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Natural propagation of freshwater mussels (*Lamellidens marginalis*) using vegetation in captive environment

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Abstract
Natural breeding of freshwater mussel, *Lamellidens marginalis* in captive environment had been studied to ensure the availability of juvenile mussel. A method of reproductive influence of farm animal to suit management called controlled breeding. It is aimed at enhances the production number, survival rate as well as optimization of reproductive performance in intensive system. Brood Mussels was stocked at the rate of 80/decimal in three different captive conditions *viz* Treatment 1; High aquatic vegetation without any disturbance, Treatment-2; Light aquatic vegetation with disturbance and Treatment-3; No aquatic vegetation with disturbance for breeding. As the freshwater mussel needs host fish to fulfill its life cycle, different fish species were stocked at the rate of 150/decimal. Microscopic observation indicated the occurrence of higher glochidia in the gills and fin of *Oreochromis niloticus*, *Puntius sarana* and *Heteropneustes fossilis* during breeding season. After nine month of brood mussel culture juvenile mussels were harvested from the ponds. A total of 2425±122.01 juvenile mussels per decimal were collected from the treatment 1 followed by 750±55.97 and 370±46.70 juvenile mussels from treatment 2 and treatment 3 respectively. During the research period, all activities' water quality parameters were favorable. The study revealed that ponds with high aquatic vegetation provides suitable environment during the breeding season for the breeding of freshwater mussels and high number of juveniles was produced in captive area.

Keywords: Natural propagation, glochidia, host, juvenile mussel, aquatic vegetation

Introduction
Controlled breeding is the method of reproductive influence of farm animal to suit management. It is primarily aimed at achieving synchronized breeding and enhances the production number, survival rate as well as optimization of reproductive performance in intensive system. Among all the variety of freshwater bivalves, Unionida (Bivalvia) signifies 72% (Lopes-Lima *et al.*, 2018) [25]. Inland water features including ponds, lakes and rivers in India, Bangladesh, Sri Lanka, Pakistan and Myanmar are teeming with freshwater mussels, such as Lamellidens marginalis (Lamarck, 1819), which are members of the Order Unionida. (Ghosh and Ghose, 1972; Dan *et al.*, 2001) [14, 9]. In Bangladesh, *Lamellidens marginalis* is the most common species that are available all over the country and suitable for pearl production (Hossain *et al.*, 2004) [19]. *L. marginalis* are harvested from natural sources, and this pink pearl-producing mussel has promising possibilities for commercial pearl production. (Miah *et al.*, 2000) [26]. Freshwater bivalves known as Unionoida are found in all sorts of inland waterways across the world. During the life cycle they go through a parasitic larval stage. Distribution of freshwater mussels is constrained by a special co-evolutionary interaction with fish that characterizes the unionid group (Modesto *et al.*, 2018) [42]. Freshwater mussels must adhere their larvae (Glochidia) to appropriate fish tissues (such as the gills and fins) in order to encyst and develop into juveniles (Barnhart *et al.*, 2008) [2]. In addition to acting as carriers of unionid mussels, hosts fish also provide energy and nutrients for the formation of encysted glochidia. (Denic *et al.*, 2015) [10].

Initial contact with the host fish is necessary for successful glochidial attachment; this interaction is in turn controlled by the microhabitat preferences, behavior, and abundance; the unique invasion strategy of a specific mussel species; and favorable ecosystem circumstances for both fish and mussels (Barnhart *et al*., 2008; Donrovich *et al*., 2017) [2, 11]. Five kinds of mussels have been recorded to live on the common carp, *Cyprinus carpio* (Lefevre and Curtis, 1910; 1912; Parker *et al*, 1984) [23-24, 30]. The silver barb, *Barbodes gonionotus*, tilapia, *Oreochromis niloticus*, Catfish *Heteropneustes fossilis* and *Ompok pabda* were found highly suitable for native freshwater mussel *L. marginalis* (Sku *et al*., 2021) [32]. The sole known host for the endangered *Cyprogenia aberti* is the goldfish, *Carassius auratus* (Chamberlain, 1934) [43]. *Anodonta oregomensis* and *Lasmigona compressa* have also been observed to live on the green swordtail (*Xiphophorus hellerii*) and the guppy (*Poecilia reticulata*), respectively (Chamberlain and Jones, 1929; Tompa, 1979) [8, 37]. Due to lack of attachment, encystment, and avoidance of the host immune system, only a tiny fraction of glochidia reach the juvenile stage (Kirk and Layzer, 1997) [21]. Therefore, for the growth and development of freshwater mussel species, the existence and population size of suitable hosts are crucial (Wen *et al*., 2011) [40]. Unionid population reduction and extirpation are mostly caused by anthropogenic influences such siltation, pollution, reservoir building, channelization, changing flow regimes, and introduction of non-native species, both directly and indirectly (Bogan, 1993; Williams *et al*., 1993) [6, 41]. Recent efforts to spread and restore the species in Europe have exploded due to the declining population of several freshwater mussels. (Buddensiek, 1995, Beasley & Roberts, 1999, Hastie & Young, 2003a, Preston *et al*., 2007) [7, 4, 18, 31] and elsewhere (Strayer *et al*., 2004, Barnhart, 2006) [34, 1]. *Lamellidens marginalis* mussel breeding in captivity is required since we rely heavily on nature to gather the mussel for the manufacturing of pearls. So, the study was conducted to see the production performance of *Lamellidens marginalis* in ponds using diverse fish species as host and aquatic vegetation as the shelter for glochidia.

