morphogenesis of primary organs and systems at this stage. The larva was free from the chorion shell. The brain was present and hatching glands were present in the epidermis of the head, pectoral fin and tail. The mouth was not fully formed and was ambiguous, the fins were also indistinct and the branchial skeleton was not fully developed. The head was not distinctly separated from the yolk sac. The head and yolk sac looked like a bulb-like structure. The eyes were not

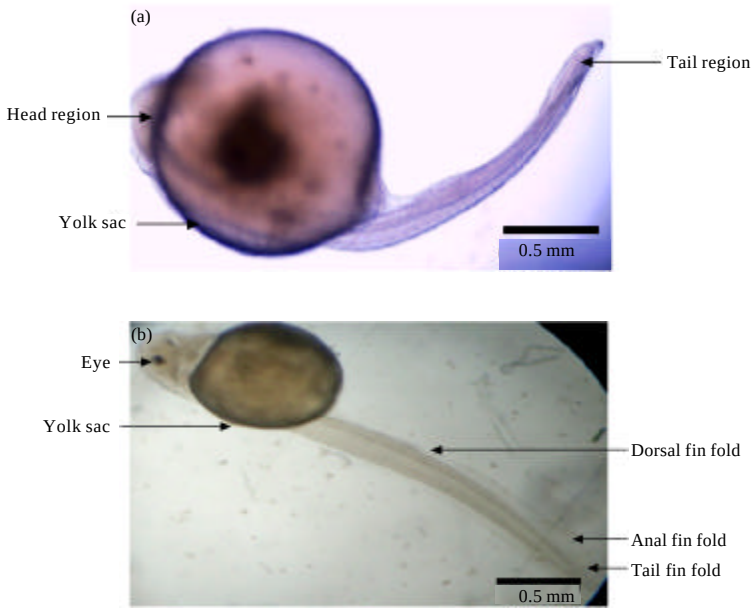

Fig. 3(a-b): Larval developmental stages of river catfish, *H. nemurus*; (a) Newly hatched larva and (b) One day old larva

pigmented and the newly hatched larvae were active. The sizes of the newly hatched larvae were 3.0±0.2 mm. The post hatchling stages were also observed during the study so as to have an insight into the early larval stage developments of *H. nemurus*. After hatching, it was observed that pigmentation of the eyes proceeded. At 24 h post hatching (ph), one day old larva had pigmented eyes (Fig. 3b). The dark spots were observed on the eye vesicles. The eyes at this stage was developed and pigmented. Fin folds were differentiated, a thin membrane fin fold was observed around the caudal region and yolk sac. Anal fin fold around the tail region was conspicuous. The mouth was still yet to be fully developed; circulation of body fluid was observed around the notochord, brain and yolk. The yolk sac was reduced and a prominent eye spot was seen on the anterior part of the head. The upper and lower jaws were formed and the mouth was not yet opened. A one-day-old larva increased in size and measured 4.5±0.1 mm in total length. There were no barbels. At three days post hatching, the larvae of *H. nemurus* in this study increased in body length and measured 6.0±0.2 mm and yolk sac was absorbed at this stage. The eyes were fully developed. The mouth was opened and fully developed. Pairs of barbels were observed.

DISCUSSION

The egg of *H. nemurus* was similar to eggs of other catfishes like *Mystus montanus*, *Mystus cavasius*, Thai pangas, *Pangasius sutchi*, channel catfish, *Ictalurus punctatus* and Indian catfish, *Heteropneustes fossilis* (Arockiaraj *et al*., 2003; Islam, 2005; Puvaneswari *et al*., 2009). However, silver catfish Jundia, *Rhamdia quelen* and striped snakehead fish, *Channa striatus* have eggs that are non-adhesive (Pereira *et al*., 2006; Marimuthu and Haniffa, 2007; De Amorim *et al*., 2009). Comparing the egg of *H. nemurus* with other catfishes, the egg of *I. punctatus* (3.5-4.0 mm) and *R. quelen* (2.46±0.11 mm) were bigger (Scott and Crossman, 1998; De Amorim *et al*., 2009).

A smaller fertilized egg diameter was observed in *M. cavasius* of 0.5 mm and the fertilized egg diameter of *P. sutchi* and *C. striatus* were similar to *H. nemurus* (Islam, 2005; Marimuthu and Haniffa, 2007).

Blastodisc of *H. nemurus* egg was formed 15 min pf. The blastula stage of *R. quelen* was observed at 3:00 h pf (De Amorim *et al.,* 2009) and was similar to *H. nemurus*. Puvaneswari *et al.* (2009) recorded the time for blastula period in *H. fossilis* as 3:30-4:00 h pf. High and low blastula stages were observed at 2:30 and 4:00 h, respectively *P. coruscans* (Cardoso *et al.*, 1995). In *C. striatus* the blastula period occurred between 5:00-6:00 h pf (Marimuthu and Haniffa, 2007). This was in contrast with *H. nemurus*.

