Bagrid Catfish Mystus nemurus仔魚における外部形態、感覚器官および行動の発達

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Development of Morphology, Sensory Organs and Behavior of Larval Bagrid Catfish *Mystus nemurus*

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**Abstract:** Development of morphology, sensory organs and behavior were described for bagrid catfish *Mystus nemurus* larvae from 0 to 15 days after hatching reared at 28-29 °C. First feeding with rotifer (*Brachionus plicatilis* sp. complex) was observed at 36 hours after hatching (hAH) when the yolk sac absorption was 66.1% and nearly completed at 72 hAH. At this time, the larvae displayed aggregation and foraging behavior as well as negative thigmotaxis and positive rheotaxis. These behaviors corresponded with the development of semicircular canals, disappearance of adhesive characteristics at the tips of the maxillary barbel, increasing growth of pectoral fin, opening of mouth and anus as well as peristaltic movement of the intestinal tract. Negative phototaxis and day time foraging coincided with the appearance of deep pigmented eye, single cone cells and optic nerve while night time feeding possibly relied more on taste buds, olfactory organ and free neuro­masts. Cannibalistic behavior was first observed at 54 hAH and this corresponded with the initial appearance of teeth. In this study, relationship among the development of morphology, sensory organs and behavior provides fundamental understanding which suggest possible improvement of larval rearing techniques for bagrid catfish.

**Key words:** *Mystus nemurus*; Cannibalism; First feeding; Larval development

Bagrid catfish *Mystus nemurus* (Cuvier & Valenciennes) (syn. *Hemibagrus nemurus*) is an important freshwater catfish especially in Southeast Asia (Amornsakun et al. 1997, 1998). It has been a favourite owing to its excellent taste and nutritional content (Mesomya et al. 2002; Widjaja et al. 2009), thus has higher market value compared to other commercially cultured catfishes such as African catfish *Clarias gariepinus* (Burchell) and sutchi catfish *Pangasianodon hypophthalmus* (Sauvage) especially in Malaysia, Indonesia and Thailand. In this regards, there is a growing attempt for culturing bagrid catfish in Southeast Asia with high potential as global aquaculture candidate in the future (Tantikitti and Chimsung 2001).

Present culture of bagrid catfish relies on the inconsistent supply of wild seedling (Tantikitti and Chimsung 2001). Although seeds can be artificially produced, early starvation and cannibalism cause low survival, hence the mass production remains unreached. This often occurs in raising larval fish in captivity as well as in other species due to insufficient knowledge of the early life history and its ecological adaptations (Kawamura and Munekiyo 1989). Understanding the morphogenesis changes provide vital information on the fish biology and environmental preferences of the larvae in establishing rearing techniques. Previous studies focused on the development of sensory organs which are particularly important in food detection and
predator avoidance (Matsuoka 2001; Uyan et al. 2006). In accordance with the development of morphology, these factors affect the changes in behavior and in turn facilitate in providing suitable rearing techniques to improve larval survival in captivity (Kawamura and Ishida 1985; Senoo et al. 1994). In this regards, the relationship among the development of morphology, sensory organs and behavior of artificially produced bagrid catfish were investigated.

Materials and Methods

Artificial reproduction was conducted in Aug 2009 at the Center of Collaborative Research in Aquaculture, Universiti Malaysia Sabah-Kinki University in Malaysia. Final oocyte maturation of 500 g female brood fish was initiated with two intraperitoneal injections of Ovaprim (0.5 ml/kg, Syndel International Inc., Canada) at 6 h interval. Male received only one hormonal injection (0.25 ml/kg) at the same time as the second injection for female. At ovulation time (about 8 h after the second injection at 28–29°C), eggs from female brood fish were stripped into a dry bowl by applying a gentle pressure on the abdomen. Milt from male were stripped and inhaled into a vinyl tube (inner and outer diameter; 4 and 6 mm, respectively) connected to a 60 ml plastic vial before mixing with the eggs to initiate fertilization. Fertilized eggs were spread onto a net with the mesh opening of 1 mm and placed in a 1,000 I incubation tank until hatching. The tank was kept under natural light condition (12 h light; 12 h dark) with temperature, dissolved oxygen and pH measured at 28–9°C, 5–6 mg/l and 6–7, respectively.

