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Larval rearing and observation of larval development of freshwater goby, *Glossogobius giuris*; a preliminary study

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Abstract

The artificial propagated larvae of freshwater goby, *Glossogobius giuris* were reared first time in the aquarium condition in Bangladesh. The larvae were reared in two different stocking densities & development stages observed from both. The larval development progress were examined under electronic microscope and stages were categorized into five distinguish stages on the basis of their ontogenetic development with time. In the first stage, the pigmented eye and large yolk sac was clearly visible within 12 hours of hatch. In the second stage within 24 hours of hatching yolk sac partially reduced, tail thickened, turbid yolk sac turned to be diluted. The yolk sac was convex interiorly and become tubular due to greater absorption of yolk sac interiorly in the third stage within 48 hours of hatching. Notochord and otolith development also noticed in this stage. In the fourth stage, within 96 hours, mandible and myomeres were developed. In the last observed stage, the yolk sac was completely disappeared within 100-120 hours of hatching.

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Introduction

Gobiidae is the second largest teleost family after Cyprinidae, and contains about 212 genera and approximately 1875 species (Nelson, 1994). The freshwater goby, *Glossogobius giuris* is an important edible freshwater fishes of Bangladesh. It is locally familiar with the name "Bele" or "Bailla" and commonly known as "Freshwater gobi". The majority of the goby fishes, being small in size, do not constitute an important fishery anywhere in Bangladesh but *G. giuris* which attains about a foot in length is notable, as it forms a fishery of some magnitude in the southern part of the country. Recent times it is one of the commercially important and highly demanded fish due to its high nutrition and palatability.

The species is widely distributed in the freshwater and estuaries in Bangladesh, India, Pakistan, Burma and Fareast. It is commonly found in the rivers, estuaries, haors, boars, beels, ponds and swamps. About 80% population is poor in the country and they depend on small size fish for their daily supply of animal protein as they are available at reasonable price (Siddique, 1985). Fish is the main source of protein in the diet of the people of Bangladesh because 60% of the animal protein comes from fish alone (Fish Week, 2012). Costa *et al.* (1984) reported *G. giuris* was one of the most important capture fisheries in our country. However, the scenario has been changed sharply and turned to highly demandable item during 2002 (Islam, 2002) which becoming exclusively for rich only. Ahmed *et al.* (1984) reported on the biochemical composition of goby fishes and noted as high protein low fat in the flesh in *G. giuris*. Though the nutritive and commercial importance of the species, very little attention has drawn to the fisheries scientists (Islam, 2004).

There are some research on the ovarian and larval development have done on some other goby fishes (*Bathygobics soporator*, Peters, 1983; *Trimma okinawae*, *T. gramistes*, *Trimatom* Sp; Sunobe, 1995; *Gobius cobitis*, Gil *et al.*, 1997; *Microscops Swinhonis*, Iwata *et al.,* 2001; *G. cruntatus,* Gil *et al.,* 2002; *Gobius paganellus*, Borges *et al.*, 2003; *Zosteisessor ophiocephalus*, Privileggi *et al.,* 2009) but no such work for the freshwater goby *G. giuris*. Considering the commercial important of the species, adequate research of the species is required. To establish breeding technique of goby, it is needed to know the detail larval biology and rearing technique. The present study was the first time trial to rear in control condition and to develop the larval developmental stages of the species.

Materials and methods

Experimental design

The experiment was conducted in the laboratory of Department of Fisheries & Marine Bioscience (FMB), Jessore Science & Technology University, Jessore, Bangladesh during July-August, 2012. The experiment was designed into two distinguish segments; i) Larval rearing and ii) Examine larval development stages. The glass aquariums (20 liters) were used for the rearing. For the rearing of larvae, there were two treatment sated i) 1000 larvae/aquarium ii) 1500 larvae/aquarium. The two different treatments were provided same feed.

Measurement of water parameters

The water parameters such as pH, DO, temperature were recorded twice in a day. The water was exchanged about 25% and wastes were siphoned out by siphoning tube every day. pH and DO was measured by pH meter (EZoDO, 7200; Taiwan) and DO meter (LTLutron YK-22DO; Taiwan) respectively.