Similarly as observed in this study, the germinal ring and embryonic shield of *M. montanus* were evident between 3:00 and 5:00 h pf (Arockiaraj *et al.*, 2003). In contrast, it took longer or shorter time for the germinal ring and embryonic shield to be evident in *P. sutchi, R. quelen, M. cavasius, C. striatus, P. coruscans* and *H. fossilis* (Cardoso *et al.*, 1995; Islam, 2005; Marimuthu and Haniffa, 2007; De Amorim *et al.*, 2009; Puvaneswari *et al.*, 2009).

The head and tail regions of *M. montanus* were distinct at 7:00 h pf which was shorter period compared to that observed in *H. nemurus* which took 10:50 h. The optic vesicles appeared in the 15-somite stage at 14:00-16:00 h pf in *M. montanus*, whereas optic vesicles appeared at 10:50 h in *H. nemurus*. At 17:45 h the embryo occupied almost all the space in the egg in *M. montanus,* whereas *H. nemurus* embryo occupied almost all the space at 18:35 h pf (Arockiaraj *et al.*, 2003). Cardoso *et al.*, 1995) reported that the optic vesicles of *P. coruscans* were observed at 7:30 h pf; and the otolith and olfactory pits were observed at 14:30 h pf. According to Islam (2005), first cleavage stage, embryonic shield, tail region, neural grooves and somites were observed within 9 h pf in *P. sutchi*. In *H. nemurus*, first somite appeared at 9:35 h, whereas it appeared at 9:00 h in *P. sutchi*. Movable tails were observed in *P. sutchi* at 12:00 h pf which was shorter time compared to that observed in *H. nemurus* at 18:30 h. Notochord was observed at 8:00 h pf and the differentiation of the head and tail region was observed at 9:30 h pf in *M. cavasius*.

First somites appeared in *C. striatus* at 10:30-11:00 h pf, the optic cups and eye vesicle were observed at 14 h and the embryo occupied almost all the egg space at 18:00 h pf. The tail began to separate from the yolk, 12-15 somites were observed, embryonic fin folds were visible and movements were observed (Marimuthu and Haniffa, 2007). De Amorim *et al.* (2009) observed that the tail of *R. quelen* was separated from the yolk at 14 h pf. In *H. fossilis*, embryo differentiation began at 8:00-10:00 h pf. The first somites appeared at 10:00 h pf. At 12 h, the number of somites increased to six or eight somites and optic cups were observed. Three-fourth of the egg space was occupied by the embryo at 13:00-15:00 h pf and the notochord was clearly observed. At 17:00 h the tail was separated from the yolk, the whole egg space was occupied by the embryo, somite increased to twelve to fifteen and blood circulation was observed. Movement of embryo was observed at 18:00 to 20:00 h (Puvaneswari *et al.*, 2009). At 20:00 h, 20 somites, olfactory placodes and heart beats were observed in *C. striatus*. Two hours later, the somites increased to 25 and the embryo occupied the entire egg space at 22:00 h pf. The tail end was free and movement of embryo were more frequent (Marimuthu and Haniffa, 2007). In *H. fossilis,* the heart beat was observed, eye lenses were formed, blood circulation was observed at 20:00 h pf and at 22:00 h pf, the embryo had 22-25 somites, the olfactory pit and auditory vesicles were visible at this period (Puvaneswari *et al.*, 2009).

Eggs of *H. nemurus* would hatch in one day. Hatching in most catfishes usually occur within 1-2 days (Cardoso *et al.*, 1995; Arockiaraj *et al.*, 2003; Osman *et al.*, 2008; Puvaneswari *et al.*, 2009;

Table 2: Body lengths of catfish larvae at one day old

Fish species	Body length (mm)	References
Mystus montanus	4.40±0.21	Arockiaraj *et al.* (2003)
Pangasius sutchi	5.57	Islam (2005)
Rhamdia quelen	4.18±0.19	De Amorim *et al.* (2009)
Heteropneustes fossilis	4.00±0.2	Puvaneswari *et al.* (2009)
Hemibagrus nemurus	4.50±0.1	Present study

Islam, 2005; De Amorim *et al.*, 2009). A similar result was observed in *M. montanus* and *H. fossilis*. Hatching occurred within 23-24 h pf, (Arockiaraj *et al.*, 2003; Puvaneswari *et al.*, 2009). On the contrary, hatching occurred in less than a day (19:00 h pf) in *P. coruscans* (Cardoso *et al.*, 1995). Hatching of *Clarias gariepinus* occurred at 40 h pf which was close to two days (Osman *et al.*, 2008). Hatching occurred in less than a day in *M. cavasius* at 19±2 h pf. Newly hatched larvae measured 1.28 mm. The size of hatchling and time of hatching were in contrast with observations in *H. nemurus*.