Materials and Method

Preparation of the Experimental Ponds

Three 10-decimal-area clay ponds within the BFRI Complex served as the site for the experiment. The experimental ponds were 1-1.5 meters deep. Sand-filled pond bottom with clear water and no pollution was chosen. The ponds were set up according to protocol. The ponds' water had been completely emptied and dry. After drying, 1 kg/decimal of lime and salt were added to get rid of the worms and insects. Freshwater was added to the ponds six to seven days after the liming process.

Experimental design

To maintain the captive environment for natural breeding of mussel three methods were followed. The prepared ponds were subjected to three treatments, *viz*, Treatment 1; High aquatic vegetation, Treatment 2; Light aquatic vegetation, and Treatment 3; No aquatic vegetation. *Enhydra fluctuans, Alternanthera philoxeroides* were planted in treatment 1 and treatment 2 to provide the aquatic vegetation which was used as floating substance for glochidia.

Stocking mussels and fish

Lamellidens marginalis live adult mussels, measuring 12 to 14 cm, were gathered from several locations in the Mymensingh division. At a rate of 80/decimal, gravid mussels were chosen and put in the experimental ponds. Due to its role as a host for glochidia, fish is the prey item that is most susceptible to mussel breeding. So, in addition to mussels, a variety of fish species were stocked in the experimental ponds at a rate of 150 fish each decimal, including *Catla catla*, *Labeo rohita*, *Cirrhinus cirrhosus*, *Heteropneustes fossilis*, *Channa punctatus*, *Cyprinus carpio*, *Barbodes gonionotus* and *Oreochromis niloticus*. So that a large number of mussel glochidia may adhere to fish bodies and generate many young fish.

Culture method

Fish were fed commercial feed at a body weight-based rate of 5%. Because mussels only eat plankton and tiny benthic organisms, lime and fertilizer were added to the pond. To improve the plankton production in the pond, lime was administered twice a week at a rate of 0.5 kg/decimal, along with both inorganic (Urea: 0.1 kg/decimal, T. S. P: 0.1 kg/decimal) and organic (3 kg/decimal) fertilization.

Sample collection

The stocked fishes were observed for glochidia attachment. To observe the attachment of glochidia, sampling was done in three different ways. Monthly sampling was done in treatment 3, quarterly sampling done in Treatment 2 and no sampling done in treatment 1. These three different methods were used to see the production number of each treatment.

Monitoring water quality parameters

The method described by Tanu *et al*., (2021) [36] was used to monitor the parameters relating to water quality. For measuring temperature, a Celsius thermometer was used. Digital oxygen meters (YSI, model 58), digital pH meters (Jenway, model 3020), ammonia and alkalinity test kits (HACH test kit, FF-3 Model), and flame photometers (Buck Scientific FPF-7) for determining calcium ions were also used.

Statistical analysis

Data were gathered and entered into Microsoft Excel 2010. To determine if there was a significant difference between the treatments, a one-way analysis of variance (ANOVA) was conducted at the 5% level of significance, and Duncan's New Multiple Range Test was applied in IBM SPSS (version 25.0).

Results

Observation of glochidia

Fig 1: Fish fin observation for glochidia presence

Freshwater mussels *Lamellidens marginalis* breeds round the year having highest peak breeding season October to November (Final Report 2019). As the freshwater mussels

(*Lamellidens marginalis*) needs host to complete its life cycle, the fishes stocked together with the mussels in the experimental ponds. Fish fin, gill was checked under microscope to observe the presence of larval stage (glochidia) in fishes during the peak breeding time. Glochidia were found in the host fishes' gills and fins after microscopic examination of their scales, slime, and fins.

Harvested juvenile mussels

Fig 2: Juvenile mussel collection

After three month of breeding periods juvenile mussels were harvested from the experimental pond. The average number of young mussels taken throughout the three treatments was 2425±122.01/decimal, 750±55.97/ decimal, and 370±46.70/ decimal, respectively. The no. of juveniles differs significantly among the treatments ($p<0.05$) and the highest no. of juveniles (2425.00±122.01) was from the treatment-1, while the lowest no. was in treatment-3 (Table 1).