Newly hatched larvae were reared in an 80 l recirculatory transparent aquarium with stocking density of 30 individuals/l. The recirculatory system was only initiated at 18 hours after hatching (hAH) when the larvae were able to maintain a 3-dimensional swimming position. The larvae were fed with rotifer (Brachionus plicatilis sp. complex) at 30 hAH followed by Artemia nauplius during 2 to 4 days after hatching (dAH), Moina (4 to 7 dAH) and artificial feed (Otohime B2 and C1, Biokyowa, Japan) containing approximately 50% protein and 10% lipid (7 to 15 dAH) at 07:00, 11:00, 15:00 and 19:00.

Ten larvae were sampled and anaesthetized with Transmore containing α-methyl quinoline as active ingredient (Nika Trading) at 6 h intervals from 0 to 3 dAH and at 1 d intervals from 4 to 15 dAH. These samples were used for observation of morphology conducted under a light microscope (Model Eclipse E600, Nikon, Japan) and measurements of total length (TL), mouth height (MH) and yolk sac volume (YSV) were taken from digital image using Image J image processing software. Mouth height was calculated as \( \sqrt{UJL^2 + LJL^2} \), where \( UJL \) = upper jaw length, \( LJL \) = lower jaw length (Guma’a 1978). Yolk sac volume was calculated as \( 4/3 \pi r_1^2 \times r_2/2 \), where \( r_1 \) = minor axis, \( r_2 \) = major axis (Fukuhara 1986). Additional 30 fish were sampled, anaesthetized and fixed in Bouin’s solution for histological analyses of eye, taste buds, olfactory organ, free neuromasts and inner ear developments. Haematoxylin-eosin staining of 6 μm cross and sagittal sections were observed under light microscope.

Changes in feeding and swimming behavior were monitored from the outside of the transparent rearing tank at every feeding time for 10 min. Immediate phototaxis, rheotaxis and thigmotaxis responses were also observed on 50 larvae placed in a 1,000 ml beaker at every sampling time (6 h intervals from 0 to 3 dAH and at 1 d intervals from 4 to 15 dAH). Phototaxis behavior was determined by observing the swimming response when an LED light was flashed at one side of the beaker. Rheotaxis behavior was determined by observing the swimming response to a weak current created by a glass pipette. Thigmotaxis behavior was assessed by observing the swimming response towards a mechanical stimulus by a glass pipette.

Results

Development of morphology, sensory organs and behavior

Only prominent developments were presented. Changes of total length, mouth height,
yolk sac volume and morphological characteristics of bagrid catfish larvae were presented in Figs. 1-4, accordingly. In newly hatched larvae (mean ± SD, 4.61 ± 0.09 mm in total length, TL), pectoral fin was unapparent. The digestive system was also undeveloped with unapparent mouth and anus cavity as well as thin-walled, narrow and straight intestinal tract. First pair of barbel (maxillary) was apparent with adhesive characteristics at the tips. At this stage, sensory organs were undeveloped where the eye was unpigmented and the retinal cells were undifferentiated (Fig. 5a). In addition, larvae had

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**Fig. 1.** Changes in total length of *Mystus nemurus* larvae from 0 to 15 dAH.

**Fig. 2.** Changes in mouth height of *Mystus nemurus* larvae from 0 to 15 dAH.

**Fig. 3.** Changes in yolk sac absorption of *Mystus nemurus* larvae from 0 to 72 hAH.

**Fig. 4.** Changes of morphological characteristics of *Mystus nemurus* larvae. a, 0 hAH; b, 6 hAH; c, 12 hAH; d, 18 hAH; e, 24 hAH; f, 30 hAH; g, 36 hAH; h, 54 hAH; i, 72 hAH; j, 5 dAH; k, 10 dAH; l, 13 dAH. Bars = 1 mm.
The eye became slightly pigmented but only at a small part of the outer layer (Fig. 5b). At this stage, the larvae showed disoriented movement with twirling manner on yolk sac or sideways. Some larvae showed negative phototaxis and swam away from the light source while all of them had mild positive rheotaxis response towards water current. No thigmotaxis response was observed at this stage.