Larval rearing and observation of development

Larvae were collected from the same laboratory which was produced by inducing first time in the country. By using glass jar and scoop net larvae were separated from their parents as soon as they were hatched. From the fourth day of stocking when noticed yolk sac was absorbed, boiled egg yolk was supplied as feed. The boiled egg yolk was homogenously mixed with water by filtering with cotton net before supply.

The larval developments were observed under electric microscope (BoEco, Germany). The pictures were taken by a digital camera (Sony, Model DSC-W520) from the eyepieces of the microscope. In addition, pictures were drawn manually by the microscopic observation. Larval development stages were categorized according to their ontogenetic development and followed mostly to Tavarutmanegul and Lin (1988), Iwata *et al.* (2001) and Privileggi (2009).

Results

Water parameters

Water parameters such as temperature, pH, DO were measured twice a day and the mean values of parameters are shown in table 1. The value range of temperature, pH and DO were 28-29^{o}C, 8.0-8.5 and 3.5-5 mg/l respectively in both treatments. There were no significant differences ($p>0.05$) among the water parameters between two treatments.

Table 1. Water quality parameters (mean ± SD) of different treatment aquarium during rearing of *G.giuris* larvae.

Water parameters	Treatment1	Treatment2
Temperature (^{o}C)	29.26 ± 0.768	29.28 ± 0.66
pH	7.5 ± 0.17	7.86 ± 0.36
DO (mg/l)	4.16 ± 0.15	4.14 ± 0.21

Rearing of larvae

The larvae of the aquarium showed similar survivability rate between two treatments. Thus the stocking density did not impact on the survival of the larvae. In the fourth day of rearing, larvae movement showed a little bit lazy in both treatments. Immediately observed under microscope and found attack of *Costia* parasite. The healthy larvae though found small amount transferred to a separate aquarium. However at the fifth day, unfortunately all larvae died in all the aquariums and settle on the bottom.

Larval development stages

The development of the larvae was examined under electronic microscope. On the basis of different morphological changes with time, the development stages of *G. guiris* were categorized into five distinctive stages (Fig. 1). The distinguish characteristics developed for each stage with time duration are mentioned in table 2.

Table 2. The prime distinguishing characteristics of each development stages of larvae of *G. giuris* in the aquarium.

Stage No.	Hrs to develop	Distinguishing characteristics
01	6-12 hrs	The pigmented eye and large outside yolk sac.
02	15-36 hrs	Progress in caudal fin development along with the pigmented eye and reduced yolk sac.
03	40-55 hrs	Progress in notocord and otolith development along with the gas bladder.
04	60-85 hrs	Development of mandible and myomeres.
05	90-120 hrs	Development of caudal fin rays. Absorption of yolksac completely.

The newly hatched larvae (12 hours) were capable of swimming but they were not effective swimmer. They tended to attach on the wall of the aquarium. The larvae swam with spiral movements to the surface, with successive swimming impulse. Yolk sac was swellen and bigger than the head, dorsal and anal finfold started to develop. Blood vessels reached to near the tail tip (Fig. 1A).

Within 36 hours of hatching, caudal fin of the larvae started to develop (Fig. 1B). The larvae increased in size though could not measure the length. Eyes of the larvae were fully pigmented. The yolk sac of the larvae was reduced. Front tip of the lower jaw extended to beyond of the front head.

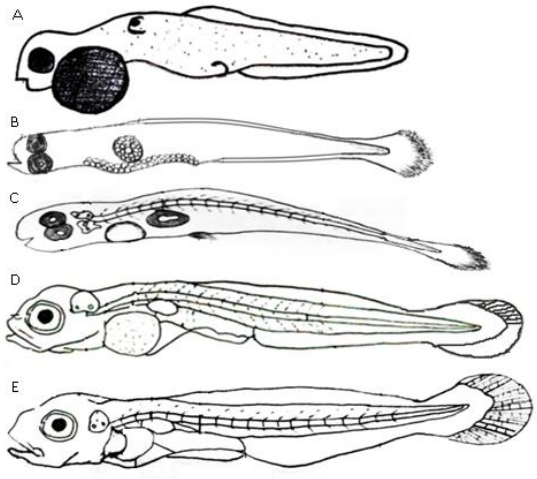

Fig. 1. Larvae development of *Glossogobius giuris*. A) just after hatching (12 hours); B) 24 hours; C) 48 hours; D) 96 hours and I) 120 hours.