Table 1: No. of juvenile mussels harvested from experimental ponds

Treatments	Descriptions	No. of juveniles (Mean ± SD)/Decimal
1	High aquatic vegetation	(2425±122.01)^a
2	Light aquatic vegetation	(750±55.97)^b
3	No aquatic vegetation	(370±46.70)^c

Mean no. of juveniles with the different superscript letter was significantly different ($p<0.05$)

Water quality parameters

The experimental ponds' water quality was checked every two weeks. Throughout the duration of the investigation, the water's temperature, dissolved oxygen, pH, ammonia, and alkalinity varied from 18.2 °C-28.4 °C, 3.37mg/l-6.8mg/l, 7.1-8.61, 0.002 mg/L-0.04mg/L and 110-210, respectively (Table 2) throughout the entire period of study.

Table 2: Variations in the experimental pond's water quality metrics on a monthly basis

Months	Temp.(C)	DO (mg/l)	pH	Ammonia (mg/l)	Alkalinity
July	26.60±0.7	6.19±1.30	7.10±0.21	0.003±0.06	180±17.32
August	27.60±0.6	5.79±0.04	7.33±0.21	0.002±0.03	170±15.28
September	26.30±0.4	5.31±0.42	8.61±0.49	0.03±0.02	180±26.46
October	28.40±0.6	3.37±0.84	7.73±0.64	0.004±0.04	190±26.46
November	26.10±0.7	3.73±0.78	8.04±0.37	0.002±0.07	180±10.00
December	24.40±0.6	5.80±0.26	8.06±0.52	0.002±0.03	200±10.00
January	18.20±0.5	6.70±0.12	8.24±0.31	0.01±0.02	190±15.28
February	25.80±0.4	6.80±0.42	7.98±0.55	0.01±0.03	140±10.00
March	27.05±0.6	4.60±0.57	8.09±0.42	0.04±0.02	110±15.3
April	26.05±0.4	5.50±0.51	8.02±0.22	0.006±0.007	120±15.3
May	25.03±0.5	6.00±0.42	7.88±0.49	0.007±0.006	150±11.5
June	24.10±0.6	5.10±0.27	8.25±0.40	0.03±0.02	210±10.0

Discussion

Significantly higher number of juveniles was obtained from treatment-1 which was designed with high aquatic vegetation compared to treatemtn-2 and treatment-3 which have light aquatic vegetation and no vegetation, respectively. Although the effects of aquatic vegetation on breeding of *Lamellidens marginalis* has reported that the branches of aquatic plants provide substrate which helps glochidia to float in water for long time and grab suitable fish host. It's the matter of concern that the higher number of glochidia found in treatement 1 where aquatic vegetation was kept high.

The term "controlled propagation" refers to a variety of practices, typically carried out in a controlled environment, such as the collection of gravid females or wild glochidia, inoculation of host fish, recovery and care of juveniles, captive grow-out, and captive breeding (Lacy, 1995; USFWS and NMFS 2000; George *et al*., 2009) [22, 39, 13]. Unionid mussels must attach their larvae (called glochidia) to a typically limited variety of fish hosts in order to breed before developing into juveniles (Barnhart *et al*., 2008; Berg *et al*., 2008) [2, 5]. Naturally, in order to undergo metamorphosis and transform into young mussels, the majority of bivalve glochidia must parasitize a particular host fish for a brief period of time (Mostly on the gill). Bivalve glochidia's ability to metamorphose and develop depends heavily on their ability to parasitize a particular host fish, since most glochidia will perish if they are unable to do so. Different strains of glochidia favor various hosts. According to Haag and Warren (1999) [17], certain glochidia can parasitize several hosts, whereas others can only parasitize a small number or one host. Through microscopic examination, it was discovered that the glochidial infestation was more prevalent in the gills and fins of Oreochromis niloticus, *Puntius sarana*, and *Heteropneustes fossilis* among the fish stocked with mussels in this study Most unionid mussel species have a high level of host specificity and can only utilize one or a few, while some are known to use over 30 species, according to Strayer (2008) [33] and Trdan and Hoeh (1982) [38]. Glochidia will encyst (Fisher and Dimock, 2002; Nezlin *et al*., 1993) [12, 29] if connected to a suitable fish host, which mostly occurs, in the gills (Hookless glochidia) and fins (hooked glochidia). Glochidia are expelled from the gravid female by the siphoning process, and depending on the species and the abiotic environment, they can survive in the water column for a short period of time or up to 14 days (Bauer, 1994; Haag, 2012; Jansen *et al*., 2001) [3, 15, 20]. Drifting glochidia have the capacity to cling to a variety of surfaces, including several living things (Haag, 2013) [16].

Conclusion

Breeding of *Lamellidens marginalis* in captive condition is possible if favorable environment is provided with suitable fish host. In this study, glochida of *Lamellidens marginalis* was observed higher number in the gills and the fins of *Oreochromis niloticus* and *Puntius sarana* and *Heteropneustes fossilis*. Significantly higher number of juvenile *Lamellidens marginalis* was harvested from the ponds with high aquatic vegetation. So it can be concluded that high vegetation in breeding pond can increase the production of juvenile mussel.

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