At 12 hAH (5.03 ± 0.10 mm), rudimental hypural, third pair of barbel (mandibular) and a pair of nostril became apparent. Lower jaw showed slow movement and the intestinal wall thickened with increased lumen width. At this time, two pairs of free neuromasts were observed at the anterior and posterior part towards the eye. For the inner ear development, the auditory vesicle enlarged and formed two prominent semicircular canals. At this stage, more larvae exhibited negative phototaxis response while stronger positive rheotaxis response was observed. All larvae showed mild negative thigmotaxis response when they were stimulated with the glass pipette.

At 18 hAH (5.32 ± 0.22 mm), yellowish fluid was observed along the intestinal tract. The retina of the eye differentiated into pigmented epithelium, outer nucleus, outer plexiform, inner nucleus, inner plexiform and ganglion layer (Fig. 5c). At the same time, single cone cells (Fig. 5d) and optic nerve (Fig. 5e) were also observed. Small taste buds can also be seen on the lips and barbels of the larvae. The protrusion of the semicircular canals of the inner ear was further divided into three sections namely anterior vertical, horizontal and posterior vertical canals (Fig. 9b). In accordance to these developments, the larvae started to show aggregation behavior by gathering at the bottom corner of the tank. At 24 hAH (5.75 ± 0.12 mm), rudimental dorsal fin was apparent. Mild peristalsis was observed with discharged yellowish fluid which indicated the opening of the anus. At this stage, some of the larvae showed vertical swimming behavior along the bottom corner wall of the tank.

At 30 hAH (6.09 ± 0.21 mm), thick folds of intestinal wall with frequent peristalsis was
observed. The adhesive characteristics at the tips of maxillary barbel disappeared. At the same time, taste buds were observed inside the mouth and on gill arches, and their numbers on the lips and barbels increased (Figs. 6a-6d). The sensory epithelium of the inner ear thickened and had sunk deeper from the nasal surface together with its cilia, forming deeper pit with larval growth which began to fold at 30 hAH (Fig. 7a). At this stage, the larvae started to show foraging behavior at the bottom of the tank. At this time, rotifer was offered to the larvae as first feeding. The average of mouth height and absorbed yolk at this time was 500 μm and 65.4%, respectively. However, no food was detected in the digestive tract of the larvae at this time.

At 36 hAH (6.35 ± 0.06 mm), rudimental anal fin was apparent. For the olfactory organ, one lamella with increased cilia was recognized and continued to fold to four lamellae by 4 dAH. At
this stage, feeding activity was often observed at the bottom of the tank with rotifer detected in the intestine. At the first intake of food, the mouth height and absorbed yolk were 550 \( \mu \text{m} \) and 66.1%, respectively. At 48 hAH (6.79 ± 0.19 mm), a pair of nasal barbel was evident. At 54 hAH (6.90 ± 0.22 mm), teeth started to develop and flexion of notochord was observed. The utricular and saccular maculae of the inner ear were also differentiated (Fig. 9c). At this stage, the larvae started to show cannibalistic behavior by biting the posterior body of their conspecifics. However, no mortality associated with cannibalism occurred. At 72 hAH (8.24 ± 0.27 mm), rays of dorsal, anal and caudal fins started to develop. Yolk sac absorption was 99.2% at this time and could not be visibly measured at 4 dAH onwards. However, from the histological sections, small traces of yolk can still be observed at 4 dAH. Throughout daytime, the larvae aggregated at the bottom of the tank when they were not feeding. The larvae exhibited active swimming and foraging behavior in the water column at night or when food was provided. Some of the larvae swam against water current from the water inlet.

At 5 dAH (11.38 ± 0.76 mm), dorsal and anal fin started to separate from the fin-fold and caudal fin started to fork. Pars superior and pars inferior of the inner ear developed and are fully functional by 5 dAH (Fig. 9d). As the larvae grow, the appearance of free neuromasts had increased especially on the head and trunk of the larvae (Fig. 8). However, the exact type, numbers and distribution of the free neuromasts were not able to be determined through the use of only histological sections in this study. At 6 dAH, the nostril formed two pits and became recognizable as anterior nasal pit and posterior nasal pit (Fig. 7b). At the same time, seven lamellae with increased cilia were observed in the nasal pit (Fig. 7c). Throughout the experimental duration of 15 dAH, rod cells were not able to be detected. In accordance to these developments, mortalities due to cannibalistic behavior were frequently observed from 5 dAH onwards. At 13 dAH (23.07 ± 1.86 mm), adipose fin was fully separated from the fin-fold and the larvae metamorphosed into juvenile stage.