Larvae swam to the surface, where they seemed to gulp air, likely to fill the gas bladder. The gas bladder visualized after 48 hours of hatching, this was pigmented and not completely filled (Fig. 1C). Caudal fin support apparently begun to form. Pigmentation of eyes was reduced greatly. Notochord and otolith started to develop within second day of hatching. The yolk was reduced to the same size as the eye and the notochord started to develop.

The gas bladder was filled during this stage (96 hours). Undeveloped anal, dorsal fin fold was observed. Caudal fin was much developed. Sagittae and lapilli otoliths were visible. Mouth, undeveloped ventral margin, myomeres (muscle bands) and mandible lower jaw were visible (Fig. 1D). Some caudal fin rays were differentied, pectoral finfold appears and the middle part of the dorsal and anal finfolds began to drop lower.

Within 120 hours of hatching larvae increased in length. The anal and dorsal fin fold was yet to develop. However, the caudal fin rays were developed. The lower jaw was projected. The yolk sac was fully absorbed (Fig. 1E) and they were suppose to take external feed. Gut and nasal pores were clearly visible.

Discussion

Water parameters

The values of water parameters were suitable for the rearing of larvae of *G. giuris* by comparing other goby fishes. There is no available information for the recommended temperature, pH and DO for the larvae of *G. giuris* to confirm the findings. Tavarutmaneegul and Lin (1988) showed temperature 26-28^{o}C, pH 5.8 – 9 and DO 7. 4-5.5 mg/l are suitable for the species of sand goby (*Oxyeleotris marmoratus*). By considering the tropical and polar region, the effect of temperature on the development of larvae of different species can clearly be shown from various research findings. Higher temperature increase larval development and lower temperature decrease the development of larvae. Gibson (1996) reported temperature 13.5- 17.5^{o}C good for the survival of the giant goby *G. cobitis*.

The water exchanged followed by Islam (2002) and exchanged at a rate of 20% daily and at a rate of 50% weekly. The water was regularly siphoned by siphoning tube to remove the wastes from the bottom of the aquarium. During rearing sand goby (*O. marmoratus*), Tavarutmaneegul and Lin (1988) were observed that exchanging water at a rate of 25% showed better growth of the larvae. To maintain hygienic condition, Gil *et al.* (2002) suggested to shiphon during the rearing of larvae.

Rearing of larvae

In the present study, newly hatched *G. guiris* showed that they are not capable for effective swimming and feeding which is similar with sand goby, *O. marmoratus* (Tavarutneegul and Lin, 1988). At the 4th day of rearing, boiled yolk was supplied in the larvae rearing tanks when it was noticed yolk sac partially or fully disappeared. However, it was not possible to notice whether the feed is taken or not. In the present study, *G. guiris* showed complete yolk sac absorption within 4-5 days which is similar with *O. marmoratus*. Sand goby (*O. marmoratus*) completed their larval stages within 3-4 days and transfer from endogenous feeder to exogenous feeder at a temperature of 26-28°C. The effect of temperature is well known for the development of larvae in many fish species (Blaxter, 1969). The *G. cobitis* larvae developed and started to take external feed within 10 days at the temperature of 12-14°C (Sparta, 1950). Despite the relatively large fecundity and high hatching rate of *G. giuris*, juvenile production was reduced by severe mortality during larval developmental stage. Mortality estimated as high as 90% larvae during 5th day of rearing which turned 100% on the 6th day. The similar higher mortality (>80%) also observed in sand goby (Tan and Lam, 1973; Tavarutmaneegul and Lin, 1988). The mass mortality of *G. giuris* may be due to attack of parasite *Costia* at the 4th day of rearing. The sources of parasite could not be identified. Apart from this, mass mortality could be occurred due to they shifted from endogenous to exogenous food sources. The larvae of *T. okinawae and T. grammistes* were also died of starvation by 3rd day after hatching (Sunobe, 1995).