Temperature can affect embryonic developmental period of fish. Lower temperature tends to increase the duration of embryonic development. For instance, in *P. sutchi,* hatching will normally occur at 24 h post fertilization at 26°C (624 degree-h). However, at lower temperature of 20°C, hatching will be around 32 h (640 degree-h) (Islam, 2005). Based on the observations on *H. nemurus*, hatching occurred between 23-24 h post fertilization at 27°C (621-648 degree-h). Temperature changes can affect the time of hatching of *H. nemurus*.

The observations on one day old larvae of some catfish species were similar to *H. nemurus* (Table 2). A day old larvae of *M. cavasius* were smaller than those of *H. nemurus*. The yolk sac was reduced and two pairs of barbels were observed. De Amorim *et al.* (2009) reported that one-day-old *R. quelen* had optical cups, notochord, embryonic fin, myomeres and developed circulatory system similar to *H. nemurus*. Barbel-like structures were also observed in one-day-old *R. quelen* at one day which was in contrast with this study. No barbel-like structure was observed in *H. nemurus* at one day ph. Puvaneswari *et al.* (2009) observed similar features in one-day-old larvae of *H. fossilis*. Also, barbels were noticed in *H. fossilis*; but this was contrary to observation on one-day-old larvae of *H. nemurus*, no barbels were observed at this stage. Ogunji and Rahe (1999) reported that mouth opening in *Heterobranchus longifilis* (Valenciennes) occurred at 3-4 h ph. This was not evident in *H. nemurus*.

Yolk sac was absorbed on the third day in *H. nemurus*. Similarly, yolk sac absorption was completed on the third day in *P. sutchi, C. striatus, M. montanus and Mystus macropterus* (Bleeker) (Wang *et al.*, 1992; Arockiaraj *et al.*, 2003; Islam, 2005; Marimuthu and Haniffa, 2007). Some fish species may take longer period to absorb the yolk sac. *I. punctatus* could take up to ten days for completion of yolk sac absorption. Long period for yolk sac absorption might be due to the effect of water temperature on the yolk sac which is usually large (Scott and Crossman, 1998). *R. quelen* completed yolk sac absorption in 5 days (De Amorim *et al.*, 2009). Some fish species could also absorb the yolk sac in less than three days. This was observed in the *H. longifilis*. Yolk sac absorption occurred at 55 h post hatching Ogunji and Rahe (1999).

The larvae of *C. striatus* at 3 day ph measured 5.8 mm. Pectorals were visible, the head was more conspicuous, free movements of the eye balls were noticed, blood circulation was observed, yolk sac was completely absorbed and the mouth was opened (Marimuthu and Haniffa, 2007). Mouth opening was observed in *C. striatus* at 36 h ph. At three days ph of *R. quelen*, the larvae

measured 5.19±0.17 mm. The yolk sac was reduced and not fully absorbed. It took 5 days for the yolk sac to be completely absorbed (De Amorim *et al*., 2009). According to Puvaneswari *et al*. (2009), 3 day post-hatched larvae of *H. fossilis* conforms to the observation on *H. nemurus* except that the larvae of *H. fossilis* were smaller in size.

The transition from endogenous feeding which occurs when the yolk sac is not yet absorbed into exogenous feeding which occurs when the mouth is opened and yolk sac is absorbed is a very important stage in larval development. This is because fish farmers would be able to know the appropriate time for feeding of the larva. Larvae mostly begin exogenous feeding when the yolk sac absorption is complete and the mouth is fully developed and opened. High larval mortality rate could occur during the transition period i.e., change from endogenous to exogenous feeding which is a critical stage in the development of larvae (Sarasquete *et al*., 1995). In *H. nemurus,* exogenous feeding started after three days ph when the yolk sac was fully absorbed.

CONCLUSION

H. nemurus has a short embryonic development period and sense organs develop rapidly. This makes it a suitable fish species for aquaculture. Yolk sac was completely absorbed at three days post hatching and fish farmer are expected to start feeding the larvae at this time. Developmental studies would enable us to understand the early embryonic developmental stages and reproductive behaviour of *H. nemurus* as a whole. The study provides essential information on the embryonic and larval development of *H. nemurus* and such information could also be beneficial for comparative studies and as a basis for further studies on the ontogeny of *H. nemurus*.

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