**Discussion**

Generally, bagrid catfish showed rapid development of morphology and functionality of sensory organs, similar to other catfishes such as African catfish (Mukai et al. 2008) and sutchi catfish (Mukai et al. 2010). These developments are strongly related to the changes of behavior.
which can fundamentally suggest the possible improvement in the rearing techniques for higher survival.

From this study, a promising method to predict the commencement of first feeding can be indicated by the behavioral changes from being at a static position to swimming at the bottom of the tank corresponded with the developments of specified morphology and sensory organs while the yolk reserves are still partially absorbed. Various studies have emphasized the importance of first feeding where delay in the commencement will affect their survival (Gisbert et al. 2004; Zhang et al. 2009) and growth (Shan et al. 2008), increase the risk of deformity (Dou et al. 2005; Pena and Dumas 2005) and starvation that might lead to increased cannibalism at early larval stages. In this study, first feeding was observed at 36 hAH which was earlier than that reported by Amornsakun et al. (1996) at 52 hAH for the same species. The delay could have contributed to the negative effects shown in other studies and should be further researched for bagrid catfish.

The expression of cannibalistic behavior in bagrid catfish was first observed at 54 hAH with chasing and biting of the posterior body part (but rarely consuming whole). This behavior corresponded with the development of teeth and increased mouth height which proves early initiation at larval stages affecting the survival. In addition, cannibalistic behavior was often observed at night despite not having rod cells for better night visual. This is probably aided by the appearance of taste buds on barbels, lips, inside of the mouth and gill arches which increased its foraging sensitivity corresponded to its nocturnal behaviour. Although bagrid catfish do not refuse to feed during the day as commonly conducted, this feeding practice might cause starvation at night leading to increased risk of cannibalism. Therefore, manipulation of photoperiods (light and dark) and feeding schedules (continuous, day or night time feeding) should be considered to improve the larval survival by decreasing the cannibalism.

Through the behavioral observation in this study, the larvae tend to aggregate closely at the bottom corner of the tank during the day but showed active swimming behavior throughout the tank during the night. Aggregation behavior could have been related to the free neuromasts distributed on the head and trunk as suggested by Faucher et al. (2010). However, it is unknown whether it reflects its natural behavior or avoiding from light during the day since functions of free neuromasts were not evaluated in this study. Aggregation behavior among the larvae could have caused more physical contacts between individual which increase the risk of cannibalism as observed in African catfish (Applebaum and Kamler 2000). Hence, suitable larval density considering more on the bottom surface area to maximize the resting place for the larvae could reduce the risk of cannibalism. In addition, providing shelter through the use of 3-dimensional space area of the whole tank could be used for the same purpose and should be further investigated.

In conclusion, development of morphology and sensory organs affected the behavior of the larvae. Through the behavioral observation, specific requirements of the larvae towards the improvement of the rearing techniques could be implemented to increase the larval survival of bagrid catfish in the future.

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Bagrid catfish *Mystus nemurus* 仔魚における外部形態、
感覚器官および行動の発達

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孵化後15日目のbagrid catfish *Mystus nemurus* 仔魚の外部形態、感覚器官および行動の発達に
について調べた。仔魚はシオミズソバワシの稚魚を卵黄を66.1%吸収した孵化36時間後に（36 hAH）に
開始し、36時間の外部栄養への移行期を経て72 hAH には卵黄吸収を完了した。この時点で凝集およ
び採食行動とともに正の走形性および負の走流性がみられた。これらの行動は、半規管の発達、上顎
と下顎の歩行段階の発達、卵巣の成長に伴い、不明に成長を伴うと成長を伴う。卵巣の成長は
負の走形性および卵巣の採食行動は、卵巣への抽出量、卵巣の細胞および卵巣の出現を一致して
おり、卵巣の生长はおそらく味覚、嗅覚および感覚段階に依存していると推測される。共食い行動
は54 hAH に最初に観察され、これらが卵巣に卵巣に一致していた。以上のようなbagrid catfish 仔魚の
発育に関する基礎的研究は仔魚飼育技術の開発に有用であると考えられた。