Development stages

The larval developmental stage of *G. giuris* has never been identified before the present study. The present data can be compared with those of closely related *Gobius* species. The newly hatched larvae showed the typical feature characteristic of the Gobiidae (Neira & Mickiewicz, 1988; Gil *et al.*, 1997; Leis & Rennis, 2000; Iwata *et al.*, 2001). The basic larval development sequence was similar to that known for other goby species: *G. cobitis* (Gil *et al.*, 1997), *G. paganellus* (Borges *et al.*, 2003) and *Zosteriessor ophiocephalus* (Privileggi *et al.*, 2009).

The eyes and the pectoral fin were fully developed at hatching which is similar with *G. cruentatus* (Gil *et al.*, 2002) but the newly hatched larvae of *G giuris* was not capable of active swimming like *G. cruentatus*, they were attached on the aquarium wall. The mouth and anus were opened of 12 hours newly hatched larvae. The newly hatched larvae of *G. cobitis* (Gil *et al.*, 1997) and *G. paganellus* (Borges *et al.*, 2003) also showed an open mouth and anus. The newly hatched larvae had external yolk sac which was larger than the head and this phenomenon is common in goby (*G. cobitis,* Gil *et al.*, 1997; *Micropercops swinhonis*, Iwata *et al.*, 2001 and *Z. ophiocephalus*, Privileggi *et al.*, 2009). However, internal yolk sac noticed in some goby fishes such as in *G. paganellus* (Borges *et al.*, 2003). But the size of the yolk sac and head varied in different species. The pigmented eye in this stage was similar to that *G. cobitis* (Gil *et al.*, 1997), and *O. marmoratus* (Tavarutmaneegul and Lin, 1988).

The pigmentation of eyes was reduced within 36 hours of hatching (Fig. 1C) which is similar to sand goby, *O. marmoratus* (Tavarutmaneegul and Lin, 1988) and frillfin goby, *Bathygobius soporator* (Peters, 1983). The size of yolk was reduced, slightly larger than the eyes which are similar with sand goby.

Within 48 hours, the larvae had pigmented gas bladder, future caudal skeleton area swelled slightly which is very similar with *M. swinhonis* (Iwata *et al.*, 2001). Borges *et al.* (2003) found caudal fin rays and pigmented eyes, filled gas bladder at the second day of larval rearing in *G. paganellus* which was also reported in the larvae of giant goby, *G. cobitis* (Gil *et al.*, 1997).

The development characteristics of larvae within 96 hours showed similar pattern with other species but in different life period. Some shown earlier and some are in late. Like, full filled air bladder and incomplete notochord flexion observed in the present study on 4th

day which found in the 1st day larvae of *T. grammistes* (Sunobe, 1995) and at 9th day old larvae of *G. cobitis* (Gil *et al.*, 1997). Mouth, undeveloped gut, myomeres (muscle band) and mandible lower jaw were present in *G. giuris* larvae at this stage which is similar with naked goby (Johnson and Allen, 2005). The middle part of the dorsal and anal fin folds began to drop lower which occurred same in *M. swinhonis* (Iwata *et al.*, 2001) but at the age of 2nd days.

The caudal fin support apparently began to form at 5th day which was similar to *G. cobitis* (Gil *et al.*, 1997) but took longer period (6-10 days) in *M. swinhonis* (Iwata *et al.*, 2001) and in *Bathygobius soporator* (Peters, 1983). Snout, operculum, dorsal fin fold, anal fin folds, caudal fin rays observed in the present study which is very similar with naked goby (Johnson and Allen, 2001). However, Monteiro *et al.* (2008) found larvae with otolith capsule and caudal fin pigmentation, exhibiting all anal, second dorsal and caudal fin bud at the age of 5th day in *G. xanthocephalus*. The lower jaw was projected clearly and supposed to begin exogenous feeding which was reported in two day larvae of *M. swinhonis* (Iwata *et al.*, 2001).

Conclusion

Though the larvae of *G. guiris* was first time possible to rear until their larval development stage and showed mass mortality just their transition period to larvae to post larvae. Further study required for the rearing technique of the larvae and more precise examine needed for the recognized of the development stages.

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